The University of Adelaide
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Genetic variation in *Triticum aestivum* and *T. turgidum* ssp. durum for bicarbonate toxicity associated with high pH soils in South Australia.

by

Alison Millar
B. Ag. Sci. (Hons.)
University of Adelaide, South Australia

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School of Agriculture, Food and Wine
Waite Campus, University of Adelaide
Glen Osmond, South Australia

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Abstract

In South Australia alkaline soils comprise over 80 percent of the cropping region, with topsoil (0-10cm) varying from acid to alkaline, although the subsoil (>30cm) is almost universally alkaline, typically ranging from pH_w 8.0 to 10.5. In most soil solutions, the pH of alkaline soil is directly related to the concentration of HCO_3^- and CO_3^{2-} and these become toxic at a concentration of approximately 3-5mM HCO_3^- or pH 8.5. Moderate levels of tolerance had been identified in locally adapted bread wheat (*Triticum aestivum*) varieties, with the widely adapted variety Krichauff identified as the most tolerant. Commercial durum wheats (*Triticum turgidum* L. ssp. durum), however, were less tolerant to bicarbonate, which may contribute to their poor adaptation to alkaline soils in comparison to bread wheats.

Bread and durum wheat varieties and populations were grown at several alkaline sites in 2004 and 2005 to determine the influence of high pH on grain yield. At three sites increasing subsoil pH (>8.5) was found to decrease grain yield in the bread wheats, however, at six sites increasing subsoil EC was also found to significantly decrease grain yield in both the durum and bread wheats. At all the field sites, except two, soil EC was measured at toxic levels ($EC_{1:5}$ >400µS/m, Rengasamy 2002, Cooper 2004), but generally soil pH and EC failed to account for a significant percent of the variation in grain yield. In 2004 and 2005, all sites suffered from low soil moisture, particularly in 2004, and multiple abiotic stresses, such as salinity and boron, and biotic stresses, such as crown rot in the durum wheats and stripe rust in the bread wheats. The multiple yield-limiting factors likely inhibited the identification of a single soil constraint at the field sites, particularly a dynamic character such as soil pH.

In many cases the poor performance of durum wheats in comparison to bread wheats on alkaline soils has been associated with poor uptake and nutrient use efficiency by the commercial durum wheats. Durum landrace lines selected for tolerance to bicarbonate solution were generally found to take up less Ca^{2+}, Mg^{2+}, Na^+, and possibly Mn^{2+}, and more Zn^{2+}, K^+, and possibly Fe^{3+} and Cu^{2+} in the field than those lines selected with lower tolerance to bicarbonate solution. Tolerance to bicarbonate, or high pH soils, in durum wheats appears to be strongly related to the ability to extract adequate nutrition from

alkaline soils, as either HCO_3^- toxicity decreases root elongation and reduces the surface area for nutrient absorption, or HCO_3^- prevents the absorption of nutrients and depress their translocation.

A simple screening method was developed to evaluate the tolerance to bicarbonate toxicity in commercial durum and bread wheat varieties, advanced durum breeding lines and durum landraces. A difference of up to 30% in relative root length between bread wheat varieties was identified, yet no varieties were found more tolerant that Krichuaff. In the durum wheat varieties, Kalka was found to be the most tolerant, although had a significantly shorter root length than Krichauff. Several advanced durum lines, however, were found to exceed Krichauff, with parentage that included bread wheat and previously selected bicarbonate tolerant durum landraces. Bicarbonate screening of a further 484 durum landraces from mainly west and south Asia, Europe and north Africa, identified eight percent of landraces as exceeding Kalka for bicarbonate tolerance, offering a potential for improvement in the level of bicarbonate tolerance in commercial durum varieties.

Landrace lines identified as the most tolerant were crossed with Kalka, Tamaroi, or the fixed breeding line Na49/Kalka#4, for the screening of F_2 and F_3 populations. Transgressive segregation was identified in five of the seven populations, indicating that several genes were responsible for the bicarbonate tolerance response in the durum wheats. Further genetic analysis of more closely related bread and durum wheat mapping populations identified a highly significant QTL for root length in bicarbonate solution on chromosome 7A for the RAC875/Cascades and Berkut/Krichauff populations. Other possible loci identified in marker analysis, included regions on chromosome 4A (Cascades), 6D, 3D and 2B (Krichauff) and 7B (Berkut). Quantitative analysis of the Frame/Yarralinka//Pugsley and Wk/TmWLYY9//WLYY9Tm populations, suggested that at least three genes were segregating for root length in bicarbonate solution. Bicarbonate tolerance appears simply inherited, although the response may involve a number of tolerance mechanisms, including tolerance to HCO_3^- toxicity, Zn or Fe efficiency, root vigour or morphology.

Three populations, RAC875/Cascades, Frame/Yarralinka//Pugsley and Wk/TmWLYY9// WLYY9Tm were assessed in the field in 2003-2006 to determine if the difference in root

length when exposed to bicarbonate in high pH solution screens corresponded to grain yield. A general positive correlation for root length in the mildly alkaline control and grain yield was found, but unexpectedly, a general negative relationship was identified between the root length in high pH solutions (bicarbonate tolerance) and grain yield. A negative association was also identified between boron tolerance and grain yield in 2003 and 2005 for the Wk/TmWLYY9//WLYY9Tm population. The negative response of grain yield to bicarbonate and boron tolerance is likely associated with the increased absence of available subsoil moisture throughout consecutive growing seasons, from 2003-2006. Bicarbonate tolerant types may have initially provided a higher number of tillers and greater biomass, but with consistently dry finishes to the growing season, may have been prematurely water stressed, reducing the final seed set and grain fill. Further investigation is needed to understand the effect of bicarbonate toxicity and tolerance on the growth of crops throughout the growing season, particularly in relation to the soil water content.

Statement of Originality

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Alison Millar

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Chapter 1

General Introduction

The grains industry in Australia contributes in excess of $8 billion to the economy from the production of 37 million tonnes of grain (ABS 2005). South Australia accounts for 14% of production, grown on over 3.9 million hectares (ABS 2005). The majority of the grain production area is farmed as broad-acre, dryland enterprises with an average farm size of 960 hectares (5700 grain and mixed farms in SA) and rainfall ranging from 250mm to 500mm per annum. Bread wheat is the largest grain crop in Australia, with production averaging 26 million tonnes per annum, and worth $4.3 billion (ABS 2005). South Australia grows over 1.8 million hectares, producing about 25% of Australia's tonnage (ABS 2005). Annually, approximately 1.4 million hectares of SA's wheat production is grown on the highly alkaline soils of the Eyre Peninsula, Yorke Peninsula, Upper North, and Murray-Mallee (Appendix 1).

Soil mapping studies by Maschmedt (2002) identified 80% of South Australia's cropping region as having dense clay subsoils with high sodicity, salinity and pH>8.5. Subsoils (>30cm) typically ranged from pH 8.0 to 10.5, while topsoils (0-10cm) varied from acid to alkaline. Salinity (EC>4dS/m) is commonly associated with sodic soils (ESP>6) and in southern Australia the accumulation of boron also occurs naturally at depth. The prevalence of carbonate, boron and sodium toxicity, combined with various deficiencies and poor physical characteristics can, under certain conditions, severely reduce plant growth and yield. Furthermore, the presence of these constraints under minimal rainfall often means limited crop choice, due to poor adaptation of alternative crops to these conditions, and reliance of cereals, such as wheat and barley.

Significant research has been conducted on increasing wheat yield in areas affected by boron (Paull 1990, Moody *et. al.* 1993, Campbell *et. al.* 1994), salinity (Munns *et. al.* 2003, Cooper 2004), sodicity (Naidu and Rengasamy 1993, Rengasamy 2002), nutrient deficiencies (Rengel and Graham 1995, Cakmak *et. al.* 1996, McFarland 1999, Rengel 2001, Genc *et. al.* 2006, Price 2006), and various soil physical conditions (Rengasamy 2002), yet these low-rainfall, highly alkaline areas have had the lowest yield gains in South

Australia for the last half century. For example, in areas of the Eyre Peninsula and Murray Mallee (rainfall <400mm/y), wheat yield gains have only equated to 4-5 kg/ha/year from 1950 to 1991, whereas the high rainfall areas (>450mm/annum) have had yield gains of 11 kg/ha/year (Hamblin and Kyneur 1993).

In South Australia it was recognised that some varieties, notably the bread wheat *cv.* Krichauff and sister line Worrakatta, were widely adapted in South Australia, performing well on highly alkaline soils (Rathjen *et. al.* 1999). The improved adaptation of these varieties was attributed, in part, to their ability to withstand high pH conditions by tolerating the presence of high concentrations of bicarbonates. Initial bicarbonate solution screens by Lui and Rathjen (1998) found moderate levels of tolerance in many of the locally adapted bread wheat varieties, with *cv.* Krichauff appearing to be the most tolerant. No attempts have been made since this time to identify genetic variation for tolerance to bicarbonates in bread wheats, and only a few reports exist of genetic variation in other grain crops (Coulombe *et. al.* 1984), although the variation is often confounded by other genetic mechanisms, such as, root vigour, root morphology or Zn and Fe efficiency (Hajiboland *et. al.* 2003).

The analysis of soil-based problems is often hampered by the complexity of physical and chemical constraints, which often occur simultaneously, and can vary under different seasonal conditions. Recognising what is limiting crop growth in a particular environment is essential in order to identify possible solutions.

Durum wheat has a short breeding and production history in South Australia, only grown commercially since 1991, but has considerable economic benefits to farmers. Generally, durum wheat has received a $30-40/t price advantage over Australian Hard (AH) or Australian Premium White (APW) wheat, although the price advantage reached $150/t in 2007, and is currently $100/t above AH and APW in 2008. With the price advantage, durum wheat has the potential to become a reliable high value option to bread wheat. Durum has mainly been grown on the higher rainfall areas of the northern Mt Lofty Ranges on alluvial soil types (Blanchtown clay), where durum has had a higher gross return than any other crop. The price advantage saw production increased steadily in South Australia from 1991, reaching a high in 2001/02 of 350,000t, but has since declined to

approximately 150,000t per annum. The recent decline in production has been attributed to a succession of lower than average rainfall years, the fear of crown rot (*Fusarium pseudograminearum* and *F. culmorum*), inadequate price projections, and poorly adapted varieties incapable of tolerating South Australia's soil conditions.

The release of the South Australian bred variety Kalka, with improved crown rot tolerance and boron tolerance has been considered a step towards overcoming durum's adaptational problems. However, further improvements are necessary if the durum industry is to achieve its national production target of over one million tonnes per annum (Gordon 2006). To reach this amount, production has to increase in traditional growing areas of the mid North, but also expand into new production areas, such as the Mallee areas of South Australia. Unfortunately, durum wheat is poorly adapted to the low rainfall, sandy surface soils and highly alkaline subsoil areas of the state, and is unable to maintain yield when challenged by environmental stress.

In areas where the annual rainfall is below 400mm bread wheat generally substantially out-yields durum wheat, reducing or eliminating durum's economical advantage. The poorer yield of durum, relative to bread wheat on alkaline soils, has been attributed to its greater sensitivity to water and heat stress (Zubaidi *et. al.* 1999), poor efficiency for nutrient deficiencies such as Zn and Mn (Rengel and Graham 1995), susceptibility to root and crown diseases (Kirkegaard *et. al.* 2004), accumulation of higher concentrations of Na (Munns *et. al.* 2003, Cooper 2004), and added sensitivity to toxicities, such as boron and bicarbonates (Jamjod 1996, Lui and Rathjen 1998, Brooks 2004).

Toxicity to bicarbonates has received little research attention compared to the other nutritional, toxicity and disease problems, yet durum's poor tolerance to bicarbonate toxicity may have the potential to severely inhibit the expansion of durum production across much of South Australia. Few agronomic options exist for reducing the severity of bicarbonate toxicity, either through amendments to the soil or plant avoidance due to the ubiquitous nature of bicarbonates. The genetic variation previously identified in bread and durum wheats (Lui and Rathjen 1998), however, suggests genetic improvement is a feasible option for improving tolerance to soil bicarbonates.

This thesis aims to develop a simple, reliable method to screen for bicarbonate tolerance, identify genetic variation for bicarbonate tolerance in durum wheat equivalent or better than bread wheat, determine the genetic control of the bicarbonate tolerance identified, and confirm the laboratory screening results with field data from yield trials. This thesis also aims to determine the extent of bicarbonate toxicity in South Australia, across a number of seasons and sites, and in relation to other soil constraints.

Given the complexity of the bicarbonate/alkalinity response on plant growth, the methodology is strongly field based in an attempt to identify material that translates to a yield advantage in the target environment. The overall aim is to identify genetic material that can potentially increase the yield of bread and durum wheats grown in low rainfall, alkaline environments, develop a low-cost bicarbonate screening method that can be employed routinely in a breeding program, and identify breeding strategies for the incorporation of bicarbonate tolerance into commercial varieties.

Chapter 2

Literature Review

2.1 Introduction

Understanding the limitations to crop growth in any one season at a particular location requires the thorough understanding of all issues associated with the crops environment. While this is outside the scope of this literature review, the endeavour here is to explain the abiotic soil factors encountered in South Australia and how they may influence the growth of broadacre dryland crops, particularly in reference to genetic solutions for abiotic stresses affecting bread and durum wheat production.

Knowledge of the formation of the landscape and soils is important for the understanding of the diversity that exists between locations, how the soil physical and chemical problems have developed or changed over time, and how emerging agricultural practices can be influenced. Furthermore, detailed knowledge on the individual abiotic soil factors and how they behave and interact is necessary for the understanding of how these limitations may change their degree of influence on crop growth throughout the season and from year to year, depending on changes to both the environment (temperature, rainfall, biotic stress) and agronomic practices. By understanding the circumstances under which yield is limited, a more targeted breeding approach can be undertaken through the identification of suitable genetic material for enhanced tolerance or resistance to specific stresses.

2.2 Origin, characteristics and management of South Australian landscapes

The soils and landforms of Australia have evolved over many millions of years. In South Australia landscapes range from the Archaean outcrops on the Eyre Peninsula, to Aeolian sand dunes in the Mallee, to vast salt lakes in the interior (Figure 2.01). Many of the soils are pedologically very old, developed from deep weathering and movement by wind and water to bring the continent to a state of low relief and often, poor fertility.

Figure 2.01: Geological map of South Australia (Geological survey of South Australia 1969).

2.2.1 Origin of landscapes along the South Australian Cereal Belt

The earth's crust evolved some 4500 million years ago and is continuously undergoing change through crustal movement, folding and uplifting, depressions and troughs, and weathering and deposition. For most of Australia's geological history it has been part of the larger continent Gondwanaland. The southern coast of Australia was linked to Antarctica, so that much of the formation of South Australia occurred as an internal land area until the late Jurassic (150Ma), when a rift began to develop between the continents (Figure 2.02) (McKenzie *et. al.* 2004). Prior to the separation of Gondwanaland many of the current cratons and sedimentary basins had already evolved (Figure 2.03).

The Gawler Craton was formed Precambrian comprising of Archaean to Mesoproterozoic rocks that stabilised as a crystalline basement, with little deformity since about 1450Ma (Parkin 1969). East of the Gawler Craton tectonic movement in the Neoproterozoic (or Adelaidean) (1000-500Ma) resulted in the down-warping and folding to form the Adelaide Geosyncline. The erosion of nearby cratons during this time formed a thick sedimentary layer along the developing trough. In the early Ordovician (500-480Ma) sediments of the Adelaide Geosyncline were folded and uplifted to form a major fold belt from Kangaroo Island to the northern regions of the state. The western edge of the Adelaide Geosyncline suffered only gentle folding to form the Spencer Shelf in the south and Stuart Shelf in the north, which overlies the north-eastern part of the Gawler Craton.

By mid to late Devonian time (350-380Ma) most of the Australian continent had become a relatively stable crustal unit and basin development in South Australia was entirely intracratonic (Parkin 1969). During the late Jurassic (150Ma) a rift began to develop along the southern margin of the continent beginning the separation of the Australian and Antarctic crustal plates. As Australia drifted northward the gap widened, creating a continental margin along the southern coastline around 96 million years ago (McKenzie *et. al.* 2004). Shallow seas flooded much of the continent during the Cretaceous period, covering large areas with marine sediments and flattening the continent, before eventually receding.

Figure 2.02: Geographical time scale, including chronostratgraphic intervals associated with South Australia (Drexel *et. al.* 1993).

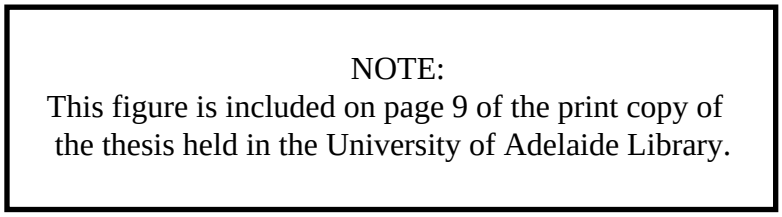


Figure 2.03: Geological provinces of South Australia (Geological survey of South Australian, Dept. Mines and Energy 1993).

In the late Cretaceous and early Tertiary Australia's vegetation was dominated by rainforests and most of the continents climate was humid. The wet climate produced widespread and strong, deep weathering, forming soils that still cover large parts of Australia (McKenzie *et. al.* 2004). The northward drift of Australia and other tectonic movements of the earth's plates triggered a change in the ocean currents, leading to a sudden series of cooling events, marking the beginning of the Tertiary period.

Marine sedimentation commenced in the St Vincent and Murray Basins during the Eocene and dominated during the mid-Tertiary. The Mt Lofty and Flinders ranges continued to form along the Adelaide Geosyncline throughout the Tertiary, as the land to the west began to sink along the Torrens Hinge Zone. The resulting north-south faults produced the rift valleys extending north to Lake Torrens and led to the formation of the present Spencer and St Vincent Gulfs (Northcote 1983).

Tertiary cooling gave way to dramatic oscillations of global climate with the alternation between cold or glacial periods with warmer interglacial periods throughout the Quaternary, with each period lasting approximately 100,000 years (Twidale and Campbell 2005). The northward drift of Australia to milder latitudes led to the continents escape with minimal glaciation. However, during this period, fluctuations in sea-level and periods of severe aridity and strong winds formed many of the present day alluvial and aeolian landscapes. The last glacial phase ended around 10,000 years ago to give a climate similar to the present climate (Twidale and Campbell 2005). The relative stability of today's climate has resulted in the establishment of constant coastal margins, increased vegetation cover, rivers and streams reach equilibrium, and soils develop into their present condition.

2.2.2 South Australian regional soil characteristics

South Australian soils have formed predominately from activities during the Quaternary (1.6Ma to present) and to a lesser extent, the Tertiary period (65Ma – 1.6Ma). Numerous layers of clays, calcretes, silts and sands were formed, and subjected to erosion, deposition, weathering and leaching, under fluctuating climatic conditions and the repeated rise and fall of the sea level. The 15 million hectares of agricultural land in South Australia range from the brown calcareous soils of the Murray Basin, to the red-brown duplex soils of the Adelaide ranges, and the mottled-yellow, sandy soils of the Eyre Peninsula (Appendix 1).

2.2.2.1 Eyre Peninsula

The soils of the Eyre Peninsula formed on the remnants of the Gawler Craton with mainly younger Pleistocene and Holocene soils overlaying Archaean, Palaeoproterozoic and Mesoproterosic rocks. Three soil groups dominate across the region, the weakly developed Tenosols along the south-west coast, the strong texture-contrast sodosols around Cleve and Cummins, and the highly calcareous calcarosols in the north and central areas (Isbell 1996, McKenzie *et. al.* 2004). Inevitably, all these soils types have high amounts of calcium carbonate, particularly as most were formed on calcrete or rubbly calcareous sediments. Generally, the calcrete layers are thought to be formed by downward leaching of calcium carbonate in solution from surface materials, with re-deposition at depth as a carbonate-rich layer, under semi-arid conditions (Milnes and Hutton 1983). The origin of the calcium carbonate was most likely from calcareous dune complexes along the coastal regions and the exposed Continental Shelf during the Pleiostocene, which was carried inland as loess with the prevailing SW to S wind (Milnes and Hutton 1983).

The south-western region of the Eyre Peninsula supports little cereal production, with mainly shallow, red-brown, non-calcareous soils on calcrete. Around Elliston relict coastal dunes dominate the low, hilly terrain with numerous calcrete outcroppings (Maschmedt 2002). Further inland loamy calcareous soils, formed over rubbly calcareous sediments, intermixed with deep sands and dune systems, which occur more frequently in the north, represent the vast expanse of area used for cereal production. Much of this area has been shaped by more recent aeolian activity in the Quaternary, similar to the Molineaux formations of the Murray Basin. The constant reworking of soil with minimal leaching has generally led to a low level of fertility, and concentration of salts, boron, and carbonates in a highly alkaline and poorly structured subsoil.

North of Kimba, on the northern margins of cereal production, brown calcareous sands diminish to expose sodic, red duplex soils, formed in the clay alluvium laid down before the sandy calcareous deposits (Northcote 1983). This deeply weathered Blanchetown clay type soils offer many challenges to cereal produces. In areas where the shallow loamy topsoils have thinned, ‘magnesia patches’ (surface NaCl) are common across the highly sodic, alkaline soils of this low rainfall (<300mm/year) region.

Similar texture-contrast soils are present in the east around Cleve and Cummins, except soils were formed on basement rock and outwash sediments on a Palaeoproterozic fold belt (Drexel *et. al.* 1993). The low permeability, sodic subsoils, experience periodic waterlogging and hard-setting, but have a greater inherent fertility than the rest of the Eyre Peninsula. The Cleve and Cummins areas have mainly deeper sandy loam to loamy topsoils, which are able to support a high level of cereal production. The red-brown earths of the Cleve district have many similarities with the Adelaide ranges while the area south of Cummins has high levels of ironstone, little calcium carbonate, and is prone to acidity in surface soils (Maschmedt 2002).

The Eyre Peninsula is generally characterised by low and variable rainfall, and soils with difficult chemical and physical problems. The majority of the area is of gently undulating terrain on sand and clay sediments, rarely more than 90m above sea level (Atlas SA, 2008). Yet, the region typically produces around 45 percent of South Australia's wheat, with over 50 percent of the regions gross value of agricultural production accounted for by wheat (ABARE 2008). Significant long-term research efforts in the region have seen the effects of applied phosphorus, and later nitrogen and trace elements, changes in management practices leading to the reduction in severity of wind erosion and root diseases, and changes in crop type and crop varieties, assisting in boosting productivity.

2.2.2.2 Adelaide Ranges (Upper, Mid and Lower North)

The Adelaide ranges provide some of the most productive soils for cereal growth in South Australia, stretching from Gawler in the south to Quorn in the north, encompassing both the upper Mt Lofty and lower Flinders ranges. Within the region north-south, sub-parallel ridges and valleys represent uplifting and folding events mainly in the Proterozoic period, with soils formed on basement rock and outwash sediments (Northcote 1983). Red-brown duplex soils (chromosols) dominate in the valleys and hill slopes, extending beyond the limits of cereal production (<250mm annual rainfall) north along the Flinders ranges. In the southern areas the red-brown earths have non-sodic subsoils, but further north towards Eudunda and Burra, sodicity increases and ridges become interspersed with brown sodosols with gradational or clayey soils overlaying calcareous subsoils (Maschmedt 2002). As rainfall rapidly decreases to the east of Peterborough, calcareous soils begin to dominate.

In the nineteenth century the mid north supported most of the colony's productivity, with the areas fertile duplex type soils and rainfall varying between 350 to 650mm/year, supporting high yielding wheat production along with other crops and livestock (Atlas SA, 2008). As a testament to early settlement, hundreds of old stone buildings are scattered widely across the area and beyond the present day outer fringes, built from the abundant stone outcrops, due to a scarcity of suitable timber. A further legacy to the intensity of early agriculture, are the scores of gullies cut by water erosion across the many of the undulating slopes. Erosion was particularly apparent on the red-duplex soils during periods of bare fallow, with their surface sealing and hard-setting nature (Atlas SA, 2008). Improved tillage and management practices in the last half a century have seen water erosion reduced to a minimum and many of the gullies disappear.

Further limitations to crop production arise from various other soil properties. As rainfall decreases to the northeast and the terrain becomes more undulating, the prevalence of subsoil sodicity and transient salinity increases. Characteristically, the impermeable subsoil underlies a lighter-textured, thin topsoil layer. At times the restriction of water movement and limited root penetration into the subsoil can lead to periods of waterlogging or water deficiency because of the surface soil's low storage capacity and low permeability of the subsoil (McKenzie *et. al.* 2004). The ability to manage these landscapes, through decades of changes to farming practices, fertility management, changes in rotation and crop varieties, has led to some of the highest productivity gains in the state.

2.2.2.3 Yorke Peninsula and St. Vincent Basin

West of the Adelaide ranges, low rises and plains stretch around the Gulf of St Vincent and on the Yorke Peninsula. The region was once covered by sea in the Miocene period, but more recently soils have been formed from re-sorted carbonate-rich sandy sediments from Pleistocene aeolian deposits (Northcote 1983). Subsoils surrounding the St Vincent Gulf are inherently calcareous, highly sodic, highly alkaline and contain toxic amounts of boron and salts. Along the St. Vincent Basin soils vary considerably from deep texture contrast formed on outwash sediments, to loamy calcareous soils of Woorinen type, and reworked loess forming deep Molineaux type sands in dune systems (Figure 2.04). These latter siliceous sands are particularly prominent on the upper Yorke Peninsula near Port Broughton.

Figure 2.04: Stratigraphic sequences in the Mallee zone of South Australia (McCord 1995).

Further south along the Peninsula soils, are mainly shallow, calcareous or loamy calcarosols, except for an area to sodosols around Maitland. Where minor uplifting and weathering in the area has exposed Blanchetown clay texture contrast soils. Areas of Blanchetown clay also intermittently appear throughout the peninsula. Most of the Yorke Peninsula, however, is characterised by gently undulating terrain with calcareous soils. The region has much in common with the central Eyre Peninsula and Mallee region although the milder coastal climates have generally provided a more reliable growing season and a greater level of productivity.

2.2.2.4 Murray Mallee

The Murray Mallee region of South Australia is commonly characterised by its semi-arid environment, mallee scrub vegetation, and east-west dune systems. The dune systems represent events both in the Tertiary and Quaternary periods. Throughout the Tertiary period the region alternated between marine and non-marine conditions, with fluvial and carbonaceous sediments typical of a marsh environment, laid down intermittently with fossiliferous limestones and other marine sediments. By the mid-Tertiary the sea had begun to retreat forming stranded NW-SE beach dunes from shallow sands deposited from the weathering and erosion of the surrounding ranges (Parilla sands).

At the beginning of the Quaternary, gradual uplifting had enclosed the Murray Basin drainage system. The wet, humid climate eventually formed a series of lakes, collectively called Lake Bungunnia. Clays and fine grain silts, known as Blanchetown clays, were deposited in the fluvial environment, usually between the sand ridges formed from the stranded beach dunes (McCord 1995). The advance of the sea and erosion of landforms about one million years ago again opened up the Murray Basin and allowed Lake Bungunnia to drain. The subsequent drying of the lakebed caused the concentration and precipitation of carbonate forming Bungunnia limestone (McCord 1995). The advance of the sea across the basin eroded and reworked sediments, leaving a series of calcareous beach dunes when the sea eventually receded, called the Bridgewater formation. Erosion on ridges and slopes deposited alluvium on valley floors and flats of the calcareous dune systems, forming the Pooraka formation of clayey, fine, silty sand.

From about one million to 10,000 years ago the climate oscillated between predominately arid glacial conditions and wetter inter-glacial periods, with each glacial/inter-glacial cycle lasting approximately 100,000 years (Nickolls and Angel 2003). Arid conditions with very strong, hot, dry, south-westerly winds blew across the exposed sea floor, resulting in a layer of fine calcareous loess, called Ripon calcrete, spreading across the entire region. Aeolian processes eroded and reworked the calcareous loess and exposed Tertiary sands into linear dunes of Woorinen formation (McCord 1995). During wetter phases high lakes and groundwater levels persisted, however when conditions became drier freshwater lakes shrank to become shallow saline environments, and the longitudinal dunes of Woorinen formation were simultaneously reactivated (Bowler 1980). The present day parallel east-west dune systems were formed and stabilised at the end of the last glacial cycle some 30,000 to 15,000 years ago (Nickolls and Angel 2003). The typical landscape consisting of earthy sands at the crest of the dunes, sodic brown soils on their flanks, and red-brown loams to clays on the adjacent flats (Butler *et. al.* 1983). In the past 10,000 years, following the stabilisation of the sea level, further aeolian processes of erosion and reworking formed the Molineaux sand dunes from Tertiary sands and Bridgewater formation (Parkin 1969).

Most of the evolutionary history of the basin throughout the Tertiary and Quaternary periods can be observed in various landforms and soils across the region. Along the western margins, shallow calcareous soils were formed on the calcrete layers of both marine and aeolian origin. East of the Murray River, Woorinen soils are mixed with

Molineaux soils, forming a region of high alkalinity, sodicity, carbonates and boron, in a dry environment of less than 300mm annual rainfall.

Between Wanbi and Bordertown erosion and reworking has resulted in areas of often deep, infertile siliceous sand dunes. Molineaux (or Lowan) sands dominate, with exposed areas of Blanchetown clay around Lameroo and Pinnaroo, and exposed Parilla sands north of Bordertown. Significant areas of these less fertile, easily eroded sands remain covered with Mallee vegetation, forming the Ngarkat and Billiat Conservation Parks. Those soils that are cultivated tend to contain less carbonate, boron, and sodium and are less alkaline, but the significant absence of trace elements has caused considerable problems to cereal production. Additionally, some sandy soils have developed water repellent characters, which are not readily wetted by rain due to hydrophobic organic films produced by fungi that coat the sand grains (Hubble *et. al.* 1983).

Cereal production ceases to the south-west of Coonalpyn and Keith as soils are mainly younger coastal dunes and beach ridges formed on carbonate sediments, with inter-dunal lagoons and highly saline land closer to the coast, and include the Coorong. In the upper South-east, neutral to alkaline, sandy texture contrast soils formed on lagoonal clay or limestone, provide the landscape for some of South Australia's most productive high-rainfall pasture and livestock industries.

2.2.3 Historical and current management of the agricultural landscape

In terms of geological history the impact of humans on the landscape and soils has been quite recent. The arrival some 60,000 years ago of Aboriginals led to major changes in vegetation and wildlife, mainly through low-intensity burning (McKenzie *et. al.* 2004). A more intense impact came with the arrival of Europeans to Australia in 1788 and later to South Australia in 1836, with farming beginning on the Adelaide Plains in 1840 (Atlas SA, 2008). The area of cultivated land expanded rapidly on the near treeless plains surrounding Adelaide and in the valleys of the nearby ranges. Exploitation and exhaustion of soils occurred rapidly and agriculture steadily moved inland from the higher rainfall regions along the coast and southern Mount Lofty Ranges to the more arid and impoverished soils of the interior.

Expansion of agriculture across the state followed a series of boom and bust cycles brought on by drought, pest and disease infestations, World Wars, economic recessions, intermittent with periods of prosperity. At the turn of the 20th century wheat farmers had reached as far north as Hawker, including the Willochra Plain, between the ranges, spurred on by the exhaustion of much of the land in the older districts (Atlas SA, 2008). The discovery of the widespread benefits of the use of superphosphate in the early 1900s and the development of methods for clearing mallee scrub, allowed further expansion in the Murray Mallee and Eyre Peninsula, and restoration of many of the older cropping districts.

In an attempt to maintain income during the 1930s farmers were urged to cultivate more land, more intensely. A system of wheat-fallow was generally employed, whereby after a year of fallow with frequent cultivation to suppress weeds, wheat was sown with superphosphate on the first autumn rains. However, decades of excessive bare fallowing proved exploitive, and by the end of the 1940s soils had become severely eroded with poor structure and depleted fertility. The improvement of the general national prosperity following the second World War and during the wool boom in the 1950s, saw the move towards conservation, through the reduction in land clearing and reduced farming intensity.

One of the most significant land development schemes occurring at the time was the Ninety Mile Desert (Tiver 1988). The Ninety Mile Desert comprises about 1.5 million hectares in the upper southeast of South Australia, representing extensive tracts of siliceous sands derived from aeolian, resorted, leached A-horizons of the coastal calcareous sands (Tiver 1988). Research had begun in the region on trace element deficiencies in the 1930s to explain Coast Disease in sheep, but it was not until the field experiments of Dr David Riceman of the CSIRO during the late 1940s near Keith, which revealed that crops benefited from trace amounts of copper and zinc in addition to superphosphate (Tiver 1988), that extensive land settlement schemes commenced. Later research also indicated a deficiency of manganese, molybdenum and cobalt in some areas across the region. The application of trace elements with superphosphate on the cropping and pasture land of the Ninety Mile Desert transformed the infertile desert sands into the productive farmlands, now commonly known as the Coonalpyn Downs (Tiver 1988).

Further advancement to productivity across the state followed the increased viability of the livestock industries and subsequent development of the ‘Ley farming’ system of legume-

based pastures rotated with crops. Higher rainfall areas with acidic soils mainly used subterranean clover, while drier alkaline areas incorporated medics into the rotation. The ley farming system offered a cheap and effective method of raising wheat yields through reducing the frequency of bare fallowing prior to sowing and consequent erosion, improving the soil nitrogen status and soil fertility, and reducing the intensities of root diseases. Unfortunately, the falling profitability of the wool industry, problematic weed infested, medic pastures, and the significant wheat yield advances arising from the semi-dwarf types, converted many farmers back to higher intensity cropping.

By the 1970s wheat quotas had been introduced and with declining prices for cereal and livestock products, alternative crops and more efficient production methods were sought (Bos *et. al.* 1995). The introduction of grain legume and oilseed crops, the increasing use of nitrogen fertilisers, the development of a wider range of herbicides, and increased awareness of the need to maintain soil structure resulted in the trial of minimum tillage and continuous cropping techniques in the late 1970s and 1980s. The extent of adoption of minimum and no-till methods varied considerably across the state due to the numerous problems encountered with uptake of the technology. These problems included the incidence of CCN (Cereal Cyst Nematode), a lack of suitable machinery to handle stubble at sowing, the absence of specific herbicides and later herbicide tolerance, the unsuitability of most legumes and oilseed crops to drier regions, lack of management skills and understanding of crop-weed-disease interactions, and root problems, such as, take-all and RLN (Root Lesion Nematode) from reduced cultivation.

The eventual widespread uptake of minimum tillage cropping by the early 2000s came about mainly through new varieties, changes in management and agronomy, particularly the greater use of nitrogenous fertilisers, and from outside expertise. New varieties of wheat were released with CCN resistance, and a greater adaptation to poorer soils, with the most notable improvement being tolerance to boron toxicity. On average, the improvement of cultivars, from the early 1970s to the mid-90s, has been estimated to contribute half the total overall yield increase (Hamblin and Kyneur 1993). The increased resistance of wheat varieties to CCN and other biotic and abiotic constraints contributed to the eventual uptake of minimum-till and continuous cropping regimes though the ability to repeatedly and reliably include more years of cereals within the rotation. Extension officers and advisors had become more readily available and utilised due to the increasing scientific research

input, the greater complexity of machinery and computerisation, the wider range of chemicals, fertilisers and other products, and the increased number of market options.

As agriculture moves forward into the 21st century, many avenues for improvement still exist in terms of both productivity and sustainability. In the past 150 years dramatic changes to farming systems have occurred in a relatively short period of time. Today, dryland cereal farmers across the state still face various environmental problems such as sodicity, salinity, compaction, surface sealing, hard-setting, waterlogging, low water availability, erosion, wind blasting, alkalinity and acidity, boron, aluminium and carbonate toxicity, soil nutritional deficiencies, changing weed populations and herbicide resistance, nematodes, fungal and bacterial diseases, frost and heat, insects, snails and feral animals. History has shown that vast improvements to production can be made through ingenuity and understanding, although not always smoothly. Continuous boom and bust cycles have plagued farming since its beginning and at times changing production systems have led to serious environmental consequences. One of the great challenges for the future of agriculture is to gain a level of reliability of production, by maintaining yield under drier conditions, reducing the severity of diseases and pests, managing fertiliser requirements, reducing erosion, and increasing crop adaptation to various soil physical and chemical conditions.

2.3 Acidity and Alkalinity

The pH of the soil is an important parameter in understanding and assessing the soil condition, soil structure, possible nutrient deficiencies and toxicities, and crop suitability. Soils are described as either acid or alkaline and are measured by the term pH, which is defined as the negative logarithm to the base 10 of the hydrogen ion activity.

$pH = -\log\{H^+\}$, where $\{H^+\}$ denotes the activity of hydrogen ions.

The logarithmic relationship means that for each unit change in soil pH, there is a tenfold change in the amount of acidity or basicity. In pure deionised water the dissociation constant is 10^{-14}, such that:

$$K_w = \{H^+\}\{OH^-\} = 10^{-14} \text{ and,}$$
$$\{H^+\} = \{OH^-\} = 10^{-7}$$

A pH value of 7 is therefore regarded as neutral, values below 7.0 are acid, and those above 7.0 are alkaline or basic (Figure 2.05).

Figure 2.05: Soil pH range (Peverill *et. al.* 1999).

The pH of soil is influenced by several factors, including the type of parent material (acid or basic rocks), rainfall (leaching), decomposition of organic matter (release of H^+), nitrogen fixation by legumes (acidification), plant species (nutrient removal), nitrogen fertilisation (increase plant residues), and flooding (neutralised pH) (Glendinning 1999). Acid soils are found in areas of high precipitation, and in South Australia these are generally restricted to the Mt Lofty Ranges, South East, Kangaroo Island and Lower Eyre Peninsula, where rainfall exceeds precipitation for several months of the year (Figure 2.06). Under high rainfall conditions exchangeable cations, mainly calcium, magnesium, potassium, and sodium, are leached from the soil, allowing increased adsorption of hydrogen (H^+) and aluminium (Al^{3+}) to soil colloids (McKenzie *et. al.* 2004). In strongly acidic soils (pH_{CaCl_2}<4.5), the exchangeable cations of H^+ and Al^{3+} dominate the exchange sites. The decomposition of organic matter and addition of ammonium or sulphur fertilisers can also induce acidification, since the accumulation and breakdown of organic matter produces organic acids, commonly carbonic acid, and fertilisers with ammonium or elemental sulphur can increase the H^+ activity in the soil.

Low pH soils potentially suffer numerous growth limiting problems, particularly in soils were pH_{CaCl_2} is below 4.5. The solubility of elements such as aluminium, iron and manganese increases with increasing acidity and can reach toxic concentrations. Hydrogen ion activity may directly affect plant growth, but only at a pH_{CaCl_2} of less than 3.7 (Glendinning 1999). Aluminium (Al^{3+}) toxicity is generally considered the most significant impediment to plant growth in acid soils and has been intensively researched (Section 2.5.3). Other nutrients, such as calcium and potassium, are more readily leached since many of the exchange sites are occupied by H^+ and Al^{3+} ions, leading to deficiencies.

Figure 2.06: Area susceptible to acidity in South Australia (Maschmedt 2002).

Further problems associated with acid soils include the reduced activity of rhizobia bacteria responsible for nitrogen fixation in legumes, and lower numbers of organisms responsible for the decomposition of organic matter and mineralisation of nitrogen, phosphorus and sulphur (Glendinning 1999). Highly acidic clay soils are also generally less aggregated, causing low permeability and reduced aeration. In broadacre dryland cropping areas, acidity is managed either by using acid tolerant species or varieties, or by surface applications of lime ($CaCO_3$) or dolomite ($CaMg(CO_3)_2$). Lime reacts with the acid soil and consumes hydrogen ions, raising pH, and reducing the solubility of Al^{3+} and Mn^{2+}.

Alkaline soils account for over 80% of the cropping area in South Australia, occurring where evaporation exceeds rainfall for most of the year (Figure 2.07). In these semi-arid areas rainfall is insufficient to leach base forming cations, such as calcium, magnesium, potassium and sodium that are slowly released from the weathering of rocks and minerals, or deposited in rainfall. These cations occupy a greater percentage of the exchange sites, allowing more OH^- ions into solution. Soils dominated by calcium carbonate have a pH_w no greater than 8.3, with higher values usually caused by sodium carbonates or bicarbonates, or magnesium carbonates (Section 2.5.4). Strongly alkaline soils (pH_w >9.2) can occur when sodium carbonate is present, with pH often increasing with depth.

High pH soils, similar to low pH soils, suffer numerous physical and chemical problems. Soil structure problems associated with high pH soils tend to be related to either the calcium carbonate or sodium concentration. Highly calcareous soils tend to drain readily and have low water holding capacities, while sodic-alkaline soils tend to be impermeable with low porosity, poor water-soil relations and are dispersive in nature (Rengasamy 2002). The options for correction of alkalinity in soils used for large-scale crop production are limited. Gypsum ($CaSO_4.2H_2O$) is commonly used to reclaim sodic-alkaline soils, providing high levels of free Ca^{2+} that lowers pH and improves soil structure. However, alkaline sodicity is generally present in the subsoils (>30cm depth), making amendment a difficult and costly process. Furthermore, gypsum has no effect on calcareous soils, since the pH_w would not be lowered below 8.4. The addition of elemental sulphur has also been effective in reducing pH, but its cost limits its use to highly intensive production systems (Maschmedt 2002).

Figure 2.07: Area of alkaline soil types in South Australia (Maschmedt 2002).

Plant growth on alkaline soils is limited directly by high OH^- concentrations and indirectly by nutritional disorders and HCO_3^-/CO_3^{2-} toxicity. The direct effect of high OH^- concentrations is relatively unknown, but is thought to limit root growth (Kopittke and Menzies 2004). Excess levels of HCO_3^-, however, have been identified in several studies as adversely affecting the growth of a number of plant species (Section 2.5.4). More commonly, growth inhibition on alkaline soils has been observed as nutrient disorders, particularly iron deficiency (lime-induced chlorosis), but also deficiencies of zinc, manganese and copper.

The availability of P in the soil solution depends largely on soil pH. At low pH phosphorus ($H_2PO_4^-$) forms insoluble complexes with aluminium oxides, and at very low pH iron oxides (Figure 2.08). As pH rises above 7.2 the HPO_4^{2-} ion becomes the dominant ion in solution, however, the abundance of calcium generally associated with mildly alkaline soils (pH 7.5 –8.5) reverts the soluble phosphorus to low-solubility calcium phosphates (Troeh and Thompson 1993, Bertrand *et. al.* 2003). The low solubility of calcium phosphates means that phosphorus deficiency is common in calcareous soils, even though total soil phosphorus may be high. A rise in pH_w above 9 generally indicates the dominance of the sodium cation in the soil solution, and as such, phosphorus availability increases due to the formation of the more soluble sodium phosphates.

Iron deficiency occurs readily in alkaline soils even though total soil Fe is abundant due to the low solubility of most Fe species. At pH 3 Fe is plant available as Fe^{3+} and to a lesser extent Fe^{2+}, but for each unit rise in pH Fe solubility decreases about a thousand-fold (Miller and Gardner 1998). Inorganic Fe in soil solution hydrolises to yield $FeOH^{2+}$ and $Fe(OH)_2^+$ in the acid soil range, and $Fe(OH)_{3(aq)}$ and $Fe(OH)_4^-$ at alkaline pH (Figure 2.09). Soluble Fe is at a minimum in the alkaline pH range with the soluble Fe only reaching a concentration required to meet plant needs in strongly acidic soils. However, many organic substances, such as root microbial exudates or organic decomposition, can react with Fe to form soluble Fe compounds (James 1984, Miller and Gardner 1998). Chelates of Fe^{3+} and Fe^{2+} are the dominant forms of soluble Fe in soils solutions and often capable of supplying plant needs. Fe deficiency is most commonly observed in soils in the pH range 7.5 to 8.5, with more than 20% $CaCO_3$ due to high concentrations of HCO_3^- and the formation of relatively insoluble iron salts (Section 2.5.4).

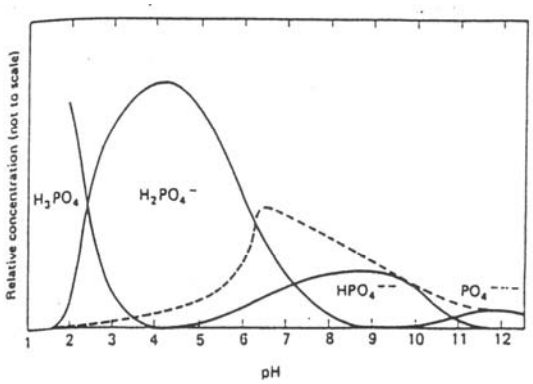


Figure 2.08: Relative proportions of phosphate ions in solution at different pH levels in a Ca-H_3-PO_4 system. The dashed line shows the upper limit on available P in solution imposed by the solubility of calcium phosphates above pH 6.5 or of iron and aluminium phosphates below pH 6.5 (Troeh and Thompson 1993).

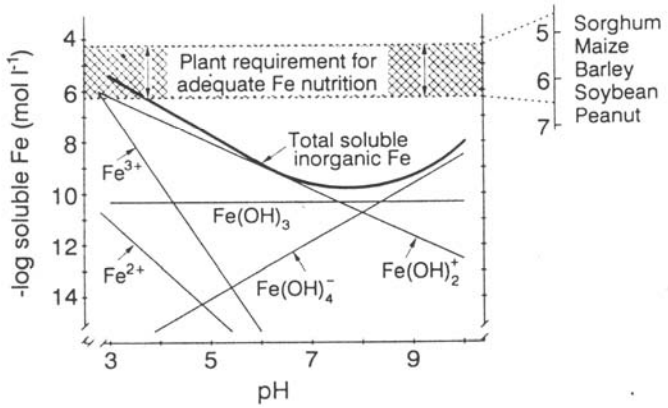


Figure 2.09: Solubility of inorganic iron species in equilibrium with iron oxides (synthetic Fe (III) in well-aerated soils in comparison to the requirement of soluble iron at the root surface of various plant species (Marschner 1997).

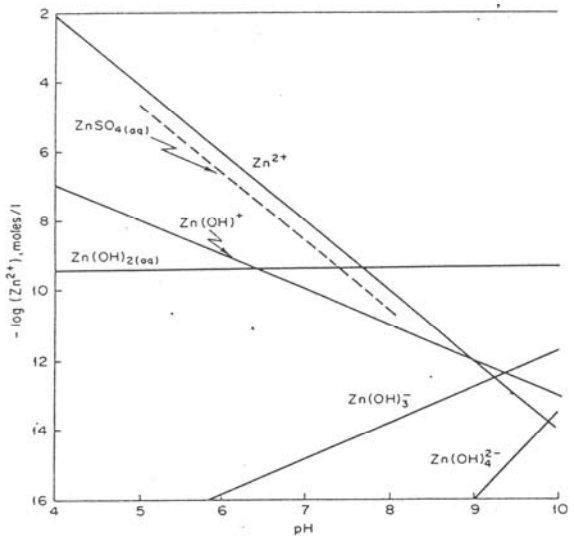


Figure 2.10: Soluble zinc species in soils in equilibrium with soil $ZnSO_4 = 10^{-3}$ (Lindsay 1972).

Similar to Fe deficiency, the occurrence of Zn deficiency is also highly pH dependent and commonly occurs in alkaline soils. The solubility of Zn^{2+} decreases with increasing pH at the rate of 100-fold per unit increase in pH (Figure 2.10). Zn deficiency is often the result of a low Zn^{2+} concentration and mobility in the soil solution, not total soil Zn, and at any given pH can depend on adsorption and desorption processes occurring in the soil matrix (Marschner 1997). In high pH calcareous soils, Zn^{2+} mainly adsorbs to clay or $CaCO_3$, with plant uptake further reduced at the root surface by high concentrations of bicarbonate in soil solution (Forno *et. al.* 1975, Trehan and Sekhon 1977). In highly alkaline soils with increased $NaHCO_3$, plant availability and uptake of Zn^{2+} are inhibited by both increased HCO_3^- activity and impaired root growth through the soil (Section 2.5.4).

Manganese can exists in multiple oxidation states, although in soil solutions Mn^{2+} is the dominant form and is associated with exchange sites on soil surfaces, while Mn^{3+} and Mn^{4+} exist predominantly in numerous oxide-rich solid phases (Norvell 1988). Soil pH influences the rates of biological and chemical reactions, such as solubility equilibria, adsorption, desorption and oxidation of Mn^{2+}, and the reduction of oxide-Mn in soils (Reisenauer 1988). The solubility of Mn decreases with increasing pH and the Mn^{2+} uptake rate by plants is more closely related to the soil pH than is the uptake of any other micronutrient (Figure 2.11). In acid soils Mn^{2+} is the predominant solution species and as soil pH decreases the proportion of exchangeable Mn increases with a decrease in Mn oxides and Mn bound to Mn and Fe oxides (Marschner 1988). In alkaline soils the solubility of Mn decreases with increasing pH and with increasing contents of carbonates and Mn oxides (Reuter *et. al.* 1988). In calcareous soils Mn is adsorbed on $CaCO_3$, oxidised on MnO_2 surfaces, or coprecipitated with Ca^{2+} forming Mn-Ca-CO_3 solid phase compounds (Jauregui and Reisenauer 1982, Marschner 1988, Reisenauer 1988). In highly alkaline soils chemical oxidation of Mn^{2+} occurs (biological oxidation at $>pH_w8.5$), but the ability of exudates from plant roots to reduce and dissolve oxidised forms of Mn in the soil decreases (Reisenauer 1988, Reuter *et. al.* 1988). Mn deficiency is common in soils of high pH, particularly in the presence of high concentrations of free carbonates, or combined with high organic matter content (Farley and Draycott 1973).

Copper is very versatile and numerous forms are likely to exist in soils. Cu has the ability to chemically interact with soil minerals and organic components, and to a lesser extent, form precipitates with sulfide, carbonate, hydroxide and other anions (McBride 1981). In

most soils environments, little Cu^{2+} exists as exchangeable ions on clays or adsorbed on external surfaces of clay silicates of oxides, instead the bulk of Cu is complexed by organic matter, occluded in oxides and substituted in primary or secondary minerals (McBride 1981). The concentration of Cu^{2+} in the soil solution is controlled by sorption/desorption reactions, with the surface of most minerals capable of adsorbing Cu ions from solution. The adsorption of Cu ions depends on the surface charge, which is strongly controlled by pH (Kabata-Pendias 2001). Only hydroxy and carbonate complexes are expected to exist commonly as significant species in solution, although sulfate and chloride may form complexes with Cu^{2+} in saline soils (McBride 1981). In soil solution below pH 8 the hydrolysis products $CuOH^{+}$ and $Cu(OH)_2^{2+}$ are the predominant Cu forms (Figure 2.12). At pH 7.5 to 8.0 the solubility of Cu^{2+} reaches a minimum, then increases above pH 8 as carbonate and anionic hydroxy complexes form (McBride 1981). At high pH, $Cu(OH)_4^{2-}$ and $Cu(CO_3)_2^{3-}$ become the predominant soluble forms of Cu. However, at both acid and alkaline pH the most common forms of Cu in soil solution are soluble organic complexes (Marschner 1997).

The control by pH of solubility equilbria and sorption/desorption reactions is a common attribute for a number of essential plant nutrients, as previously discussed, but pH may also indirectly relate to the deficiency of other macronutrients and trace elements. In calcareous soils (pH_w 7.5 – 8.5) the presence of soil carbonates are generally sufficient to suppress the availability of phosphorus, zinc, manganese, copper and iron, but saturation of the cation exchange complex by Ca^{2+} can also reduce the availability of other cations, such as Mg^{2+} and K^{+} (Naidu and Rengasamy 1993, Price 2006). Saturation of the cation exchange complex has an even greater affect on nutrient availability at pH higher than 8.5. In highly alkaline soils Na^{+} is invariably the dominant cation on exchange sites, displacing Ca^{2+} and Mg^{2+} and increasing the incidence of deficiency (Naidu and Rengasamy 1993, Tan 1998). High pH soils may also display multiple physical constraints, such as poor soil aeration and water holding capacity, which can inevitably reduce the ability of plant roots to obtain adequate nutrition and moisture. In low rainfall areas with high pH soils, crops often fail to meet potential yields based on available soil water at sowing and growing season rainfall (van Rees 1997, Nuttall *et. al.* 2005). Subsoil constraints such as salinity, sodicity (section 2.5.1), and high levels of extractable boron (section 2.5.2) restrict root growth and impede water uptake.

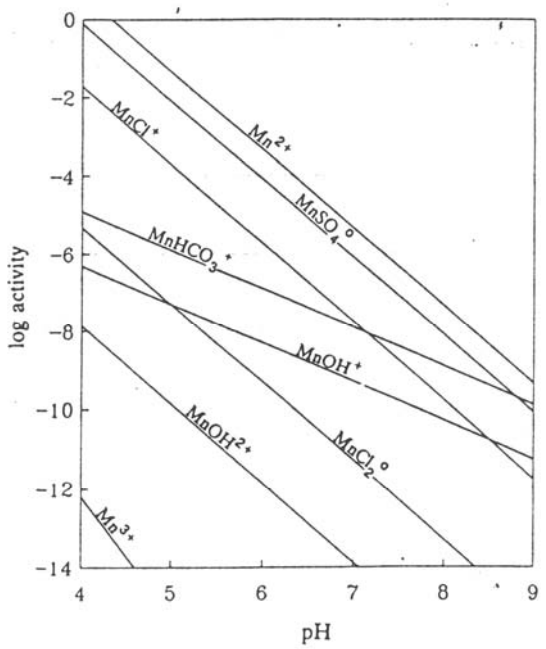


Figure 2.11: Solution species of Mn in equilibrium with manganite and pyrolusite at pe + pH 16.6, when Cl^- and SO_4^{2-} are at 10^{-3}, and CO_2 is at $10^{-4.52}$Mpa ($10^{-3.52}$ atm) (Lindsay 1972).

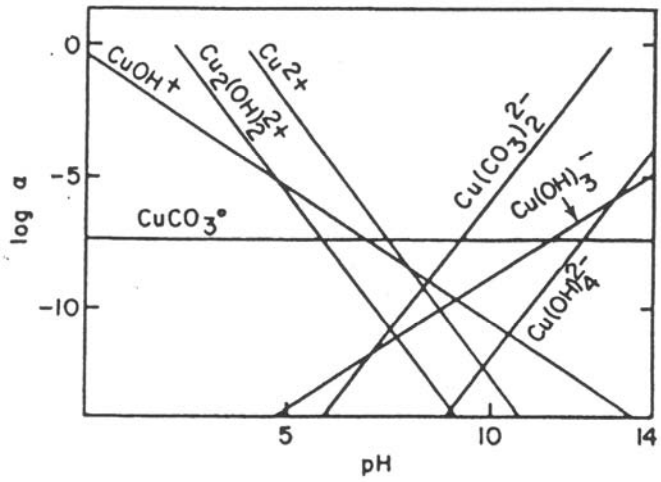


Figure 2.12: Activities of Cu^{2+} species in equilibrium with CuO as a function of pH (25ºC, ionic strength = 0, logP_{CO2} = -3.52) (McBride 1981).

The ability of plants to obtain adequate nutrition and tolerate toxic elements differs between plant species and often reflects their natural distribution and diversity (Baar and Roelofs 2002). Plant species that preferentially grow on calcareous soils (calcicoles) and highly alkaline soils have evolved adaptive mechanisms, such as a higher capacity for iron,

zinc and phosphorus acquisition and tolerance to high bicarbonate concentrations, while those growing under acid conditions (calcifuges) may have a greater tolerance to Al toxicity and Ca deficiency (Marschner 1997). For example, plants such as coffee, pineapple, tea, oil palm, and some tropical grasses and legumes are Al tolerant (Glendinning 1999) and bean, maize and apple are sensitive to zinc deficiency (Marschner 1995), and many perennial and annual dicotyledons such as citrus and lupins are prone to Fe deficiency, therefore all three groups prefer a more acid pH range for optimum growth. On the other hand, cotton, sorghum and lucerne are Al-sensitive (Glendinning 1999), most grasses (Strategy II) such as wheat and barley are less prone to Fe deficiency, and wheat, oat and pea are less sensitive to Zn deficiency (Marschner 1997), therefore allowing optimum growth at a higher pH level. Generally, most plant species prefer neutral to slightly acid soils (Table 2.01) due to the numerous nutrient deficiencies in calcareous or highly alkaline soils, although significant genotypic variation can exist within a species allowing growth over a much wider pH range (Shukla and Raj 1974, Graham *et. al.* 1992).

Table 2.01: Optimum pH range for various crops with pH measured in a 1:5 soil:water suspension (Maschmedt 2002).

The ability of plant species to tolerate specific pH ranges can also be related to the extent at which a plant can change the rhizosphere or regulate root pH. Typically, in soils ranging from pH 3 to 10, rhizosphere pH can differ from the bulk solution by up to 2 units, whereas the root apoplast and root vacuoles are strongly buffered in the mildly acid range (Grignon and Sentenac 1991, Guern *et. al.* 1991) and the root cytoplasm is in the pH range 7.2 to 7.6 (Guern *et. al.* 1991). Cellular regulation of pH is an essential feature of all living systems. For normal cell growth pH homeostasis must be maintained by an interactive balance between the proton-consuming and the proton-generating processes within the cell (Gerendas and Ratcliff 2002). The intracellular pH homeostasis is achieved by a multitude of pH regulatory mechanisms operating within the cell, and it is only under extreme stress,

such as flooding, drought, salt or very high or low external pH, that significant changes in root cytoplasmic or vacuole pH occur (Gerendas and Ratcliffe 2002). Oxygen deprivation from flooding causes acidification of the cytoplasm and eventual cell death, particularly in acid soils (Roberts *et. al.* 1984, Felle 1996). In saline soils osmotic stress can lead to slight increases in root cytoplasm and vacuole pH, and increased Na^+ influx into the vacuole can lead to a substantial increase in vacuole pH (Katsuhara *et. al.* 1997). At an external pH below 4.5 some studies have indicated that the passive influx of protons can overwhelm the capacity of the plasma membrane H^+-ATPase leading to acidification of the cytoplasm and poor root growth (Yan *et. al.* 1992). At the other end of the pH scale, high external pH has been found to only slightly increase (<0.1 pH unit) root cytoplasmic pH (Gerendas and Ratcliffe 2000).

Unlike intracellular pH, soil pH at the root surface may fluctuate considerably and differ from that in the bulk soil a few millimeters away (Pineros and Kochian 2002). The ability of plant roots to interact and actively modify the rhizosphere has a direct effect on the uptake of essential nutrients and phytotoxic elements (Pineros and Kochain 2002). Root-induced changes in the rhizosphere pH arise from imbalances in the cation/anion uptake ratio and the corresponding differences in net release of H^+ and HCO_3^- (or OH^-), and the excretion of organic acids. The extent of the change is also influence by the initial bulk soil pH and the soils pH buffering capacity (Marschner 1997). The form of nitrogen supply has a significant impact on the cation/anion uptake ratio, since the nitrate supply correlates with the net release of HCO_3^- (or H^+ consumption) and vice versa with ammonia supply (Marschner 1997). The application of ammonia to plants growing on alkaline soils can lead to acidification of the rhizosphere and enhanced mobilization of phosphorus, iron, zinc and manganese, although this affect can vary significantly between species (Gahoonia *et. al.* 1992, Marschner 1997). In alkaline soils acidification of the rhizosphere can also occur in response to nutrient deficiencies, such as from the release of organic acids in response to phosphorus, iron or zinc deficiency (Hoffland 1992), although rhizosphere acidification is limited in high carbonate soils (Hauter and Mengel 1988).

Soil pH is undoubtedly the most important parameter in assessing plant growth responses, and in southern Australia extremes of acidity and alkalinity are not uncommon. What must be remembered though, is that pH is a dynamic parameter with significant spatial and temporal differences (Rengel 2002). Soil pH can be highly variable across a small area and

change with depth, but can also fluctuate throughout a season, from year to year, or over decades. High evapotranspiration, flooding, transient salts, cation leaching, and fertilizer and vegetation type can all affect soil pH. Soil pH values are also highly dependent on methods of pH measurement, with time in storage, mixing intensity, soil to water ratio, measurement of supernatant versus soil sediment, and type of pH instrument, affecting final pH readings (Slattery *et. al.* 1999). The determination of soil pH may not be accurate measure of the specific soil solution pH in contact with plant roots, however, its use as a reference or index measurement is still one of the most important indicators of soil chemical processes and the availability of many essential nutrients or solubility of toxic ions.

2.4 Nutrient availability

Australian soils are mostly old, strongly weathered and deficient in many plant nutrients. Nitrogen and phosphorus deficiencies are common, and often associated with other nutrient deficiencies, such as sulphur, potassium and the trace elements, molybdenum, copper, zinc, manganese, cobalt, calcium, magnesium, boron and iron. Australia's native vegetation is generally well adapted to the low soil fertility, particularly phosphorus. However, with land clearing and the growth of wheat crops from the early days of South Australia's settlement, virgin land quickly became exhausted of nutrients. Initially, land settlement took place in areas of higher fertility, from the relatively fertile Adelaide Plains, along the ranges and finally pushing out onto the infertile Mallee plains of the Yorke and Eyre Peninsulas and Murray Basin. The correction of nutrient deficiencies, beginning with the discovery of the benefits of phosphate fertilisers at the turn of the 20th century, played a major role in the development of farming across the state.

2.4.1 Phosphorus

Phosphorus (P) is deficient in almost all South Australian soils in their natural state. The low P status of these soils has mostly been attributed to the low P content of the parent material and from leaching and erosion associated with intensive weathering (Williams and Raupach 1983, Moody and Bollard 1999). The weathering of P containing minerals and the mineralisation of organic-P releases P into the soil solution in the form of several

compounds, including the two orthophosphate ions, $H_2PO_4^-$ and HPO_4^{2-}, which can be taken up by plants (Price 2006).

The availability of P varies considerably depending on the P status of the soil, amount and type of clay, the soils P buffering capacity, soil pH, moisture, aeration, compaction, temperature and other nutrients (Price 2006). Soils with high clay contents are able to adsorb more P than sandy soils, with clay types varying in their affinities for P adsorption. Soils with higher affinities for P adsorption have a higher buffering capacity to released soil P. In alkaline soil P forms insoluble compounds with Ca and to a lesser extent Mg, resulting in reduced P availability. As pH falls P is released from calcium phosphates, with P availability the greatest between pH_w 6.0-7.0 (Price 2006). Availability is again reduced in acid soils as P forms compounds with Fe, Al, and Mn.

The application of P as precise fertiliser blends is widely researched and presented as the most efficient and effective means of overcoming P deficiency. Repeated fertilisation with granular P fertilisers has built up soil P reserves, with many soils now reaching a P status that requires only maintenance P applications to replace the P removed in farm products and lost by fixation and leaching (McLaughlin 2004). P deficiency has, however, proved endemic in some areas, despite regular applications of granular P fertilisers. The low rainfall areas of South Australia, such as the Eyre Peninsula and Mallee, where calcareous soils predominate, crops tend to suffer from poor early growth and other symptoms of P deficiency, even with high levels of soil P (McLaughlin 2004). P behaviour in highly calcareous soils is largely controlled by soil calcium carbonates, with most of the P precipitated as Ca phosphates in the soil in forms relatively unavailable for plant uptake (McLaughlin 2004). Research on the improvement to P nutrition of calcareous soils has generally focused on the use of fluid P fertilisers (McLaughlin *et. al.* 2003).

Adequate fertiliser application may provide a suitable answer to P deficiency, however, high soil P levels in many South Australian cropping soils provide the potential to reduce the need for fertilisers by improving the efficiency of uptake and utilisation of P in crop cultivars. Genotypic variation has been identified in cereal species and genotypes in their ability to both take up and utilise P (Osborne and Rengel 2002, Mingtan *et. al.* 2004). The limited mobility of P in the soil means that the uptake rate is generally governed by the nutrient supply rather than the capacity of root cell to take up more nutrients (Rengel and

Marschner 2005). P efficiency mechanisms to increase the availability and uptake of P tend to include, allocation of a greater proportion of assimilates to root growth, finer roots giving a larger root surface area, interaction with mycorrhiza fungi, or exudation of a range of organic compounds to increase the mobilisation of P from sparingly soluble sources (Rengel and Marschner 2005). Little is known about the reactions and processes occurring in the rhizosphere from exuded organic compounds, or the role of micro-organisms in P solubilisation. Regardless, the differences found in plant growth and P uptake efficiency in a range of cereal genotypes, suggest that improvements to the P uptake efficiency of cereal varieties are possible.

2.4.2 Nitrogen

Soil N exists in three main forms, organic-N, ammonium-N, and nitrate-N. Most N in the soil in its natural state is bound in organic matter, with very little that is plant available. Since the rocks and minerals on which the soils were formed do not contain nitrogen, the total amount of N in the soil depends on the organic matter concentration of the soil (Price 2006). The historically low levels of N in virtually all of South Australia's soil have been because of their inherently low organic matter status. However, in the last decade increasing use of nitrogen fertilisers has increased the N status of many of South Australia's cropping soils (McDonald 1989, Anderson and Hoyle 1999).

The direct response to N application on crop biomass production, when water is not limiting, has led to N fertiliser becoming one of the most expensive inputs used in present day wheat production. Numerous attempts have been made to improve the efficiency of wheat varieties for N use, with limited success, due to the complexity of the biomass, grain yield, grain protein relationship. For improvement to be made to N use efficiency (NUE) the processes of uptake, translocation, assimilation, and redistribution need to be considered. Genetic variation for nitrogen uptake efficiency and utilisation efficiency has been identified in bread wheat, however, the outcomes of breeding for NUE are complicated by the often negative relationship between grain protein and grain yield, and the need for adaptation to low N while retaining an ability to respond to high N conditions (Le Gouis *et. al.* 2000, Laperche *et. al.* 2007, Williams *et. al.* 2008).

Increasing the uptake of N, either by more prolific root systems (Liao *et. al.* 2006), or altering root transport systems (Crawford 1995, Forde 2000), has led to higher grain yield and grain protein concentrations. However, under increasing soil water deficits that restrict the uptake of N from the soil, most grain N is derived from pre-anthesis stored N (Simmons and Moss 1978). The remobilisation of N from vegetative to reproductive tissue depends largely on environmental conditions, although genetic variation has been identified in wheat cultivars giving higher grain protein levels (Desai and Bhatia 1978, Termen 1979, Halloran 1981, Cregan and Berkum 1984, van Sandford and Mcknown 1987, Cox *et. al.* 1985, Rostami and O'Brien 1996).

2.4.3 Potassium

Most of South Australia's soils contain adequate levels of potassium (K), except for some of the sandy soils in the higher rainfall districts, such as the southern coastal regions, and areas where large amounts of K have been removed through the harvesting of grain or hay (Gourley 1999). The naturally high levels of soil K in South Australia originate from rocks and sediments containing clay minerals with large amounts of K (Maschmedt 2002).

Potassium deficiency can, however, appear intermittently under certain seasonal conditions, with high NaCl concentrations. Soils containing marginal concentrations of K^+ and high levels of Na^+ may experience some degree of K deficiency due to the competitive relationship between K^+ and Na^+, restricting K^+ uptake and effectiveness in biological processes (Amtmann and Sanders 1999, Cooper 2004). Under marginal K^+ soil concentrations, improvements in the ability of plants to uptake greater amounts of K^+, avoiding deficiency, are generally approached through the selection for greater uptake and utilisation efficiency (Woodend and Glass 1993). However, the prevalence of saline conditions (both transient and dryland) in South Australia and the increasing levels of both grain and hay production, means any plant improvement to K^+ uptake must occur in the presence of high levels of Na^+.

Genotypic variation has been identified in the Triticeae for tissue K^+/ Na^+ ratios and uptake selectivity, at both moderate and high levels of salinity (Dvorak *et. al.* 1994, Dubcovsky *et. al.* 1996, Tester and Davenport 2003). Generally, advances made it reducing the uptake of Na^+ by plants also offer benefits to improving tissue K^+ concentrations. The selection of

low Na^+ accumulators, through reducing the activity of non-selective K^+/Na^+ membrane channels, increasing efflux of Na^+ from the root, enhanced compartmentalisation of Na^+, or increased re-circulation of Na^+ out of the shoot (Schachtman and Liu 1999, Tester and Davenport 2003), can reduce the incidence of potassium deficiency (Section 2.5.1).

2.4.4 Sulphur

Sulphur (S) deficiency is common in South Australia, and although less widespread than P or N deficiencies, the occurrence of S deficiency is increasing in cropping programs, especially where canola is included (Williams and Raupach 1983). The widespread use of single superphosphate fertilisers for P deficiency, which can contain over 11% S, had essentially suppressed the occurrence of S deficiency (Williams and Raupach 1983). However, with increased use of high analysis fertilisers containing little or no S, combined with higher yielding crops, greater additions of nitrogen and phosphorus fertilisers, and the immobilisation of S in organic matter from conservation tillage practices, S deficiency has been accentuated (Price 2006).

The grain S concentration and N:S ratio are particularly important in dough quality, with low S supply changing the protein composition, leading to dough with increased resistance to extension and lowered extensibility (Moss *et. al.* 1981, McRitchie and Gupta 1993). The increased S-utilisation efficiency in wheat has therefore been a target for improvement. Genotypic variation in S uptake has been found to significantly affect quality parameters in bread and durum wheat (Luo *et. al.* 2000). Research has focused on improving the uptake of S resources through the manipulation of root transporters, increased accumulation of S reserves through increased activity of the assimilatory pathway, and improved mechanisms for the remobilisation of S reserves by increasing the efficiency of the remobilisation processes, particularly the remobilisation of vacuolar sulphate (Hawkesford 2000). Generally it has been the inability to over accumulate S, and subsequently effectively remobilise S-reserves, that has restricted optimum S-use efficiency.

2.4.5 Calcium and Magnesium

Calcium (Ca) and Magnesium (Mg) are present in large amounts adsorbed to the clay minerals of most soils with absolute deficiencies in South Australia rare (Maschmedt

2002). However, problems with Ca and Mg can occur in soils of low pH, sandy to sandy loam soils in high rainfall areas, and soils were imbalances in the cation ratio exist (Williams and Raupach 1983). Calcium and magnesium concentrations are governed by the cation exchange phenomenon and held in exchange sites on the negatively charged surfaces of clay and organic matter. Ca is often the most dominant cation in many of South Australia's soils and may occupy over 70% of the available cation exchange sites (Price 2006). Other cations competing for exchange sites are potassium, magnesium, sodium and in some circumstances, manganese, ammonium and aluminium. In acidic soils, where Al^{3+}, H^{+} and Mn^{2+} are rhizotoxic, Ca^{2+} and Mg^{2+} can alleviate toxicity by displacing the cations on the plasma membrane, restoring membrane integrity (Kinraide *et. al.* 2004).

For most South Australian soils the relative concentrations of cations in soil solutions may bear no relationship to relative amount of exchangeable cations, with the proportions of the various cations on the exchange sites of more importance. The relative affinities of ions for exchange sites ($Ca^{2+}>Mg^{2+}>K^{+}>Na^{+}$) often means that in many soils sodium (Na) is the dominant cation in solution and Mg often exceeds Ca in the soil solution (Cooper 2004). When sodium becomes dominant on the exchange site, Ca deficiency can be induced. Low soil Ca:Na ratios can lead to reduced tissue Ca concentration and inhibited growth by the displacement of Ca from the membrane of root cells (Cramer *et. al.* 1985, Ehret *et. al.* 1990). The addition of Ca compounds, such as lime, gypsum and Ca fertilisers, often improves the structure of these soils by displacing the weakly held Na ion, and has been shown to improve root elongation (Kurth *et. al.* 1986), shoot growth (Cramer *et. al.* 1984), and eliminate symptoms of Ca deficiency (Ehret *et. al.* 1990, Reid and Smith 2000).

The interaction of Mg with other cations is a major factor affecting Mg availability. High concentrations of the other cations creates competition and inhibitory effects to the uptake of Mg by plants (Aitken and Scott 1999, Cooper 2004). A high Ca:Mg ratio in coarse-textured soils with low cation exchange capacity (CEC) may accentuate magnesium deficiency, along with high K rates, high Na rates, or high availability to ammonium-N (Price 2006). Generally, provided the exchangeable Mg is high enough, the ratio of Ca to Mg can vary over a wide rate because plants are able to maintain adequate balances through the absorption mechanisms of their roots (Aitken and Scott 1999, Maschmedt 2002).

2.4.6 Trace elements

Trace element research is South Australia has had a major impact on the development and success of various agricultural industries. From the mid 1920s onwards deficiencies were identified for manganese in oats (Samuel and Piper 1929), boron in apples (Atkinson 1935), copper and zinc in cereals (Riceman and Donald 1938, Piper 1938), molybdenum in clover (Anderson 1942), iron in trees and crops (Baxter 1957), and cobalt in subterranean clover (Powrie 1960). Out of the eight micronutrients essential for the growth of higher plants (Mn, B, Cu, Zn, Mo, Fe, Ni and Cl), six have been identified as being deficient in multiple soils types in South Australia, in varying amounts, and in combination with each other and macronutrient deficiencies. Deficiencies in manganese (Mn), boron (B), copper (Cu), zinc (Zn), molybdenum (Mo), and iron (Fe) are a consequence of a deeply weathered landscape, and an insufficient amount of any of these trace elements in the soil can limit plant growth.

Chloride (Cl) tends to be ubiquitous in nature with most soils containing sufficient quantities of chloride salts naturally or receive adequate amounts from rainfall or sea sprays (Marschner 1997). Cobalt (Co), while deficient in large areas of the southeast and in coastal regions of South Australia, is not considered an essential element of higher plants, but is necessary for the *Rhizobium*-legume symbiosis (Reuter *et. al.* 1988). No clear evidence exists of nickel (Ni) deficiencies is South Australia, although pot experiments using calcareous soil have shown that wheat supplied with Ni enhanced growth (Singh *et. al.* 1990).

Boron deficiency is limited in South Australia to acidic, sandy soils with low organic matter, where B is highly mobile and readily leached. Few reports exist of B deficiency being a problem in cereal production in South Australia, with deficiency mainly isolated to pasture and vegetable production (Bell 1999). B toxicity is of far greater importance, occurring in arid to semi-arid areas where leaching is limited and in soils with alkaline, sodic subsoils (Cartwright *et. al.* 1986).

Molybdenum deficiency was first reported in the Mount Lofty Ranges of South Australia in mixed pasture grasses (Anderson 1942). Since the initial observations, Mo deficiency was identified along the Fleurieu Peninsula, Kangaroo Island, Southeast, and lower Eyre Peninsula, on sandy, acid or ironstone soils in higher rainfall areas (Reuter *et. al.* 1988).

The essential requirement of Mo in nitrogen metabolism, particularly the legume *Rhizobium* symbiotic process, has resulted in Mo deficiency commonly occurring in pasture production (Srivastava 1997). Mo deficiency has not been detected in cereal crops in South Australia, but readily occurs on the naturally acidic sandplains in areas of south-western Australia (Riley 1984, Brennan 2006).

Iron deficiency occurs spasmodically in cereal crops in South Australia, mainly on highly calcareous soils, deep siliceous sands or poorly drained soils (Maschmedt 2002). In the southeast, on black and grey alkaline clays over limestone, peas, linseed and cereals, particularly oats, are prone to Fe deficiency (McFarland 1999). Fe deficiency has also been identified in cereals, particularly regrowth after hay cutting, and more severely in pulses and pasture legumes, on the southern Yorke Peninsula and deep sands of the Eyre Peninsula (McFarland 1999).

Copper deficiency is found extensively in South Australia mainly on acid and calcareous sands, but also intermittently on a range of other soil types (Maschmedt 2002). The availability of Cu may be reduced by increased pH, competition with other cations, increased soil nitrogen, type and amount of organic matter, and binding to iron and aluminium oxides (Brennan 1998b).

Manganese deficiency is widespread in South Australia since it is commonly observed on highly calcareous soils, particularly sand with more than 60% free carbonate (Maschmedt 2002). Mn deficiencies have also been recorded on some ironstone soils, acid sands, and soils that have been over limed. Where soils are highly acidic, Mn can become toxic, although toxicity is rare in South Australia. Deficiencies in Mn are often associated with high soil pH, with symptoms often developing in cereal crops when topsoils dry out in spring and moisture is sort from the highly alkaline subsoil (Price 2006). An imbalance of other nutrients such as Ca, Mg, and Fe can also exacerbate Mn deficiency.

Zinc deficiency is the most widespread nutrient deficiency in South Australia, occurring on calcareous soils of all texture ranges, including clays. Zn deficiency has also been identified on acid sands, and acid to neutral sandy or loamy texture contrast soils (Maschmedt 2002). The availability of Zn is influenced by several factors, including

decreased availability from rising pH, high P levels, adsorption on soil colloids, and adsorption by carbonates (Price 2006).

The primary method of overcoming micronutrient deficiencies has been the addition of small quantities of fertilizers, which has proven highly successful. Although, the addition of adequate micronutrient nutrition does not always ensure the absence of deficiency symptoms due to various environmental conditions, such as moisture and temperature, or interactions with other nutrients. Often deficiency symptoms can still appear throughout the season if the topsoil (0-10cm), which contains the bulk of the fertilizer at the cultivation layer, has dried out and plants are acquiring water from the micronutrient deficient subsoil. Nutrients may also interact by affecting the availability of another in the soil or by its status in the plant, through adsorption, distribution or utilization.

Generally, N and P enhance the plant requirements for micronutrients through enhanced growth, which may cause deficiency symptoms to occur where the micronutrient status of soils are marginal (Marschner 1997, Brennan 1998a,b). More specifically, high levels of P can decrease the solubility of Fe, while K fertilizer can increase Fe mobility and solubility (Brennan 1998a). High P can also depress the adsorption of Zn by plant roots either through inhibiting *Vesicular arbuscular mycorrhiza* infection or the immobilization of Zn in roots through the formation of Zn pyrate, although the impact the P-Zn interaction has in crop production has been minimal (Armour and Brennan 1999). High levels of Zn, however, can strongly depress grain yield where Cu is marginal by inhibiting Cu absorption, and vise versa when Cu levels are high, due to the competition in uptake between Cu and Zn (Brennan 1998b). Available soil Zn also shares a relationship with Fe, whereby Zn deficiency increases the Fe concentration in grasses, and similarly Fe deficiency increases the Zn concentration in grasses (Marschner 1997). The relative absence of either Zn or Fe has been associated with the plant's release of phytosiderophores (PS), acidification of the rhizosphere or release of reductants, increasing the mobilisation of both ions.

The complexity of micronutrient availability has resulted in the existence of numerous mechanisms for tolerance to deficiency between and within species. Large differences have been identified between cereal genotypes for Mn, Fe and Zn in both acquisition and internal utilization. Efficiency mechanisms identified have included enhanced internal

utilization or a lower functional requirement, a greater capacity to solubilise non-available forms into plant-available forms, or increased transport across the plasma membrane (Graham 1988, Rengel 2001). Mn efficiency in plants generally arises from their ability to extract more Mn from deficient soils, rather than internal utilization. Variation in Mn efficiency has been identified as occurring from differing levels of root exudates such as, H^+ reductants, Mn binding ligands and microbial stimulants, increased root to soil contact, and increased rates of adsorption, although little is known about the genetic control of these traits (Graham 1988, Rengel 2001).

Zn efficiency has been attributed to a complex set of plant responses that operate at varying levels within the plant (Genc *et. al.* 2006). Mechanisms identified for Zn efficiency have included a longer length and greater amount of fine root hairs (Dong *et. al.* 1995), the release of higher amounts of PS (Cakmak *et. al.* 1996, Rengel 1997, Rengel 2001), internal utilization efficiency (Rengel 2001, Genc *et. al.* 2006), differential influence on rhizosphere microbial populations (Rengel 1997), and increased carbonic anhydrase and superoxide disutase (SOD) activity (Cakmak *et. al.* 1997a), with often more than one of these being responsible for the level of Zn efficiency in a genotype. The most important aspect of Zn efficient genotypes appears to be their ability to extract and uptake more Zn from the surrounding soil.

The widespread commonality of Fe deficiency has evolved complex systems by plants to acquire adequate levels of Fe from soils from low plant-available forms. In the case of graminaceous species, PSs are released with plants taking up the ferrated PS complexes through a specific uptake system that is activated under Fe deficiency. Romheld and Marschner (1990) showed that the rate of release of PS was positively related to the Fe efficiency of different species and wheat genotypes.

Generally, studies have shown that nutrient efficiency is simply inherited, with relatively few genes involved (Scott *et. al.* 1998, Rengel 2001). Seberi *et. al.* (1999) identified two genes that segregated for Mn efficiency in a durum population with moderate tolerance to Mn deficiency. In chromosome addition lines of wheat, Mn, Zn and Cu efficiency were identified as independent traits carried by different rye chromosomes. Transferring 1R and 7R into wheat increased Zn efficiency (Cakmak *et. al.* 1997b), 5R increased Cu concentrations (Schlegel *et. al.* 1997), and 2R and 7R increased both Mn and Fe

concentrations (Rengel 2001). Efficiency has also been attributed to the A and D genomes of wheat and has been found to exist in wild relatives of wheat (Cakmak *et. al.* 1999).

The variation in micronutrient efficiency in South Australian varieties, wild relatives and rye, and the existence of simple selection methods for tolerance or deficiency opens the possibilities for the inclusion of efficient genotypes into breeding programs. However, the influence of such traits on adaptation and yield has been difficult to assess except where micronutrient deficiency had a major inhibitory influence on yield, due to the highly erratic environment of southern Australia, particularly the inconsistent rainfall, variable fertilizer use and fertilizer contamination, and interaction effects of other soil ions. Far greater yield responses have been identified in the poorly adapted durum wheats than in bread wheats for Mn (Seberi *et. al.* 1999), Zn (Graham *et. al.* 1992, Cakmak *et. al.* 1996, Genc *et. al.* 2006), and Cu (Brennan and Bollard 2004), offering a potential for increasing durum yields on South Australia's alkaline, sodic soils, where nutrient imbalances are common (Zubaidi *et. al.* 1999).

2.5 Chemical and physical properties of South Australia's agricultural soils.

The chemical and physical characteristics of soils play a crucial role in overall plant performance. The presence of multiple physical and chemical constraints, often occurring simultaneously, covers almost all of South Australia's cropping areas. Many of these constraints occur naturally in the soils, but have often been exacerbated by continuous farming practices. The most common result of physical constraints, such as compaction, surface sealing, hard-setting, waterlogging, and erosion, is reduced access of plant roots to available soil moisture and nutrients, substantially reducing final crop yield. Nutrient deficiencies are a major problem for crop growth in South Australia, and substantial gains to productivity have been achieved through the application of fertilisers and genetic improvement (Section 2.4). Unlike nutrient deficiencies, soil toxicities such as sodicity, salinity, boron, aluminium and carbonates, are far more difficult and costly to ameliorate. The only feasible method to date has been the identification of genetic mechanisms for tolerance and their subsequent incorporation into crop varieties. However, the presence of multiple stresses, with largely unpredictable interactions that are generally non-additive, produces a complex system that is not always easily understood.

2.5.1 Sodicity, salinity and sodium toxicity

The majority of South Australia's arable areas are affected by salinity and/or sodicity, with NaCl (sodium chloride) the dominant salt. The accumulation of sodium salts into Australia's soils has been through the weathering of rock during soil formation and atmospheric transport. Significant quantities of Na salts were present in rocks of both terrestrial and marine origin (Gunn and Richardson 1979), however, it is now generally accepted that the more substantial accumulation of salts has been through the deposition of minute quantities carried by wind and rain over thousands of years (Rengasamy 2002). Hutton (1976) was able to demonstrate that the deposition of Cl^- (or NaCl) decreased markedly with greater distance from the ocean, supporting the notion that marine salts, incorporated into water vapour, are precipitated inland (Isbell *et. al.* 1983, Chartres 1995, Herczeg *et. al.* 2001). Further transport of salt can then occur through aeolian activity, involving the transport of salts from eroded soils and inland playas (Chartres 1995). Aeolian activity has had a significant impact on Australia's landscape during periods of severe aridity and changing ocean depths. However, even today, dust movement and deposition leading to NaCl accumulation may still be significant in arid and semi-arid environments (Middleton 1984, McTainsh 1989).

The development of sodicity and salinity in southern Australia has been attributed to factors such as, weathering/leaching ratios, mineralogy of the soil clay fraction, permeability of the soil, the presence and type of vegetation, the amount and seasonal distribution of rainfall, the chemical composition of waters interacting with the soil profile, and the presence of soil carbonates and other materials such as oxides and hydroxides of iron and aluminium (Chartres 1995, Shaw 1999). While the affects of Na^+ accumulation and behaviour within the soil profile are complex, the development of soil Na^+ problems can be broadly characterised as sodicity, seepage salinity (dryland salinity), and transient salinity and 'magnesia patch' (dry saline land).

Sodicity in Australian soils is caused by the adsorption of Na^+ by the negatively charged sites on soil particles, leading to crusting, hardsetting, and waterlogging through the processes of swelling and dispersion. Leaching of free Na salts, such as NaCl, Na_2CO_3, $NaHCO_3$ and $NaSO_4$ from soil layers by rainfall or irrigation leave exchangeable Na^+ absorbed to soil particles, particularly clay minerals (Rengasamy 2002). In soils where Na^+

is the principle absorbed cation, continuous wetting and drying cycles increases the inter-particle distance between clay particles until completely independent of each other, resulting in clay dispersal, which can clog soil pores reducing soil permeability (Rengasamy and Olsson 1991). Soils can exhibit structural problems with as few as 6% of exchange sites occupied by adsorbed Na^+, leading Northcote and Skene (1972) to define sodic soils as those with and Exchangeable Sodium Percentage (ESP) >6.

$$\text{ESP (\%)} = \frac{\text{(Exchangeable Na)}}{\text{Cation exchange capacity}} \times 100$$

(ie. Ca^{2+}, Mg^{2+}, K^+, Na^+, Al^{3+})

However, no universally accepted value for sodicity exists due to the variability between soils. In soils where free salts are also present (saline-sodic soils), the electrolyte concentration in the soil solution counteracts the dispersive effect of adsorbed Na (Rengasamy and Olsson 1991). Furthermore, the degree of sodicity can be affected by the nature and content of organic matter, soil pH, clay mineralogy, the dominance of Ca^{2+} versus Mg^{2+}, and the soil biology (Churchman *et. al.* 1993). Instead, it is often more accurate to define a soil as being sodic when the adsorbed Na^+ reaches a concentration where it starts to affect the soil structure.

In sodic soils the swelling and dispersion of soil aggregates severely degrades soil structure by reducing the porosity and permeability, and increasing the soil strength (Rengasamy 2002). The increased density of the soil has significant consequences to water, oxygen and nutrient transport. Reduced water infiltration, movement and storage, often results in plants exhibiting early water stress and soils being either too wet or too dry. Waterlogging occurs readily after rainfall, further reducing oxygen availability, and can lead to surface crusting or hard-setting of the soil (Naidu and Rengasamy 1993). Where waterlogging or surface crusting occur, crops often suffer poor emergence and establishment, appearing as patchy growth or barren patches across a paddock (Rengasamy 2002). Poorly structured soils and bare soils are susceptible to erosion, such as gully and tunnel erosion following heavy rainfall.

Crop growth is further inhibited in sodic soils by reduced nutrient availability through the direct inhibition of root elongation and expansion due to high soil strength, reduced water movement and root uptake, and changed redox potential leading to ionic transformation potentially causing the deficiency or toxicity of ions (Naidu and Rengasamy 1993). The lack of organic matter, limited biological activity, and low mineralisation, generally

associated with sodic soils, further minimises nutrient availability. Unfortunately, the application of fertilisers to sodic soils without correcting the underlying physical and chemical problems has often not led to improved productivity (Blaike *et. al.* 1989, Naidu and Rengasamy 1993).

Improvements to the physical condition of sodic soils are often limited due the extent of the area experiencing sodicity, the cost of reclamation, and the lack of recognition of subsoil sodicity hidden under more friable, fertile topsoil. In southern Australia many dense, impermeable sodic soils are overlain with shallow (10-30cm), sandy to loamy topsoil. During prolonged dry periods, common throughout southern Australia's growing season, the subsoil is an essential source of water and nutrients. In soils with sodic subsoils crops suffer early water stress due to reduced root penetration and limited availability to water, oxygen and nutrients (Naidu and Rengasamy 1993). Crop growth is further reduced in sodic soils, where a significant reduction in root growth is expected due to $CaCO_3$ accumulating during pedogenesis, generating $NaHCO_3$ and Na_2CO_3, and raising pH above 9.2 (Naidu and Rengasamy 1993). In SA approximately 80% of the cropping region has dense clay subsoils with high sodicity and highly alkaline pH (Figure 2.13 and 2.07).

Management of subsoil sodicity has generally been aimed at improving water storage and transport, by adding ameliorants such as lime and gypsum, increasing soil organic matter, and simulating biological activity and diversity (Rengasamy 2002). Gypsum acts as a source of cations (Ca^{2+}) for the displacement of adsorbed Na^{+} on soil particles. In alkaline sodic soils gypsum also leads to the precipitation of HCO_3^{-} and CO_3^{2-} complexes, lowering pH to 8.5 (Gupta and Abrol 1990, Rengasamy and Olsson 1991, Naidu *et. al.* 1995). However, the addition of gypsum to saline-sodic soils can often have little effect or even suppress productivity due to the predominance of osmotic effects associated with salinity (Jarwal and Rengasamy 1998, Rengasamy 2002). Over 50% of South Australia's arable area suffers some form of salinity (Maschmedt 2002), reducing the effectiveness of any soil amendments for sodicity.

Figure 2.13: Proportion of land affected by sodium toxicity in South Australia (Maschmedt 2002)

In southern Australian dryland farming systems the accumulation of soluble salts (salinity) manifests as either seepage salinity, associated with a watertable at or near the surface, or dry saline land, which is not associated with a watertable. In the semi-arid conditions, common of South Australia, the salts derived from rainfall and soil weathering reactions are unable to be leached to the deep groundwater. Insufficient rainfall and dense subsoil clay layers have hindered the movement of salt, resulting in a 'bulge' of salt accumulating in soil layers below 2-10m depths from the soil surface (Figure 2.14) (Rengasamy 2002).

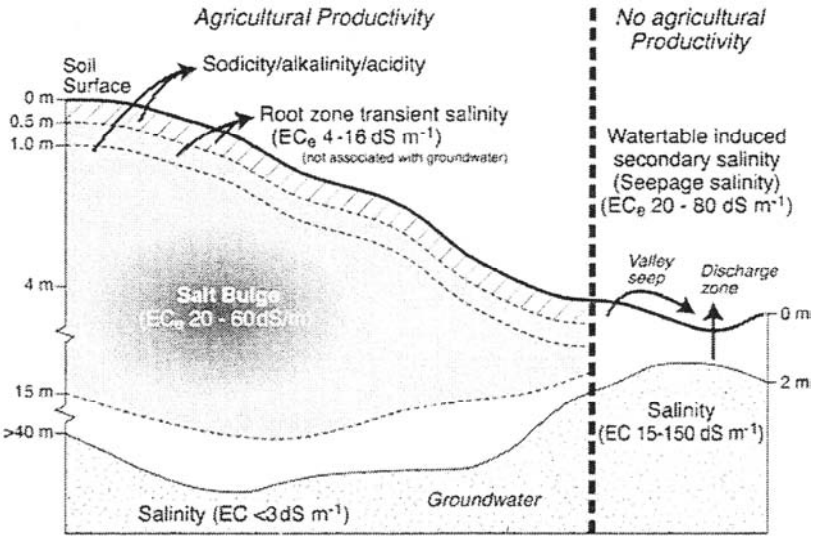


Figure 2.14: Different forms of dryland salinity in the landscape (Rengasamy 2002, 2003).

Even in the presence of high concentrations of salt in mid-level soil layers, the groundwater below a 30m depth can often be low in salts, with an EC<3dS/m (deciSemens per metre) (Rengasamy 2002). In areas were the groundwater is closer to the surface, leaching of salts has led to the accumulation of salts in the shallow groundwater, reaching an EC ranging from 15 to 150dS/m (Rengasamy 2002). Clearance of deep-rooted native vegetation and the introduction of shallow rooted pastures and annual crops has reduced water utilisation and increased water movement down the profile, leading to a higher, shallower watertable. When the watertable is within 2m depth of the soil surface, salts are able to reach the surface by capillary rise of saline water creating a discharge zone (Rengasamy 2002). In lower lying areas, as the groundwater approaches the soil surface, the surface becomes salinised and waterlogged. Seepage salinity, associated with a shallow watertable, affects 370 000 ha in South Australia (Figure 2.15), mainly in the Upper South East (Aust. Dryland Salinity Ass. 2000). The obvious visual land degradation associated with seepage salinity evokes considerable public concern.

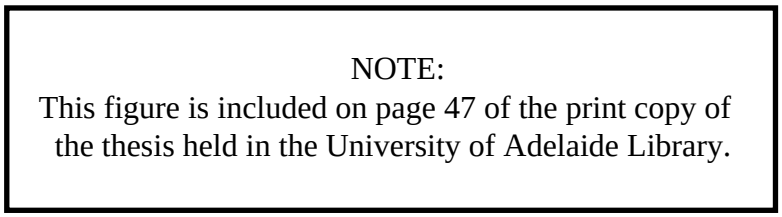


Figure 2.15: Area of watertable induced salinity in South Australia (Maschmedt 2002).

Figure 2.16: Area of dry saline land in South Australia (Maschmedt 2002).

In South Australia, however, the total affects on the State's crop productivity are limited in comparison to the less obvious affects of 'transient salinity', which is not associated with the watertable and affects a much larger area (Figure 2.16). The extensive occurrence of subsoil sodicity in southern Australia has led to the restriction of leaching through the dense subsoil, and the accumulation of detrimental quantities of salt in the root zone for optimal crop growth. The prolonged water infiltration through the subsoil layers causes temporary waterlogging and a saturated zone, called a 'perched watertable' (Rengasamy 2002). Salt accumulates in the saturation zone and remains when the water evaporates. Species with high evapo-transporation (i.e. lucerne) can concentrate more salt in the root zone, than plants with lower evapo-transpiration (i.e. cereals), which can allow leaching of salts below the root zone (Figure 2.17) (Rengasamy 2003). The effects on plant growth of salt accumulated in subsoil layers fluctuates with depth and seasonal rainfall, leading to the use of the term 'transient salinity'. Transient salinity results from limited leaching, low rainfall, transpiration by vegetation, and high evaporation during the summer (Rengasamy *et. al.* 2003).

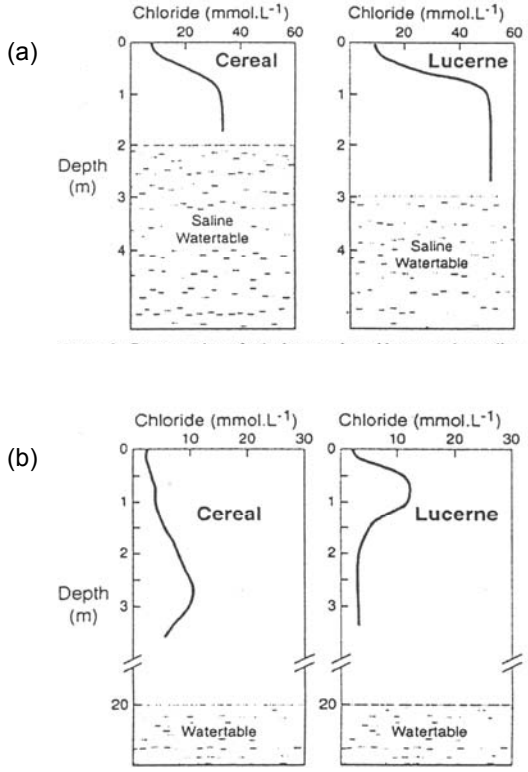


Figure 2.17: Concentration of salt by cereals and lucerne in soil layer when watertable is (a) shallow (<3m from the surface) and (b) deep (>20m from the surface) (Rengasamy *et. al.* 2003).

In areas prone to transient salinity, where topsoils are shallow and have high clay content, 'magnesia patches' may occur. The high surface salinity levels associated the magnesia patches can inhibit seed germination resulting in an often mosaic pattern of bare patches across a paddock (Kennewell 1999). Magnesia patches have been estimated to affect 35-45,000ha of South Australia, mainly on the upper Eyre Peninsula and along the coastal margins (Figure 2.18) (Kennewell 1999, Hughes and Jeffery 1994). The occurrence of magnesia patches on marginal cropping country allows few profitable management options, and reliance mainly on maintaining surface cover or the application of sand or straw mulch to more severe areas to reduce the concentration of salt in the surface soil and enhance germination (Kennewell 1999).

Magnesia patches only comprise a relatively small proportion of transient saline land, with the majority of soil types across the cropping districts having been leached of sufficient salt from the surface, even with limited rainfall, to allow initial growth. The fluctuations in the effects of transient salinity on crop growth generally follow seasonal rainfall patterns, and are therefore highly variable. After rainfall, salt in the root zone can be moved downward, or become dilute enough to have little to no detrimental affects on plant growth. As the soil dries, the salts become more concentrated and move up from deeper layers by capillary action, affecting crop growth through both increased osmotic potential of the soil solution and Na^+ toxicity (Naidu *et. al*. 1995). Under South Australia's climatic conditions, cycles of wetting and drying are common, often with prolonged dry periods during critical growth stages, such as pre-anthesis, when crops are often reliant on subsoil moisture. Subsoil (30-60cm depth) salt levels typically range from an ECe of 2 to 18dS/m with fluctuations in measurements when taken throughout the season. When soils reach an ECe of >4dS/m (40mM NaCl) crop potential declines (Rengasamy 2002).

Salinity in excess of 4dS/m can affect plant growth in multiple ways. In southern Australia salinity reduces crop productivity mainly through the lowering of the soil water potential, Na^+ and/or Cl^- ion toxicity, or the development of nutrient imbalances. The soil water potential is controlled by both the matric potential, which is the energy at which soil particles hold water, and the osmotic potential, which is the energy at which ions (such as salts) attract water (Rengasamy 2005). For plants to take up water they need to expend energy equal to the total water potential of the soil (matric + osmotic). As a soil dries the

Figure 2.18: Area of dry saline land (Magnesia patches) in South Australia (Maschmedt 2002).

energy required by plants to extract water increases, to a maximum of 1500kPa, where plants unable to draw up water, begin to wilt (Rengasamy 2005). The soil water content at which plants reach wilting point is generally dependent on the soil texture (clay fraction and type) and the soil water ionic composition (osmotic pressure). As the concentration of dissolved salts, such as NaCl, increase in the soil water fraction, the osmotic potential is raised. With a raise in osmotic potential, the energy required by plants to extract soil water is increased resulting in plants reaching wilting point at a higher soil moisture percentage (Figure 2.19). Crops growing on dry saline land tend to suffer from the rapid onset of water deficiency symptoms, particularly when extracting water from southern Australia's commonly occurring sodic, saline subsoils.

Figure 2.19: Energy (equivalent to matric plus osmotic potential) required by plants to take up water influenced by $EC_{1:5}$ and soil water content in a loamy soil (Rengasamy 2005).

The osmotic effect, associated with high salt levels in the rooting medium, has been shown to have a rapid influence on reducing cereal crop growth rates, before the onset of Na^+ toxicity from excess ion accumulation (Yeo *et. al.* 1991, Neumann 1993, Munns *et. al.* 1995, Munns 2002). The length of the initial osmotic phase is dependent on the concentration of salt in the soil, the transpiration rate, and the rate of accumulation of Na^+ in the leaves (Munns *et. al.* 1995, 2002). When high concentrations of Na^+ are able to

accumulate in the leaves, the lifespan of the leaf is severely reduced. Necrosis begins on the older leaves at the tips and margins, until the entire leaf is necrotic, effectively reducing the plants assimilate production and thus net productivity (Munns 2002).

Passive transport of Na^+ across root membranes (Cheeseman 1982), followed by transport to the shoot in the xylem transpiration stream, allows the build up of Na^+ in the cytoplasm of leaf cells. Root cells tend to be less affected by Na^+ ions, maintaining a relatively constant concentration of Na^+, by regulating efflux from the roots to either the soil or the shoot (Tester and Davenport 2003). In graminaceous species the accumulation of Na^+ in the leaf can cause a range of osmotic and metabolic problems. Metabolically Na^+ competes with K^+ for essential binding sites, but is unable to perform the role of K^+. High Na^+:K^+ ratios may reduce or disrupt enzyme activation or enzymatic processes, protein synthesis, or ribosome function (Tester and Davenport 2003). Osmotically, the accumulation of Na^+ in the apoplast of leaves can cause damage through the dehydration and turgor loss of cells and tissues, leading to leaf death (Oertli 1962, Flowers *et. al.* 1999).

In non-graminaceous species, such as grapevine, deciduous woody trees, conifers and many leguminous species, high concentrations of Cl^- have been found to be toxic (Sykes 1992, Maas 1993, Marschner 1997). Cl^- accumulation in the shoot affected plant productivity, mainly through the inhibition of photosynthesis (Flowers 1988), whereas Na^+ was retained in the woody roots and stems and had little affect on growth. Few reports exist of Cl^- toxicity in graminaceous species, except at very high tissue concentrations (Chauhan and Chauhan 1984, Weir and Cresswell 1984). In wheat (Gorham 1993), sorghum and rice (Flowers *et al* 1991, Yeo 1993), Na^+ was identified as the toxic ion when exposed to NaCl. Furthermore, many of the detrimental affects to plant growth associated with exposure to high Na^+ under saline field conditions were associated with nutrient imbalances from interactions between Na^+ and other ions.

In saline soils high amounts of Na^+ can reduce the uptake of other nutrients, such a Ca^{2+}, K^+, Mg^{2+}, Zn^{2+}, directly by interfering with transporters in the root plasma membrane, or indirectly through osmotic changes, reduced root growth, and reduced water and nutrient transport, leading to nutrient deficiencies. The role of salinity-induced calcium deficiency and application of Ca^{2+} for the amelioration of Na^+ toxicity are well documented (Marschner 1997, Cooper 2004). A high Na^+/Ca^{2+} ratio is thought to displace Ca^{2+} from

the binding sites on the outer surface of the plasma membrane of root cells, or from intracellular membranes (Lynch and Lauchli 1988), disrupting cellular Ca^{2+} homeostasis and signalling pathways (Rengel 1992). A high Ca^{2+}/Na^{+} ratio, however, restricts Na^{+} influx and transport to the leaves (Banuls *et. al.* 1991), and may stimulate the accumulation of K^{+} (Tester and Davenport 2003). Ca^{2+} is involved in several processes that may reduce the unidirectional influx of Na^{+}, such as cell wall and membrane integrity (Cramer *et. al.* 1985, Yermiyahu *et. al.* 1994, Kinraide 1999) and the modification of ion channel activity (Schroeder and Hagiwara 1989, Shubert and Lauchli 1990, Tester and Davenport 2003). The role of Ca^{2+} in reducing the affects of Na^{+} toxicity through reduced influx and transport offers evidence that exclusion of Na^{+} from the root at the initial entry site may be an important mechanism in minimising the toxic affects of Na^{+} in saline soils (Schubert and Lauchli 1990).

Minimisation of the toxic affects of Na^{+} are achieved either by the exclusion of Na^{+} from the shoots of plants, or tolerance to high leaf tissue concentrations. In South Australia, surveys of wheat varieties have shown that decades of selection of high yielding genotypes under saline field conditions has led to released varieties maintaining a low level of Na^{+} accumulation, when compared to varieties bred in other less saline areas. Cooper (2004) was able to demonstrate that the widely adapted South Australian varieties Krichauff, Halberd and Machete excluded Na^{+} to a level equivalent to the widely used Na^{+} tolerant standard Kharchia from India. These findings are supported by a range of studies on different species that identified that the ability to exclude Na^{+}, or maintain a high K^{+}/Na^{+} ratio, provided plants with a greater ability to tolerate moderately saline conditions (Gorham 1990, Colmer *et. al.* 1995, Ducovsky *et. al.* 1996, Munns *et. al.* 2000, Flowers and Hajibagheri 2001). The predominance of low Na^{+} accumulating bread wheats performing well under South Australian conditions is evidence that exclusion of Na^{+} from leaf tissue of wheat is an important mechanism in the tolerance of plants to saline environments (Rathjen *et. al.* 1999).

Low shoot Na^{+} may be achieved by a reduction in the initial entry to the root, an increase in efflux from the root, reduced loading of Na^{+} to the xylem, increased retrieval from the xylem before reaching the shoot, or increased recirculation out of the shoot to the phloem (Tester and Davenport 2003). The influx of Na^{+} into the root cortical cytoplasm is favoured energetically by differences in both concentration and voltage (Cheeseman 1982,

Tester and Davenport 2003). This passive influx of Na^+ into the roots is counteracted by the energetically expensive efflux of Na^+ out of the roots, with the balance equal to the net accumulation. The level of active efflux occurring is considered substantial, since the concentration of Na^+ in the roots remains relatively constant over time and the shoot Na^+ concentration increases more slowly than the degree that passive influx suggests (Tester and Davenport 2003). Improved salinity tolerance through a decrease in the net accumulation of Na^+ may arise from either a decrease in influx through ion channels, or an increase in efflux through a probable Na^+/H^+ antiporter (Blumwald *et. al.* 2000).

Salt tolerant plants have been identified that reduce Na^+ entry to the xylem from the root symplast, or increase the retrieval of Na^+ back out of the xylem before reaching the more sensitive shoot tissues (Tester and Davenport 2003). Evidence also exists for the recirculation of Na^+ back to the roots by the phloem to an extent where the Na^+ concentration in the shoot is reduced (Munns *et. al.* 1986, Wolf *et. al.* 1990, 1991, Tester and Davenport 2003). In many plants the exclusion of Na^+ from the actively growing young tissue is essential for salinity tolerance. In plants incapable of preventing the accumulation of Na^+ in the shoot by exclusion mechanisms, a number of internal tolerance mechanisms have developed to prevent the disruption of essential cellular functions.

Salt tolerant includers of Na^+ may redirect the accumulation of Na^+ to less sensitive parts of the plant or the cell. The accumulated Na^+ may be directed towards target leaves, such as older leaves, or to bundle sheath cells, or leaf epidermal cells, away from the critical photosynthesis cells of the leaf blade, or even secreted through salt glands onto the leaf hairs (Wolf *et. al.* 1991, Karley *et. al.* 2000). Alternatively, some evidence exists for the tolerance of Na^+ in the cytoplasm of cells in the presence of compatible solutes or damage response and repair proteins, which can act to reduce the detrimental affects of high Na^+ concentrations on protein structure and function (Cheeseman 1988, Ingram and Bartels 1996, Campbell and Close 1997).

Commonly, tissue tolerance of Na^+ is associated with the ability of plants to sequester Na^+ into vacuoles before concentrations of Na^+ increase in the cytoplasm (Greenway and Munns 1980, Blumwald *et. al.* 2000). At a cellular level the accumulation of Na^+ in the vacuoles must be osmotically balanced by the synthesis and accumulation of non-detrimental organic solutes in the cytoplasm (Hu *et. al.* 2000). The inability to osmotically

balance the accumulated Na^+ leads to internal water deficits, causing decreased cell expansion, CO_2 fixation and protein synthesis.

At the whole plant level, the exclusion of Na^+ in a saline environment requires either the increased synthesis of organic solutes such as sugars and amino acids, or the increased uptake of ions such as K^+, Ca^{2+}, or NO_3^-, to maintain the osmotic balance and prevent wilting from dehydration (Gorham *et. al.* 1985). Munns *et. al.* (2006) determined in wheat that an external NaCl concentration in excess of 50mM (5 dS/m) required a significant contribution by organic solutes to maintain turgor. The detrimental affect of osmotic imbalance between saline soils and plants often exceeds any affects associated with Na^+ toxicity. Few genotypic differences in Na^+ tolerance have been identified when the NaCl concentration in the external rooting media exceeds 150mM (15dS/m) (Husain *et. al.* 2003, Munns *et. al.* 2006). The osmotic affects associated with salinity pose a significant problem when attempting to genetically improve plant tolerance to saline conditions. Variation for tolerance to osmotic damage is limited amongst different genotypes and even within the Triticeae family (Munns *et. al.* 2006). Consequently, research has generally focused on improving tolerance to Na^+ toxicity as a method of improving productivity on saline soils.

In the Triticeae family, the exclusion of Na^+ has been one of the main mechanisms identified as conferring salinity tolerance, with large differences found between cultivars within a crop species when screened at moderate NaCl concentrations (Munns *et. al.* 2000). Low rates of Na^+ accumulation and enhanced K^+/Na^+ discrimination in bread wheat (ABD) were found controlled by a single locus (*Kna1*) on chromosome 4DL (Gorham *et. al.* 1987, Dubcovsky *et. al.* 1996). The transfer of the *Kna1* locus to durum wheats (AB) by a 4D/4B substitution produced *Kna1* recombinants with greater K^+/Na^+ discrimination, more biomass, but only under low external NaCl concentrations (Dvorak *et. al.* 1994). Other genetic mechanisms are therefore likely present in bread wheat for the control of the Na^+ tolerance.

Recently attempts have been made to improve the tolerance of durum wheats to saline conditions. Durums are less Na^+ tolerant than bread wheats, having higher rates of Na^+ accumulation and reduced K^+/Na^+ discrimination (Gorham *et. al.* 1987, Munns *et. al.* 2000). Munns *et. al.* (2000) in a screen of 54 *T.turgidum* accessions identified the durum

accession Na149 (*T.monococcum* v. C68-101/*T.turgidum* ssp durum v. Marrocos) as having low Na^+ accumulation and high K^+/Na^+ discrimination similar to bread wheat. Two dominant and interacting genes of major effect, *Nax1* and *Nax2*, where identified in the control of the Na^+ exclusion trait in Na149, which were inherited from the *T.monococcum* (Munns *et. al.* 2003, James *et. al.* 2006).

The *Nax1* gene, which mapped to chromosome 2AL, accounted for 38% of the phenotypic variation for low Na^+ accumulation (Lindsay *et. al.* 2004). *Nax1* was found to reduce the leaf blade Na^+ concentration by the increased removal of Na^+ from the xylem in the roots and by the sequestering of Na^+ to the leaf sheath as Na^+ entered the leaf (Davenport *et. al.* 2005, Munns *et. al.* 2006). The putative sodium transporter HKT7-2A of the HKT family (high affinity K^+ transporter) is a candidate gene for *Nax1*, possibly controlling Na^+ unloading from the xylem (Huang *et. al.* 2006). The *Nax2* gene has a similar mechanism to that described for the *Kna1* gene in bread wheat, having enhanced K^+/Na^+ discrimination in the leaf, while also reducing the rate of Na^+ transport to the shoot (James *et. al.* 2006). *Nax2* was mapped to chromosome 5AL, which is homoeologous to the *Kna1* locus on 4DL and coincides with the locus for a putative Na^+ transporter HkT1;5 (Byrt *et. al.* 2007).

The potential of Na149 (containing *Nax1* and *Nax2*) as a source of salinity tolerance for commercial durum wheat varieties remains uncertain. Na149 grown in 50mM NaCl solution for 10 days was able to exclude around 6 times more Na^+ from the leaf blade than the commercial durum line Tamaroi (James *et. al.* 2006). The presence of either *Nax1* or *Nax2* in near isogenic lines also reduced the Na^+ concentration, but not to the level of their combined affect in Na149 (James *et. al.* 2006). In glasshouse trials in saline soils with moderate salinity (75mM NaCl) the Na^+ exclusion trait in line Na149 had a yield improvement of 20%, although at high salinity (150mM NaCl) there was no advantage in yield (Husain *et. al.* 2003). Potentially the introgression of Na^+ exclusion genes into commercial varieties should confer a level of salinity tolerance, however, currently cultivars that have been genetically manipulated for improved Na^+ exclusion have failed to consistently compete in the field with other adapted breeding material, questioning the presumed advantage of Na^+ exclusion, particularly in high saline environments.

In South Australia extensive field testing of isogenic populations from backcrosses between Na149 and Kalka (Cooper 2004) or Tamaroi (Munns *et. al.* 2003) have failed to

consistently demonstrate an advantage in yield for the Na^+ exclusion trait. In small-scale field tests in 2003 and 2004 yields were equivocal, but in larger scale field testing over eight locations in 2005, the Na^+ excluding types in the Kalka genetic background yielded on average 9% lower, and in the Tamaroi genetic background yielded on average 14% lower, than the Kalka and Tamaroi parental types, respectively (Rathjen *pers. com*). In 2006 and 2007 the Na^+ excluding Kalka and Tamaroi lines again produced lower grain yields than the non-Na^+ excluding lines (Rathjen *pers. com*). Furthermore, advanced yield trials across NSW and SA of Na^+ excluding Tamaroi types that contain the *Nax1* and *Nax2* locus have consistently indicated that at higher yielding sites, a greater yield penalty than non-Na^+ excluding types and the yield penalty is associated with the presence of the *Nax1* locus and not the *Nax2* locus (Munns and Rathjen *pers. com*).

The large gap between the benefits in yield of Na^+ exclusion in hydroponic and glasshouse screening, and the yield penalties of Na^+ exclusion in the field, highlight the complexity of the salinity response, particularly in the field. Progress to date has been slow in the development of salt tolerant crops, even with the screening of large international collections and the identification of a wide range of tolerance (Kingsbury and Epstein 1984, Sayed 1985, Colmer *et. al.* 2005). Often gains in the field are much more modest than those estimated from glasshouse studies. Commonly, poor yield performance may be explained by the use of poorly adapted genotypes as donors of the specific trait, leading to the inclusion of large chromosome segments from 'linkage drag' into breeding lines. Alternatively, the efflux of Na^+ via membrane transporters from the xylem may be energetically expensive or exclusion of Na^+ from the roots may reduce the plants ability to tolerate osmotic stress (Deller 2007). Under field conditions salinity can also interact with numerous soil chemical and physical properties, such as high pH, sodicitiy, boron toxicity, moisture, soil type, waterlogging and O_2 availability, temperature and nutrition (Ahamad 2002, Barrett-Lennard 2003, Munns *et. al.* 2006).

For improved salinity tolerance the inclusion of a Na^+ exclusion trait alone may not be enough to confer improved yield in the field. Other traits of importance may include the maintenance of K^+ or Ca^{2+} uptake in the face of high external Na^+ (Gorham *et. al.* 1993, Cooper 2004), osmotic adjustment to counteract increased Na^+ accumulation in specific tissues, cells or around roots, improved water use-efficiency, or morphological and developmental patterns that conserve water (Munns *et al* 2006). Exclusion of Na^+ is likely

to be only the initial step in improving crop tolerance to saline conditions, and may have limited value in southern Australian soils in comparison to overcoming the osmotic effects of salinity, given that many subsoils (50-150cm depth) are over 15dS/m (150mM NaCl) (Rengasamy 2002).

2.5.2 Boron toxicity

Boron is an essential plant micronutrient, which has a small range between deficiency and toxicity. On a worldwide scale boron deficiency is a major constraint to cereal production and the most frequently encountered micronutrient deficiency in the field. In Australia, however, few reports of boron deficiency exist, except in the sandy slopes of the Great Dividing Range and sandy soils in Western Australia and South Australia's lower southeast (Jackson and Chapman 1975, Bell 1999). Far more prevalent in Australia is boron toxicity. In southern Australia boron toxicity occurs extensively in areas were annual rainfall is <400mm and minimal leaching has occurred (Cartwright *et. al.* 1986) (Figure 2.20).

Figure 2.20: Barley grain boron map (CSIRO 1991).

Boron toxicity of cereals in the field was initially recognised in barley (*Hordeum vulgare*) by Cartwright *et. al.* (1984). Brown spots frequently observed on barley, which were originally assumed to be pathogenic, were later identified as symptoms of boron toxicity and found to be widespread in semi-arid regions of southern Australia (Cartwright *et. al.* 1986). Boron toxicity was later found to affect other agronomical important crops throughout southern Australia, including bread and durum wheat, oats, field peas and annual pasture medics (Paull *et. al.* 1992).

Boron toxic soils in southern Australia have typically originated from the weathering of marine sedimentary rocks containing naturally high quantities of boron (Norrish 1975). The release of boron from these rocks and minerals forms predominantly weak acids, including boric acid ($B[OH]_3^-$) and borate ($B[OH]_4^-$). Boric acid is a relatively weak, monobasic acid that acts as a Lewis acid by accepting a hydroxyl ion to form borate (Goldberg 1997). Compared to other nutrients, boron is relatively unreactive and once released from soil minerals can be readily leached (Gupta 1979). In high rainfall, coarse textured and acidic soils, boron deficiency is common. However, where minimal leaching of boron occurs under lower rainfall, on heavier clay soils, boron toxicity is common.

Prevailing soil and climatic conditions in southern Australia's cropping region have led to the accumulation of boron in evaporative deposits in the B horizon. The accumulation of boron is a consequence of a lack of leaching through the soil profile from low rainfall (<400mm/year), highly sodic subsoils rendering them with low permeability, and continuous wetting and drying cycles that concentrate boron towards the top of the B horizon (Biggar and Fireman 1960). Generally, the concentration of boron has been found to increase with depth, with a range from 15 to >100mg/kg extractable boron, between 30-100cm of the surface (Cartwright *et. at.* 1986, Paull *et. al.* 1992). Boron deposits in the subsoil lead to boron toxicity by a combination of re-mobilisation into the soil solution, whereby movement by diffusion and mass flow bring boron to root surfaces, or by the extension of roots into the subsoil where boron has accumulated.

Soil solution boron is mainly controlled by adsorption reactions, which are affected by solution pH, soil texture, soil moisture, and temperature (Goldberg 1997). Boron adsorption increases as a function of soil pH in the range of pH 3 to 9 and decreases in the range of pH 10 to 11.5 (Figure 2.21). The increase in boron adsorption with increasing pH

up to 9, results in less plant available boron, however, in alkaline soils in arid to semi-arid areas such as South Australia, the increased adsorption of boron is compensated for by the lack of leaching.

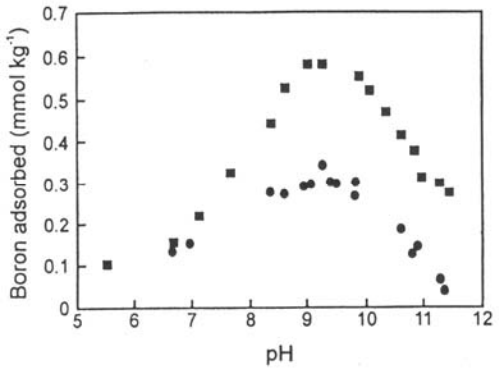


Figure 2.21: Boron adsorption on a surface (■) and a subsurface (●) sample on an arid zone soil as a function of pH (Goldberg 1997).

Apart from controlling the extent of leaching, soil moisture also affects the availability of boron by other means. Generally, a decrease in water content reduces the mobility of the soil solution, increasing the diffusion path length and reducing the amount of boron reaching plant roots by mass flow (Scott *et. al.* 1975). Limited soil moisture also reduces microbial activity, reducing the release of plant available boron from organic matter (Parks and White 1952). Boron deficiency has therefore been associated with drying soils, where plants encounter reduced amounts of available boron when extracting moisture from greater depths (Fleming 1980). In semi-arid areas, however, increased exploration of deeper soils under drying conditions often expose plant roots to higher boron concentrations than encountered in surface soils (Gupta *et. al.* 1985), with the amount of boron uptake related to the proportion of the root system exposed to boron (Bingham and Garber 1970).

Adsorption of boron onto soil surfaces is related to soil oxides, clay minerals, calcium carbonate and organic matter (Goldberg 1997). Aluminium and iron oxide minerals absorb boron by a ligand exchange mechanism on reactive surface hydroxyl groups. Boron adsorption on oxides increases with increasing pH up to an adsorption maximum of pH 6 to 8 for aluminium oxides and pH 7 to 9 for iron oxides (Goldberg 1997). However, competing anions at the reactive surface sites of oxide minerals have the ability to leach

adsorbed boron from oxides in the increasing order of silicate<<chloride<sulfate<arsenate<phosphate, where silicate has a slight and phosphorous a substantial effect on decreasing the magnitude of boron adsorption on oxides (Goldberg 1997).

Calcium carbonate increases boron adsorption by increasing soil pH from lime application, or by acting as an adsorption surface in calcareous soils. Adsorption has been attributed to an exchange mechanism with carbonate groups (Goldberg 1997). Layer silicate clay minerals are also strong boron adsorption surfaces. Initial adsorption occurs via ligand exchange with surface hydroxyl groups on the clay edges, followed by a slow fixation reaction, where boron is incorporated structurally into tetrahedral sites replacing structural silicon and aluminium (Couch and Grim 1968). Boron adsorption for clay minerals is kaolite < montmorillonite < illite (Goldberg 1997). Generally, coarse textured soils contain less available boron than fine textured soils, since adsorbed boron increases with increasing clay content (Elrashi and O'Conner 1982).

The uptake rate of boron by plants is linearly related to the external boron concentration, where increased available soil boron leads to a greater potential for plant toxicity (Marschner 1997). The uptake of boric acid [$B(OH)_3$; pKa = 9.25] at high boron supply occurs via passive diffusion across the lipid bilayer and by facilitated transport through major intrinsic proteins (MIPs) (Dannel *et. al.* 2002). A third uptake process for boron occurs via an energy-dependent high affinity transport system, however, the process is only induced by low boron supply, establishing a concentration gradient for boron between the root symplasm and the xylem (Strangoulis *et. al.* 2001, Dannel *et. al.* 2002).

At adequate boron supply, the primary translocation of boron from the root to the shoot of vascular plants occurs by passive transport across the plasma membrane into the xylem, most likely by an identical mechanism as boron uptake by the roots (Marschner 1995, Dannel *et. al.* 2002). The distribution of boron within cereal plants is primarily governed by the transpiration stream, which concentrates boron in shoot organs where the greatest water loss occurs. A gradient in boron concentration in individual leaves under excess boron supply leads to the typical symptoms of boron toxicity. The steep boron gradient from petioles < middle of the leaf blade < leaf tip (Oertli and Roth 1969), results in light browning from the leaf tips and margins, followed by necrosis (Paull *et. al.* 1990). In some

plant species (limited in wheat) boron is also phloem-mobile and can be re-translocated to growth sinks, such as fruits, cambial tissue and stems, in substantial amounts (Hanson 1991, Brown and Hu 1996).

The critical concentration of boron reported for plants varies widely among authors, between 10 and 130mg/kg dry weight (Gupta 1979, Cartwright *et. al.* 1986, Paull *et. al.* 1988, Nable *et. al.* 1997). The discrepancies in critical values are associated with the steep gradient of boron within the leaf blades, sampling age, environmental conditions and the leaching of boron from leaves (Reuter 1986, Nable and Moody 1992). The uneven distribution of boron within the plant can lead to highly variable results if sampling consistency is not achieved, since boron concentrations in the margins of the leaf are usually four to five times higher that in the leaf blade alone, and older leaves accumulate more boron than younger leaves (Oertli and Kohl 1961).

Moderate rainfall on plants growing in soil containing toxic levels of boron has also been shown to substantially decrease the boron concentration of both whole shoots and young leaves (Nable and Moody 1992). The leaching of boron from leaves following rainfall reduces the reliability of foliar analysis under field conditions, and under such conditions, the boron concentration in the grain may be a more reliable indicator for diagnosis of boron toxicity (Nable *et. al.* 1990), with a grain concentration above 2mg/kg indicating toxicity (Rathjen *pers. comm.*). Plants grown at high soil boron sites were found to contain grain boron concentrations ranging from 5.9mg/kg to 8.5mg/kg for high yielding and low yielding lines, respectively (Cartwright *et. al.* 1986)

Under adequate boron supply, boron is an essential micronutrient in plants and exists primarily as boric acid and some borate at neutral and alkaline pH. Complexation of the relatively unreactive boron is mostly limited to compounds with two hydroxyl groups in the *cis*-conformation, classified as *cis*-diols, which include simple mono- and oligosaccarides, complex sugars, diols, and hydroxyacids (Power and Woods 1997, Reid *et. al.* 2004). Boron has therefore been associated with playing a major role in maintaining cell wall structure, particularly in dicotyledonous plants, and membrane function, as well as supporting metabolic activities (Reid *et. al.* 2004). Up to 90% of cellular boron has been found in the cell wall fraction (Thellier *et. al.* 1979, Power and Woods 1997), complexed in *cis*-diol configuration and attached to a furanoid ring (Hunt 2002). The boron complex

binds to the sugar, apiose, which is the central component of the rhamnagalacturonan II (RGII) complex in plant primary cell walls (Reid *et. al.* 2004).

Boron can also form stable complexes with the *cis*-diol on a furanoid ring complex, ribose, which is the principal sugar component of RNA, and the key component of several important metabolites (Reid *et. al.* 2004). The binding of boron with other hydroxyl-containing molecules to form weak complexes has also been associated with the function of certain metabolites (Hu *et. al.* 1997, Pfieffer *et. al.* 1999). Based on the complexation chemistry of boron, Strangoulis and Reid (2002) suggested three possible causes for the initial boron toxicity response,

(1) the disruption of cell wall development,
(2) the disruption of cell division and development by binding to ribose either as the free sugar or within RNA, and
(3) metabolic disruption by binding to the ribose moieties of ATP, NADH or NADPH.

The initial boron toxicity response is likely to occur at the root tip, since Reid *et. al.* (2004) found that inhibition of root growth occurred if excess boron was applied to the root tip region, but not if excess boron was applied to mature sections of the root . Boron toxicity in the root meristem has been associated with reduced mitotic activity, coupled with an increase in chromosome fragmentation, chromosome stickiness and micronuclei development (Lui *et. al.* 2000), which can occur within 24hrs of exposure (Klein and Brown 1981). Boron toxicity at the root tip has also been associated with the disruption of metabolism. For example, in the root tips of sugar cane, high boron concentrations resulted in a decrease in the activity of a number of enzymes, specifically aldolase and glyceraldehyde-3-phosphate dehydrogenase, which is involved in carbohydrate metabolism (Bowen 1972). Following the initial exposure of plant roots to toxic concentrations of boron, multiple toxicity responses may occur within plants, leading to numerous physiological effects at the cellular, organ or whole plant level.

The physiological affects of boron toxicity can manifest as poor root growth (Nable 1988, Paull 1990, Chantachume *et. al.* 1995), with shorter root axes and fewer lateral roots (Huang and Graham 1990), decreased shoot growth, reduced leaf chlorophyll, lower photosynthetic rates and stomatal conductance, and reduced levels of lignin and suberin

(Nable *et. al.* 1997). The boron toxicity response is still not clearly understood, with many cellular activities partially inhibited in mature tissues by high boron concentrations, and exacerbated by photo-oxidative stress (Reid *et. al.* 2004). In the long-term, exposure to high soil boron concentrations can lead to a severe retardation of growth and little to no seed set (Roessner *et. al.* 2006).

Genetic variation in response to boron toxicity has been identified across a range of species with tolerance mechanisms, which include avoidance, exclusion and internal tolerance. The absence of toxic concentrations of boron in the topsoil (0-20cm) led to the suggestion that plants with shallow root systems may be able to avoid exposure to damaging boron concentrations (Nable 1988, Paull *et. al.* 1992). However, avoidance through a shallow root system is a common, non-specific tolerance mechanism for numerous subsoil toxicities and has limited benefits in situation where topsoil drying occurs regularly (Nable 1988).

Internal tolerance mechanisms proposed for boron toxicity have included inactivation of boron in the cell wall, reduced translocation of boron from roots to shoots, increase antioxidant production, cellular compartmentalisation, or re-distribution of boron between plant organs. Inactivation of boron in the cell wall may occur via binding of excess boron to pectin (Brown and Hu 1994, Hu *et. al.* 1996, Mahboobi *et. al.* 2001). Mahboobi *et. al.* (2001) proposed that an increase in cell wall uronic acid (structural component of cell wall pectin) content occurs in tolerant cultivars. However, cell wall uronic acid was not found to contribute to detoxification of excess boron in wheat and barley, and may only partially relate to the tolerance between species with significantly different cell wall pectin contents, which reflects their boron requirement (Mahboobi *et. al.* 2001).

Increased antioxidant production may reduce cellular damage from boron toxicity in some plants (Karabel *et. al.* 2003, Gunes *et. al.* 2006). When plants are subjected to environmental stresses, such as drought, salt, heavy metals, and excessive boron, reactive oxygen species are produced, such as superoxide, hydrogen peroxide, and hydroxyl radicals, which cause membrane damage that eventually leads to cell death (Mittler 2002, Cervilla *et. al.* 2007). In barley, boron toxicity was found to induce oxidative and membrane damage in leaves (Karbal *et. al.* 2003), while in apple and grapevine, excess boron toxicity induced damage by lipid peroxidation and hydrogen peroxide accumulation

(Molassiotis *et. al.* 2006, Gunes *et. al.* 2006, Cervilla *et. al.* 2007). For protection against the effects of reactive molecules, plants produce antioxidant molecules and antioxidant enzymes, such as superoxide dismutase, catalase, ascorbate peroxidase, and glutathione reductase (Mittler 2002, Blokkina *et. al.* 2003, Karbal *et. al.* 2003). Little evidence exists on the response of antioxidant defence systems of plants to boron toxicity, although a relationship between boron tolerance and increased ascorbate (most abundant antioxidant in plants) metabolism has been recognized (Blevins and Lukaszewski 1998, Smirnoff 2000, Brown *et. al.* 2002, Keles *et. al.* 2004).

Cellular compartmentalisation and re-distribution to specific plant organs is a common internal tolerance mechanism to reduce damage by various toxic ions in plants. However, except for certain halophytes, no evidence exists for plant tissue tolerance to excessive boron (Nable *et. al.* 1997). The chemical characteristics of boric acid, such as high membrane permeability, reactivity with polyols and lack of soluble salt formation, mean that boron is present in all subcellular compartments, and deposited according to the transpirational stream (Dannel *et. al.* 2002). Alternatively, deposition from the transpirational stream and the associated lack of phloem mobility may in itself be an internal tolerance mechanism (Nable *et. al.* 1997). Minimal phloem mobility reduces boron from reaching key metabolic sites, and by accumulating boron in the leaf margins plants are able to maintain some photosynthetic area, despite extensive leaf burn along the margins (Nable *et. al.* 1997).

In barley and wheat, no differences have been found in the distribution of boron between the root and shoot, and no cultivars have been identified that have a significant level of tolerance to high tissue boron concentrations (Nable 1988). Tolerance to excessive levels of boron has been associated with a reduction in both root and shoot concentrations (Nable 1988, Nable *et. al.* 1990, Paull *et. al.* 1992, Jefferies *et. al.* 1999). Furthermore, the pattern of boron distribution amongst individual leaves and roots is similar in susceptible and tolerant genotypes, despite differences in the total amounts of accumulated boron (Nable 1988). In the boron tolerant cultivar Sahara, Hayes and Reid (2004) recorded a much lower boron concentration in roots (50%), leaves (73%), and xylem (64%), than the boron sensitive cultivar Schooner, and the plant tissue accumulation was directly related to the concentration of boron in the root medium. The lower tissue concentrations of boron, compared to the external medium, mean that boron tolerance mechanisms currently

identified have been associated with the plants ability to exclude boron from the roots (Nable 1988, Nable *et. al.* 1990, Paull *et. al.* 1992, Jefferies *et. al.* 1999, Hayes and Reid 2004).

Three methods of boron exclusion have been proposed, which include, the secretion of chelating compounds into the rhizosphere (Nable *et. al.* 1997), reduced influx of boron from changes in membrane permeability (Doras and Brown 2000, Doras *et. al.* 2000), or the active efflux of boron across the plasma membrane (Hayes and Reid 2004). The high capacity for binding of boron to various polysaccarides and organic acids led to the suggestion that the secretion of chelating compounds into the rhizosphere may reduce the amount of free boron around the root surface (Nable *et. al.* 1997), similar to reactions found for aluminium tolerance. No evidence exists however, that rhizosphere complexation occurs for boron. Furthermore, for sugar excretion to have any significant effect of alleviating boron toxicity, sugars would need to be excreted in very high concentrations at a high metabolic expense (Yokota and Konishi 1990).

The reduced influx of boron from changes in membrane permeability may occur by alternations in the phospholipid and sterol composition of the lipid bilayer. The lipid bilayer can affect the transport of molecules, such as, water, glucose and some ions, and in a similar process may inhibit boric acid [$B(OH)_3$] transport (Nable *et. al.* 1997). Doras and Brown (2000) were able to demonstrate reduced membrane permeability to boron using two different *Arabodopsis* mutants with altered membrane lipid composition. By altering the lipid composition of the membrane of the *Arabodopsis* mutants boron uptake could be increased or decreased in comparison to the wild type. Huang and Graham (1990) also associated observations made on root explant and callus growth that was exposed to toxic concentrations of boron with membrane permeability. In media with toxic boron concentrations, tolerant genotypes could produce more callus than sensitive genotypes for both root explants and in undifferentiated callus cells. However, not all reports agree with the theory of increased boron tolerance through altered membrane permeability. The membrane permeability to boron is very high on the basis of water partition coefficients and measurements in both barley and wheat have demonstrated both rapid influx and efflux in roots (Raven 1980, Garnett *et. al.* 1993, Strangoulis *et. al.* 2001). Low root concentrations of boron may therefore be an artifact of washing roots prior to

measurements whereby the high membrane permeability would allow a substantial loss of boron from the roots during the processes (Nable *et. al.* 1997).

An alternate mechanism has been proposed for the exclusion of boron from roots, which involves the active efflux of boron from root cells, either by borate efflux through an anion channel or borate efflux via an anion exchange (Hayes and Reid 2004). The energetic feasibility of borate efflux is questionable, however, since high levels of energy would be required, via H^+ efflux and ATP hydrolysis, to maintain the electrical driving force and cytoplasmic pH (Hayes and Reid 2004). An anion exchange transporter would account for the charge balance, and if the exchange anion was HCO_3^-, then the pH balance would also be explained. Although some evidence exists for active efflux of the borate anion, increases in external pH can reduce the effectiveness of the transport system by increasing the external borate to boric acid ratio, which reduces the outwardly directed electrochemical gradient, leading to a loss of tolerance (Hayes and Reid 2004).

A putative borate transporter involved in xylem loading of boron has been proposed as a possible mechanism for *BOR1* (Takano *et. al.* 2002). The *BOR1* locus in *Arabodapsis* was located on the lower arm of chromosome 2A and identified as a membrane protein with homology to bicarbonate transporters in animals. In *Arabidopsis BOR1* acts as an efflux-type boron transporter for xylem loading and is essential for protecting shoots from boron deficiency (Takano *et. al.* 2002). A *BOR1* ortholog, *Bot1*, has been identified in barley and provides superior tolerance to boron toxicity in the Algerian landrace Sahara (Sutton *et. al.* 2007). The *Bot1* gene was mapped to the major boron tolerance locus previously identified on chromosome 4H, which affects boron accumulation, root length response, dry matter production and leaf symptom expression (Jefferies *et. al.* 1999). Sutton *et. al.* (2007) found that the *Bot1* transcript levels and locations in barley tissues were consistent with the role of *Bot1* in limiting the net entry of boron into the root and in the removal of boron from leaves via hydathode guttation (Oertii 1962).

In bread and durum wheat, genetic variation and loci for tolerance to boron toxicity have been found, but little is known about their function in the boron tolerance response (Paull *et. al.* 1988, Moody *et. al.* 1988, Paull 1990, Jamjod 1996). In bread wheat, at least three major genes, *Bo1*, *Bo2*, and *Bo3*, have been identified that control boron tolerance (Paull *et. al.* 1988). The genes have been described as additive, non-maternal, and partially

dominant, based on measurements of dry matter production and tissue boron concentrations in segregating progeny (Paull 1990). Transgressive segregates in the progeny also suggested the existence of a *Bo4* locus (Paull 1990), and possibly a *Bo5* locus (Chantachume 1995, Jamjod 1996). The use of monosomic crosses and mapping populations located the *Bo1* locus on chromosome 7B, the *Bo2* locus on chromosome 4A, and the *Bo3* locus on 7D (Paull 1990, Chantuachume 1995).

The *Bo1* gene historically has accounted for much of the success of wheat varieties grown in south-eastern Australia and has been used extensively in targeted breeding for tolerance to boron toxicity in South Australia since the late 1980s (Moody *et. al.* 1993, Campbell *et. al*. 1994). Following the discovery of the extent of boron toxicity across the cereal growing districts of southern Australia (Cartwright *et. al.* 1986), subsequent screening of Australian wheat varieties under high boron conditions found an association between high soil boron in the field and the type of variety under cultivation (Rathjen and Pederson 1986). The response to boron among Australian wheat varieties was found to range from very sensitive to moderately tolerant, with virtually all moderately tolerant varieties bred in South Australia or Victoria, and the sensitive varieties being released in New South Wales, Queensland, and Western Australia (Paull *et. al.* 1990, Campbell *et. al.* 1994).

Boron tolerance in Australian varieties has been traced back to Federation and Currawa, with related varieties Ghurka, Quadrat, Insignia, Heron, Olympic, Halberd and Spear, representing the most widely grown varieties throughout the twentieth century, and accounting for greater than 90% of wheat production in high soil boron regions (Rathjen and Pederson 1986). Transfer of the *Bo1* gene from Halberd via backcrossing to moderately sensitive, but otherwise adapted breeding lines, has consistently demonstrated a yield advantage of 5-10% of boron tolerant lines when grown on high boron sites (Moody *et. al.* 1993), highlighting the impact of boron toxicity on wheat production in South Australia. Additional sources of tolerance to boron toxicity have been identified from overseas germplasm, with some genotypes expressing a greater level of tolerance than Halberd, the most tolerant Australian variety (Moody *et. al.* 1988). Most of the boron tolerant bread wheats identified had originated from the eastern Mediterranean, western Asia, India and Japan (Moody *et. al.* 1988).

The screening of international durum varieties also found that boron tolerant types originated from similar regions as the bread wheats (Jamjod 1996). However, unlike bread wheat, genetic variation was found to be limited in Australian durum varieties, ranging from sensitive to moderately sensitive (Jamjod 1996). The lack of genetic variation in adapted durum varieties may be a consequence of low exposure to high boron concentrations at breeding and selection sites, a shorter and less intensive breeding history in Australia, minimal genetic variation in parental lines, or intense quality selection (Jamjod 1996). Three independent loci were identified, *$B_{OT}1$*, *$B_{OT}2$*, and *$B_{OT}3$*, in the segregating progeny of several crosses between parents ranging from tolerant to sensitive (Jamjod 1996). Disomic substitution lines were used to locate both *$B_{OT}1$* and *$B_{OT}2$* on chromosome 7B, which corresponds to the boron tolerant gene *Bo1* on chromosome 7B in bread wheat (Paull 1990).

In South Australia, the moderately tolerant Chinese landrace Lingzhi Baimong Baidamai (AUS 14010) containing *$B_{OT}2$* and *$B_{OT}3$* was used in backcrossing to transfer the *$B_{OT}2$* locus on 7BL to the moderately sensitive Australian variety Yallaroi, which contained only the *$B_{OT}3$* locus (Jamjod 1996). Yield evaluation between selected boron tolerant backcross derivatives and Yallaroi, identified a yield advantage of up to 19% in the moderately boron tolerant lines over Yallaroi when grown in soils with toxic concentrations of boron (Brooks 2004). The success in breeding for tolerance to boron toxicity in both bread and durum wheats in South Australia has served as a model for the identification of other important abiotic traits and their subsequent transfer into adapted breeding material.

2.5.3 Aluminium toxicity

Aluminium is the most abundant metal and third most abundant element comprising 8% of the earths crust (Martin 1986). Soluble aluminium is toxic to most plants, however, the majority of aluminium is locked in soils in complexed forms. Aluminium is released from minerals by weathering processes, whereby soluble aluminium results from a sequence of events that involves the stepwise loss of free energy (Ritchie 1995). Aluminium released coordinates with 6 OH_2 groups to form $Al(H_2O)_6^{3+}$ (abbreviated Al^{3+}), which dominates in acid conditions (pH<5). As pH increases, each OH_2 group dissociates a H^+ to form cationic aluminium species, $Al(OH)^{2+}$ and $Al(OH)_2^+$ (McLean 1976). At near-neutral pH (5.5 to 8.5) the solid phase $Al(OH)_3$, or gibbsite occurs. When the pH increases above 8.5 the

$Al(OH)_3$ precipitate redissolves to form the anionic species $Al(OH)_4^-$, or aluminate (Martin 1986) (Figure 2.22). Furthermore, when the total aluminium activity is increased, particularly under conditions were the solution is partially neutralised, polynuclear forms of aluminium may form, most commonly $AlO_4Al_{12}(OH)_{24}(H_2O)_{12}^{7+}$ (abbreviated Al_{13}) (Kochian 1995).

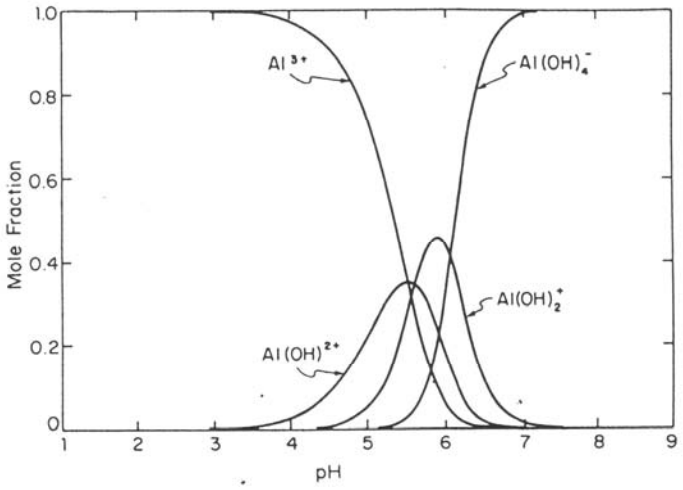


Figure 2.22: Distribution of soluble, mononuclear aluminium ion species in aqueous solutions (Martin 1986, 1988).

The chemistry of aluminium in soils has proven difficult to determine due to aluminium's complex nature. There is no single, specific form of solid aluminium that controls aluminium solubility, even within the same soil type. Before aluminium reaches the solution it may be retained and released by several solid phase sinks (Ritchie 1995). The majority of aluminium released by weathering is absorbed and then polymerised on the surfaces of clay minerals, or absorbed then complexed by organic matter (McLean 1976). Aluminium may bind to various organic and inorganic ligands, such as PO_4^{3-}, SO_4^{2-}, F^-, organic acids, proteins and lipids (Parker and Bertsch 1992). These forms of aluminium equilibriate rapidly, buffering the soil solution from the reactive aluminium species, which can be phytotoxic to plants.

The amount of aluminium in solution depends upon the amount of exchangeable aluminium, the ability of the interlayer and organic forms of aluminium to restore exchangeable aluminium and the degree of mineral dissolution (Ritchie 1995). The rate of dissolution of minerals is influenced extensively by soil pH and the presence of ions that

react with the dissolving or precipitating surface. Understanding the chemistry and phytotoxic potential of aluminium relies upon the ability to measure forms of aluminium at changing pH in soils and the ability to identify the major processes controlling soluble aluminium (Ritchie 1995), both of which have proven difficult.

Aluminium is present in the soil solution in a large array of chemical species. The majority of these chemical species are non-toxic, however some forms of aluminium have proven considerably toxic to a number of monocot and dicot species. Al^{3+} has been assumed to be the major phytotoxic species in acid conditions, but it was not until 1988, that the toxicity of Al^{3+} was confirmed (Parker *et. al.* 1988, Kinraide and Parker 1989). Al^{3+} was found to be rhizotoxic to wheat (Parker *et. al.* 1988, Kinraide and Parker 1989), but not to dicotyledonous plants (Kinraide and Parker 1990), which included red clover, lettuce, turnip, and soybean. Instead, mononuclear hydroxyl aluminium species ($Al(OH)^{2+}$ and $Al(OH)_2^{+}$) appeared toxic to dicot species (Kinraide and Parker 1990). The differences in sensitivity to Al species between monocots and dicots may be due to higher CEC in the cell walls of dicots compared to monocots, although this was not confirmed (Mutsumoto 2002).

In alkaline conditions, aluminate solutions were found to be toxic to both wheat and red clover (Rees and Sidrak 1955, Jones 1961, Fuller and Richardson 1986, Kinraide 1990). However, debate remains over the toxicity of aluminate. At pH>7.9, aluminate would constitute >99% of the mononulear hydroxyl aluminium in solution (Kinraide 1990). Additionally, aluminium appears to be toxic in alkaline fly-ash, bauxite residue and hydroponic culture (Rees and Sidrak 1956, Jones 1961, Zavas *et. al.* 1991, Piha *et. al.* 1995, Ma and Rathjen 2001, 2003). It would therefore seem plausible to associate the aluminate ion with aluminium toxicity. However, Kinraide (1990) showed that aluminium toxicity of wheat and clover decreased as the pH rose from 8.0 to 8.9 in solutions that excluded polynuclear aluminium forms (i.e. Al_{13}). The conclusion was that polynuclear Al_{13} or other toxic aluminium species were able to form in the mildly acidic root free space, even in the alkaline culture media, and the aluminate ion itself was not toxic.

Polynuclear forms of aluminium can form when the total aluminium activity is increased, particularly when the solution is partially neutralised, with the species composition being time dependent. Polynuclear aggregation occurs when $[Al^{3+}]/[H^{+}]>10^{10}$, but upon aging,

Al_{13} undergoes conversion to other aluminium species that are unreactive and may commonly comprise microcrystalline gibbsite or other solid-phase aluminium (Kinraide 1990). Al_{13} is extremely toxic to plants (Barlett and Riego 1972, Wagatsuma and Ezoe 1985, Wagatsuma and Kaneko 1987, Parker *et. al.* 1988, 1989), approximately 10-fold more toxic than Al^{3+} in wheat (Parker *et. al.* 1989).

Aluminium complexation may occur with low molecular weight ligands, such as sulfate, fluoride and organic acids (eg. citrate and malate), although most are non-toxic (Kinraide 1991). Both fluoride complexes (AlF^{2+}, AlF_2^{+}, AlF_3, AlF_4^{-}, AlF_5^{2+}, AlF_6^{3+}) and sulfate complexes ($AlSO_4^{+}$, $Al(SO_4)_2^{-}$) have been confirmed as non-toxic (Konishi and Miyamoto 1983, Kinraide *et. al.* 1985, Cameron *et. al.* 1986, Wright *et. al.* 1987, Kinraide and Parker 1987, Nable *et. al.* 1988, Parker *et. al.* 1989, Wright *et. al.* 1989), while organic acids have been implicated in aluminium tolerance mechanisms. Non-toxic low molecular weight complexes are also formed with carboxylate, inorganic phosphate and polyphosphates (Kochian 1995). Additionally, aluminium can bind reversibly to a number of macromolecules, including proteins, polynuceotides, and glycosides (Martin 1988). However, while their formation may be non-toxic, the presence of these complexes may significantly interfere with the biological systems that incorporate these macromolecules.

The confirmation of the phytotoxicity to any particular aluminium species is hindered by a number of problems. These include the difficulty of separating effects of pH from aluminium speciation, the formation of $Al(OH)_3$ and Al_{13}, the co-linearity between the concentration of certain aluminium species (Kinraide 1991), differences between ionic composition of the bulk solution and the solution present in the apoplasm (Tice *et. al.* 1992), and the identification of the aluminium species in contract with cell membranes or other points of critical contact. Conclusions can therefore be confounded, even in simple, low-ionic strength nutrient solutions. Despite volumes of research, there is still little consensus to the toxic nature of aluminium species and their influence on physiological and biochemical pathways, particularly at high pH.

The vast majority of research on aluminium phytotoxicity has been conducted under acid conditions (pH<5.5). Aluminium toxicity is generally accepted as one of the main limitations to agricultural production of acid soils, with Al^{3+} as the major phytotoxic

aluminium species. However, the increased solubility of aluminium in soil solution to an environmentally significant level at a pH>8.5, has been acknowledged for the better part of the last century (Magistad 1925). Original studies of aluminate, $Al(OH)_4^-$, toxicity were performed in fly-ash or bauxite residue (Magistad 1925, Rees and Sidrak 1956, Jones 1961, Fuller and Richardson 1986). Fly-ash is typically alkaline (pH 9-12) and high in Al, produced as a by-product after the combustion of pulverised coal (Rees and Sidrak 1956). Similarly, bauxite residue is alkaline (pH 11-12) and high in Al, produced as a waste during the extraction of alumina from bauxite (Fuller and Richardson 1986). In species calculations at a pH>7.9, $Al(OH)_4^-$ would constitute >99% of the monomeric hydroxyl-Al (Kinraide 1990).

The general findings of these studies showed a reduction in plant growth, accumulation of tissue Al and variation in tolerance between species (Magistad 1925, Rees and Sidrak 1956, Jones 1961, Fuller and Richardson 1986, Zavas *et al.,* 1991, Piha *et al.,* 1995). However, various other metals were also present in significant amounts in the growth medium to confound the results. Aluminium toxicity symptoms are also difficult to distinguish, and it is generally not possible to separate aluminium toxicity symptoms from other possible symptoms from deficiencies and toxicities occurring in the medium. What was concluded from studies using fly-ash and bauxite was that plant growth was severely affected by high pH and further affected by high Al. Furthermore, Al toxicity was governed by aluminium's solubility, which was controlled by pH.

Later studies using simple alkaline-aluminium solutions demonstrated a significant reduction in root elongation and severely affected epidermal and root cap cells in the presence of aluminium (Fuller and Richardson 1986, Eleftheriou *et. al.* 1993, Ma *et. al.* 2003). Debate remains however, as to the aluminium species causing the toxicity symptoms in high pH mediums used in previous experimental procedures. Many authors concur with the finding of Kinraide (1990), who indicated the non-toxicity of the aluminate ion, but conversion of aluminium to toxic polynuclear forms in the acidic free space of the root, in over saturated, alkaline solutions (Kopittke *et. al.* 2004). The findings were based on aluminium having no effect on root elongation when Al_{13} was excluded from the solution.

Regardless of the form of aluminium toxic to plant roots, it appears clear that the combination of high pH and aluminium does have a detrimental influence on plant growth, particularly root elongation, in solution and Al-rich media. No substantial attempt has yet been made to determine the toxicity of aluminium at high pH in field-based experiments. Ma *et. al.* (2003) was able to show a correlation between soil pH, soil aluminate concentration, and the accumulation of aluminium in shoot tissue of *T. turgidum* L. cv Tamaroi, but no relationship was made with plant growth and toxicity symptoms. Further investigation is needed to elucidate the phenomenon of aluminium toxicity at high pH in *Triticum* species.

2.5.4 Carbonate toxicity

The alkalinity or high pH of soils is directly related to the concentration of carbonate (CO_3^{2-}) and bicarbonate (HCO_3^-) in most soil solutions. However, practically all anions of weak acids might take part in the formation of alkalinity. Sulphide, phosphate and carbonate ions are the strongest bases in soils, although silicates and borates can also have a significant influence (Figure 2.23). Generally the predominance of carbonates in the anion composition of soils and their interaction with the cations Ca^{2+}, Na^+, and Mg^{2+}, can be used to explain the alkalinity or pH of most soil solutions.

In a pure carbonate equilibria system the partial pressure of carbon dioxide (Pco_2) determines the hydrogen ion activity (or pH) of the system. The concentration of CO_2 in atmosphere air is about 0.03%, while the concentration of CO_2 in soil air might reach 10% (Orlov 1992). When CO_2 dissolves in water it gives a solution of carbonic acid,

$$H_2O_{(liquid)} + CO_{2\,(gas)} \leftrightarrows H_2CO_{3(aqueous)}$$

but less than 0.3% of dissolved CO_2 is present as H_2CO_3 (Ponnamperuma 1967). Equilibria reactions operate in the solution of CO_2 in water, such that;

$$H_2CO_3 \leftrightarrows H^+ + HCO_3^-,$$

$$HCO_3^- \leftrightarrows H^+ + CO_3^{2-}, \text{ and}$$

$$H_2O \leftrightarrows H^+ + OH^-.$$

If the activity coefficients of each component are assumed to be near unity in dilute solution, H^+ activity will be;

$$(H^+) = (OH^-) + (HCO_3^-) + 2(CO_3^{2-})$$

Based on the solubility of CO_2 in water at 1 atmosphere at 25°C, the pH value of pure water in equilibrium with the air would be 5.63 (Ponnamperum 1967). If the soil solution (↑[CO_2]) is in equilibrium with the air then the pH would be near 4.5. A higher soil solution pH indicates an accumulation of bases in the soil, such as Ca^{2+}, Na^{+}, Mg^{2+} and K^{+}.

In low rainfall regions, where evapotransporation exceeds precipitation, Ca^{2+}, Na^{+}, Mg^{2+} and K^{+} are accumulated because of limited water flow. Generally, Mg^{2+} and K^{+} form secondary aluminosilicates, while Ca^{2+} remains as an exchangeable cation and precipitates as calcite ($CaCO_3$) and occasionally gypsum ($CaSO_4.2H_2O$), (Bohn *et. al.* 2001). In the soil solution Ca^{2+} activities are maintained at relatively high levels due to Ca^{2+} inputs from weathering and exchangeable Ca^{2+}. In solution the reaction

$$Ca^{2+} + H_2O + CO_2 \leftrightarrows CaCO_3 + 2\,H^{+}$$

occurs, favouring the accumulation of $CaCO_3$ under alkaline conditions by consuming hydrogen ions. Simultaneously, the reaction

$$CaCO_3 + CO_2 + H_2O \leftrightarrows Ca^{2+} + 2\,HCO_3^{-}$$

occurs, with increasing CO_2 concentrations (Bohn *et. al.* 2001). The equilibria between the two reactions depend on the soil moisture content, Pco_2, and the Ca^{2+} concentration. Soils with limited water and relatively high Ca^{2+} concentrations accumulate $CaCO_3$, despite high Pco_2 of soil air. The solubility product of $CaCO_3$ in equilibrium with H_2O and CO_2 gives a pH value of 8.3 (Bohn *et. al.* 2001). Soil solution with a pH value higher than 8.3, indicates the presence of Na^{+}, and in rare instances K^{+}.

The presence of Na^{+} on the exchange sites in soils inevitably leads to an increase in pH due to the higher solubility of Na_2CO_3 and $NaHCO_3$, compared to $CaCO_3$ (Table 2.02). For pH to rise above 8.3, Na^{+} needs to constitute >15% of the exchangeable cations in soil (Tan 1998). The ion exchange between Na^{+} and Ca^{2+} in the soil, and therefore pH, is dependent upon the ratio of Na:Ca in the soil solution, the type of exchanger (clay) and the total electrolyte concentration in the soil solution (Mashhady and Rowell 1978). The pH is therefore not directly related to the soil Na^{+} concentration, but only to that available fraction of the Na^{+} is soil, and that which is not electrically balanced by SO_4, Cl, and NO_3, the 'non-alkaline' anions (Mashhady and Rowell 1978), such that:

$$pH = 7.85 + \log[Na] - \log Pco_2 - 0.51\sqrt{\mu}.$$

where μ is the ionic strength of the solution (Ponnamperuma 1967).

pK_b		pK_a
0.2	$S^{2-} + H_2O \rightleftarrows HS^- + OH^-$	13.8
1.6	$PO_4^{3-} + H_2O \rightleftarrows HPO_4^{2-} + OH^-$	12.4
3.7	$CO_3^{2-} + H_2O \rightleftarrows HCO_3^- + OH^-$	10.3
4.6	$H_3SiO_4^- + H_2O \rightleftarrows H_4SiO_4 + OH^-$	9.4
4.9	$H_2BO_3^- + H_2O \rightleftarrows H_3BO_3 + OH^-$	9.1
6.8	$HPO_4^{2-} + H_2O \rightleftarrows H_2PO_4^- + OH^-$	7.2
7.0	$HCO_3^- + H_2O \rightleftarrows H_2CO_3 + OH^-$	7.0
7.6	$HS^- + H_2O \rightleftarrows H_2S + OH^-$	6.4

Figure 2.23: Common acid-base pairs, where $pK_a + pK_b = 14$ (Orlov 1992).

Table 2.02: Solubility of some soil carbonates (g of anhydrous substance in 100g of water) (Orlov 1992).

Compounds	0°C	20°C	50°C
$CaCO_3$	$8.1 \cdot 10^{-3}$	$6.5 \cdot 10^{-3}$	$3.8.10^{-3}$
$Ca(HCO_3)_2$	0.162	0.166	0.173
$MgCO_3.3H_2O$	0.149	0.092	0.037
$K_2CO_3.1.5H_2O$	105.3	110.5	121.3
$KHCO_3$	22.6	33.3	52.0
$Na_2CO_3.10H_2O$	7.0	21.5	—
$Na_2CO_3 \cdot H_2O$	—	—	48.5 (40°C)
$NaHCO_3$	6.9	9.6	14.5

When Na^+ is present on the exchange sites of soils and in the soil solution Na^+ can act in various reactions, such as:

$$Soil\text{-}Na_2 + CaCO_3 \leftrightarrows Soil\text{-}Ca + Na_2CO_3$$

$$Soil\text{-}Na_2 + H_2CO_3 \leftrightarrows Soil\text{-}H_2 + Na_2CO_3$$

$$Soil\text{-}Na^+ + H_2CO_3 \leftrightarrows Soil\text{-}H + NaHCO_3$$

$$Soil\text{-}Na^+ + H_2O \leftrightarrows Soil\text{-}H^+ + Na^+ + OH^-$$

The Na_2CO_3 and $NaHCO_3$ salts are water soluble and highly ionised,

$$Na_2CO_3 \leftrightarrows 2Na^+ + CO_3^{2-}$$

$$NaHCO_3 \leftrightarrows Na^+ + HCO_3^-$$

ensuring high CO_3^{2-} and HCO_3^- activity in the solution and pH values as high as 9 or 10. The interactions and equilibriums between the H_2O-CO_2, Ca^{2+}-CO_3^{2-}, and Na^+-CO_3^{2-} systems, act to control the concentrations of CO_3^{2-}, HCO_3^-, and OH^- in the alkaline solution, such that,

$$pH = \log[CO_3 + HCO_3] + 7.87 - \log Pco_2 - 0.5I^{0.5},$$

where I is the ionic strength of the solution (Chorom and Rengasamy 1997).

As previously mentioned (Section 2.3), in South Australia approximately 80% of the cereal zone has duplex soils with an A horizon ranging from acid to alkaline and calcareous, with almost inevitably a calcareous or sodic alkaline B horizon. Hence, high levels of HCO_3^- and CO_3^{2-} in the soil solution are a common feature of most of South Australia's agricultural soils. Fluctuations in HCO_3^- and CO_3^{2-} concentration, and consequently pH, occur readily in the bulk soil between sites and seasons, but more importantly in the rhizosphere, soil solution and apoplast, in response to soil changes in water content, organic matter accumulation and fertiliser application.

Water content is particularly important in influencing soil solution chemistry, nutrient availability and root morphology. The buffering capacity of most soils maintains relatively stable soil conditions when soil water values are below field capacity, however, as a soil becomes waterlogged significant changes occur to both redox potential and pH. In waterlogged soils molecular oxygen (O_2) is depleted due to the gases 10^4 times slower diffusion in water then in air (Greenway *et. al.* 2006). The consumption of O_2 by micro-organisms can lower the concentration of dissolved O_2 to trace amounts, leading to a switch from aerobic to anaerobic respiration when O_2 reaches 3×10^{-6}M (Ponnamperuma 1972). Anaerobic respiration can occur within a few days of waterlogging, and therefore may have a significant influence on plant growth even under transient waterlogging, which is common to soils with dense sodic subsoils even in semi-arid areas.

Under aerobic conditions soils maintain a highly oxidised state. Oxidation reactions persist until O_2 is at very low concentrations and anaerobic respiration by soil bacteria cause a change to reduction. After initial waterlogging, reduction follows a thermodynamic sequence, following reduction of O_2, NO_3^- is reduced to N_2, Mn^{3+} to Mn^{2+}, Fe^{3+} to Fe^{2+}, SO_4^- to H_2S, CO_2 to CH_2, N_2 to NH_3 and H^+ to H_2, with almost all reduction reactions consuming H^+ ions (Ponnamperum 1972). In acid soils the consumption of protons increases pH towards 7, but is buffered in the pH range of 6 to 7 by high concentrations of CO_2 in the soil. Under anaerobic conditions CO_2 accumulates in the soil, resulting in high Pco_2 capable of stabilising pH near neutrality via the $H_2CO_3 - HCO_3^-$ reaction (McBride 1994).

In alkaline soils the consumption of protons from reduction reactions is counteracted by the presence of carbonates, hydroxides or sulfides, which precipitate with the reduction

products to generate protons. For example, $Fe^{2+} + H_2CO_3 = FeCO_3 + 2H^+$ and $Mn^{2+} + H_2CO_3 = MnCO_3 + 2H^+$ (McBride 1994). The net effect is a reduction in pH, which is stabilised near neutral by a high Pco_2 within a few weeks of waterlogging (Ponnamperum 1972). However, once the soil water content is lowered and O_2 re-enters soil pores, the redox potential increases, reduction reactions reverse, and pH shifts back towards the soil pH prior to waterlogging (McBride 1994).

In general the pH of alkaline soils are highly sensitive to changes in the partial pressure of CO_2, which alter equilibrium reactions. High CO_2 levels (or Pco_2) may result from waterlogging, but also with greater soil depth, high biological activity, or compacted, poorly aerated soils. As earlier described for the H_2O-CO_2-H_2CO_3 system, including the reactions;

$$H_2O + CO_2 \leftrightarrows H_2CO_3$$
$$H_2CO_3 \leftrightarrows H^+ + HCO_3^-$$
$$HCO_3^- \leftrightarrows H^+ + CO_3^{2-}$$
$$H_2O \leftrightarrows H^+ + OH^-, \text{ such that,}$$
$$[H^+] = [OH^-] + [HCO_3^-] + 2[CO_3^{2-}],$$

when at constant Pco_2 the pH of the system is fixed. For example, a Pco_2 = 0.3 milliatmosphere the pH would be 5.65 (McBride 1994). An increase in Pco_2 shifts the system equilibrium, increasing H^+ activity (decreasing pH), and increasing the concentration of HCO_3^-, CO_3^{2-} and OH^- (Figure 2.24). Similarly, with known solubility constants the $CaCO_3$-H_2O-CO_2 system in equilibrium with soil air has pH 8.3, and the Na_2CO_3-H_2O-CO_2 system has pH > 9 (Ponnamperuma 1967, Bohn *et. al.* 2001), with increases in Pco_2 capable of shifting system equilibriums to a lower pH, and increasing the activity of dissolved carbonate species at a fixed pH.

Elevated levels of HCO_3^- and CO_3^{2-} can also arise from the use of poor quality irrigation water, and is commonly referred to as the sodium or carbonate hazard of irrigation water. Irrigation water containing high concentrations of free sodium ions can increase the exchangeable-Na^+ level of the soil. High Na^+ results in the displacement of Ca^{2+} from $CaCO_3$ forming Na_2CO_3 (i.e. $2Na^+ + CaCO_3 = Ca^{2+} + Na_2CO_3$) and increasing pH. With an elevated SAR (sodium absorption ratio), soils become sodic and disperse, which reduces aeration and increases Pco_2. High bicarbonate irrigation water has a similar outcome on pH, where high HCO_3^- lowers the concentration Ca^{2+} by forming $CaCO_3$ (i.e.

$Ca^{2+} + 2HCO_3^- = CaCO_3 + H_2O + CO_2$), increasing the SAR and exchangeable-Na^+ level, raising the activity of $NaHCO_3$ and Na_2CO_3 in solution, resulting in a higher pH.

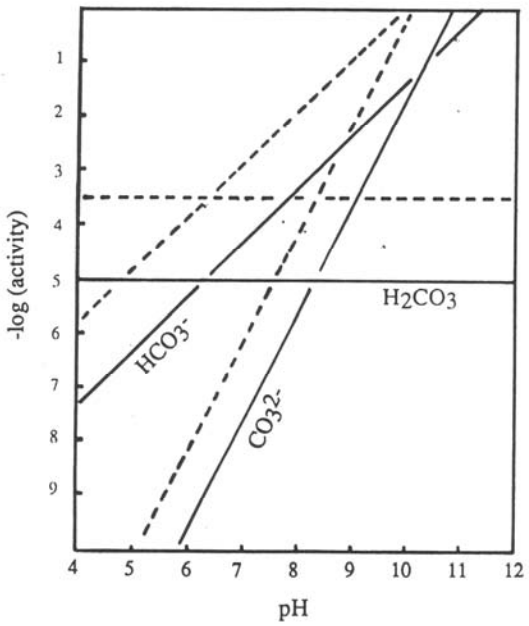


Figure 2.24: Dependence of the activities of dissolved carbonate species on solution pH, assuming atmospheric pressure (0.3 milliatmospheres) of CO_2 (solid lines). The effect of higher CO_2 (10 milliatmospheres) on each species is displayed as a broken line parallel to the original line (McBride 1994).

Regardless of whether high HCO_3^- and CO_3^{2-} levels have evolved from natural soil reactions, or induced by waterlogging or irrigation, high levels of HCO_3^- and CO_3^{2-} have numerous detrimental affects on plant growth. On calcareous soils in particular, high levels of HCO_3^- have been associated with various nutrient deficiencies, such as Fe, Zn, Mn (Shi *et. al.* 1993, Marschner 1995, Guardia and Alcantara 2002). HCO_3^- has been reported to have a number of effects on iron nutrition, although mainly on Strategy I (dicot) plants, such as, soybean (Chaney *et. al.* 1992, Inskeep and Bloom 1986), chickpea (Coulombe *et. al.* 1984), grapevine (Romheld 1987), apple (Ao *et. al.* 1987), peanut (Zuo *et. al.* 2007), and peach (Shi *et. al.* 1993). Strategy II (monocot) plants, which acquire iron via phytosiderophore release, have only a minor correlation between HCO_3^- concentration and iron deficiency (Cramer 2002). The influence of high HCO_3^- on Strategy II plants has been attributed to a decrease in root elongation reducing the capacity for uptake of Fe (Alhendawi *et. al.* 1997).

Strategy I plants have a much closer correlation between HCO_3^- concentration and iron deficiency (Cramer 2002). The relationship may arise from reduced soluble iron in the soil with an increasing pH, resulting in increased HCO_3^-. However, solution culture experiments have also shown that HCO_3^- can prevent the absorption of iron and cause Fe to accumulate in root apoplasts, depress the translocation of iron from the root to shoot and inactivate iron in the leaves (Romheld 2000, Zuo *et. al.* 2007). The accumulation of Fe in the root apoplast when exposed to high HCO_3^- has been attributed to either the reduced solubility of Fe from alkalisation of the apoplasm of the rhizodermis cells (Marschner 1995), the inhibition of Fe reduction activity necessary for absorption in the root (Romera *et. al.* 1992), or the binding of cationic Fe^{3+} with HCO_3^- in the root apoplast (Zribi *et. al.* 2002).

Reduced transport of Fe from root to shoot from high HCO_3^- concentration has been associated with an increase in root fixation of CO_2 through organic acid synthesis (Lee and Woolhouse 1969). The sequestering of iron in root vacuoles by certain organic acids inhibits iron transport to the shoot, particularly the expanding leaves, and influences the distribution of Fe within the leaf tissue (Rutland 1971, Marschner 1995). Other possible mechanisms for reduced shoot growth and low shoot Fe concentrations include the inhibition of root-pressure driven solute export into the xylem (Wallace *et. al.* 1971) or inhibition of the rate of cytokinin export to the shoot, which is necessary for chloroplast development (Marschner 1995).

Inhibited Fe acquisition and transport to the shoot due to high HCO_3^- in the rooting medium is generally regarded as the primary cause of Fe deficiency and leaf chlorosis. However, in some pot and field experiments a phenomenon called the 'Chlorosis Paradox' occurs, where higher Fe concentrations have been found in young chlorotic leaves than green leaves (Romheld 2000). The higher Fe concentration in chlorotic leaves was thought to occur from Fe inactivation in the leaf apoplast by an alkalisation processes from exposure to high external HCO_3^- (Mengel 1994). However, no substantial effects to the pH of xylem sap or apoplastic fluid of leaves has been found (Romheld 2000). Instead, Romheld (2000) proposed that the phenomenon might be the consequence of a negative dilution effect, where severe growth inhibition of chlorotic leaves with a substantially lower leaf area record a higher extractable Fe concentration per unit weight than larger green leaves. Since reduced uptake and translocation of Fe into the leaves also occurs

when the 'Chlorosis Paradox' is observed, any inactivation of Fe in the leaf is unlikely to be the primary cause of HCO_3^- induced iron deficiency.

The relationship between Fe deficiency and high HCO_3^- concentrations has mainly been studied in relation to lime-induced chlorosis of horticultural crops, such as grapes and stone fruits, where HCO_3^- has consistently had a significant impact on Fe nutrition. In dryland cereal crops, the effects of high HCO_3^- concentrations on Fe deficiency are still unclear. Symptoms of Fe deficiency in cereal varieties are observed only rarely in South Australia, and confined to poorly drained or highly calcareous soils. Some species, such as oats and durum wheat however, show a much higher susceptibility to Fe deficiency in the field than bread wheat or barley and may be more susceptible to the detrimental affects of high HCO_3^-. In solution culture, the inhibition of root growth by HCO_3^- is common to most graminaceous species, including bread and durum wheat, barley, maize, and sorghum at concentrations of 5 – 10mM of bicarbonate (Alhendawi *et. al.* 1997, Lui and Rathjen 1998). Alhendawi *et. al.* (1997) measured a significant reduction in the Fe concentration in both roots and shoots of barley, maize and sorghum, suggesting that HCO_3^- may affect root uptake and not translocation of Fe. Under field conditions the effect of HCO_3^- on Fe deficiency may not be as dramatic, although growth may still be affected by poor iron nutrition in the absence of visual symptoms. Bicarbonate has often been shown to inhibit shoot growth considerably prior to the occurrence of Fe-induced chlorosis (McCray and Matocha 1991, Shi *et. al.* 1993, Alhendawi *et. al.* 1997).

Zn uptake by graminaceous crops may be similarly affected by high HCO_3^- through the impairment of root activity (Yang *et. al.* 1993). Zn deficiency in soils occurs commonly in South Australia resulting in plant symptoms of deficiency being observed more frequently in the presence of high bicarbonate, across a range of crops. By far the most information on the relationship between Zn and HCO_3^- has come from the study of irrigated rice, where high HCO_3^- (commonly 10mM for flooded rice) under moderate to low Zn levels inhibits root growth in 'Zn-inefficient' cultivars, and stimulates growth in 'Zn-efficient' cultivars (Yang *et. al.* 1994).

The poor growth of Zn-inefficient cultivars in high HCO_3^- treatments has been attributed to the impairment of new root initiation, the inhibition of root activity caused by root injury reducing nutrient uptake (including Fe and Mn), a reduction in the transport of Zn to the

shoots, immobilisation of Zn in the roots inhibiting translocation to shoots, or the higher accumulation of organic acids (Forno *et. al.* 1975, Yang *et. al.* 1993, Marschner 1995). The accumulation of organic acids, such as malate, succinate and citrate, and insufficient compartmentalisation of the organic acids in the roots cells may chelate cations and reduce cation availability (Yang *et. al.* 1993).

Growth of Zn-efficient cultivars stimulated by high HCO_3^- treatments has therefore been associated with the better control of organic acid accumulation in roots (Yang *et. al.* 1994). Other explanations for slight increases in growth under high HCO_3^- include the enhanced supply of carbon chains for root growth by dark fixation (Bialezyk and Lechowski 1994), or enhanced cell elasticity in the root elongation zones (Ferns and Taylor 1994).

In bread wheat grown at Zn deficient levels, high HCO_3^- has been associated with a reduction in root and shoot growth in both Zn-inefficient and Zn-efficient types, although to a lesser degree than many of the studies conducted on rice growth (Cakmak *et. al.* 1996). Zn-efficiency has not been found to be closely associated with HCO_3^- tolerance, but may be related to other mechanisms such as phytosiderphore release, and differences in Zn uptake capacities, root to shoot translocation, or internal utilisation efficiency (Cakmak *et. al.* 1996, Genc *et. al.* 2006). The casual association between high HCO_3^- and Zn deficiency in *Triticum* species appears to be a general response to HCO_3^- toxicity resulting from inhibition of root growth, which decreases the plants ability to extract adequate nutrition from the soil.

In alkaline soils the concentration of HCO_3^- commonly ranges from 1-4mM in calcareous soils, but can rise to 10mM and higher in sodic soils, soils with high organic matter, waterlogged soils, or in the rhizosphere (Mengel *et. al.* 1984, McCray and Matocha 1992). The concentration of CO_3^{2-} also rises with pH and becomes the dominant carbonate species in solution when pH is higher than 9.5 (Figure 2.25). However, as previously mentioned in section 2.3, the solution pH at the soil-root interface can be up to 2 units different from the bulk soil, and root apoplasts are strongly buffered in the slightly acidic range, which can influence the form of carbonate species (increase HCO_3^-) in contact with the plant.

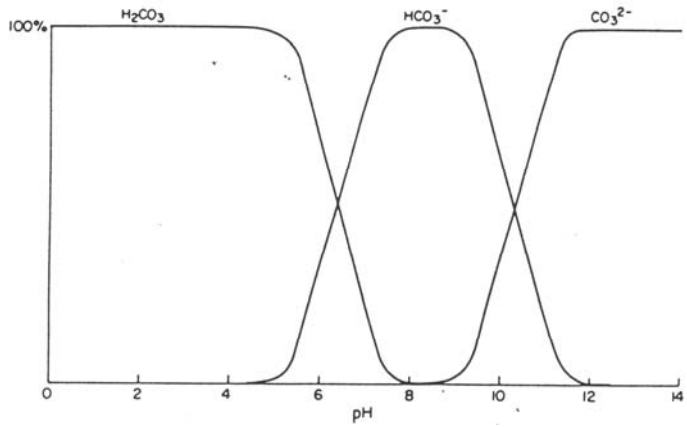


Figure 2.25: Distribution of aqueous CO_2 species with pH (Bohn *et. al.* 2001).

Alkalination of the root apoplast can occur under extreme stress, however, nitrogen uptake has also been implicated in producing high pH levels at the root surface and alkaline apoplastic pH (Hauter and Mengel 1988). At high pH plants exclusively take up nitrate due to increased nitrification and NH_3 volitisation (Paramasivam and Alva 1997). Evidence suggests that NO_3^- is transported as a symport with H^+, however, a HCO_3^-: NO_3^- antiport has not yet been ruled out, since the pH and membrane potential consequences are identical (Cramer 2002). The alkalinisation of the apoplast arises due to either the efflux of HCO_3^- with NO_3^- uptake (antiport) or influx of H^+ with NO_3^- uptake (symport) (Kosegarten *et. al.* 1999). Under high HCO_3^- and NO_3^- conditions, increased apoplasmic pH may have serious consequences for root function and metabolism, particularly in the early elongation zone, inhibiting root growth (Kosegarten *et. al.* 1999).

High HCO_3^- concentrations in contact with roots have been associated with a reduction in growth in various grass species, with species from acid soils (calcifuges) being more sensitive than species from calcareous soils (calcicoles) (Lee and Woolhouse 1969). In separate studies, root and shoot growth of barley, maize, and sorghum was inhibited by 5-10mM HCO_3^- at pH 8 (Alhendawi *et. al.* 1997), and root growth of bread and durum wheat was inhibited by 1-5mM HCO_3^- at pH 8.7 (Lui and Rathjen 1998). Reduced root growth has been associated with a reduction in length of the main root system through inhibition mainly of cell elongation (Lee and Woolhouse 1971). Observations on root growth have also described HCO_3^- as causing very short lateral roots of uniform size with large numbers of laterals failing to penetrate the cortex of the parent root (Hutchinson 1967). The inhibited root growth has mainly been associated with an increase in malate in the roots,

whereby HCO_3^- is fixed *in situ* by PEP carboxylase to form oxaloacetate, which is reduced to malate (Cramer *et. al.* 1993). As stated with Zn, malate or other organic acid accumulation can reduce cation availability and inhibit cell elongation (Lee and Woolhouse 1971).

The importance of HCO_3^- tolerance for dryland cereal crops in South Australia becomes apparent when viewed in relation to the soil environment. Almost all of South Australia's cereal cropping areas are calcareous and/or sodic, particularly in the subsoil (below 30cm), with pH values over 8, and are inherently low in nutrients, such as Zn, Mn, P, and Fe (Section 2.4). In South Australia researchers had often observed the superiority of some varieties, notably the bread wheat *cv.* Krichauff, on highly alkaline soils over a number of years (Rathjen *et. al.* 1999). Solution culture experiments with HCO_3^- concentrations ranging from 1-15mM, later confirmed that Krichuaff had a moderate to high level of tolerance to HCO_3^-, far greater than any other bread wheat tested (Lui and Rathjen 1998). Additionally, many of the varieties bred locally in South Australia on alkaline soils had a moderate level of HCO_3^- tolerance, probably from decades of selection of higher yielding types in the field under high HCO_3^- conditions (Rathjen *et. al.* 1999).

In wheat, significant genetic variation has been identified between varieties, landraces and *Triticum* species (Lui and Rathjen 1998, Das and Cooper, unpublished), with some lines exceeding the tolerance of Krichauff. The potential therefore exists for the genetic improvement for HCO_3^- tolerance in breeding programs to increase productivity on alkaline soils. Durum wheats are of particular interest due to their poor adaptation to calcareous, sodic-alkaline, and silicious sand soils, resulting in grain yields well below bread wheat. The lack of adaptation in the current varieties of durum grown in South Australia is a consequence of a relatively short breeding history in South Australia, and genetic material originating from northern New South Wales, where soils are deep and heavy. Durum wheats have consistently recorded a much lower tolerance to HCO_3^- toxicity in solution screens (Lui and Rathjen 1998), and often display symptoms to nutritional deficiency in the field. Genetic variation in the *Triticum* species for HCO_3^- can potentially be used for the improvement of grain yield on alkaline soils.

Chapter 3

General Materials and Methods

3.1 Field trials

3.1.1 Experimental design

The field trials were sown from May to June using the machinery and methods of the Waite Durum Breeding program, University of Adelaide. Each plot comprised four rows wide and 6m long, using a modified 14 row drill, with 15cm row spacing and a separation of 30cm between plots (missing row), such that, three plots are sown simultaneously (Figure 3.01). Plots are sown in 15 bays, and with 6m lengths, the total length of the experiments are 90m. The width of the experiment varied with the number of plots/lines in the population, so 450 plot experiments were 30 columns (22.5m) wide and 225 plot experiments were 15 columns (11.25m) wide. The sowing rate for the bread wheat was 30g and 35-40g for durum wheat (larger seed size), which was sown over the full 6m x 4 row plot, giving an approximate seeding rate of 60kg/ha and 70-80kg/ha, respectively. Experiments contained check plots at set intervals that varied between experiments, along with the number of replicates and sites sown. Between head emergence and anthesis pathways of 1.8m were sprayed with a knockdown herbicide (glyphosate) to allow for the automatic cleaning of harvesters between plots to reduce contamination between different lines. The total plot length at harvest was therefore 4.2m, or 2.52m^2 total plot area.

3.1.2 Trial management

The experiments were sown on farmer's paddocks, and as such, management of the field sites was in accordance with local practice. Fertiliser was applied with the seed at a rate of 80kg/ha of DAP (N:P:K:S 18:20:0:0). Herbicides were applied as required, depending on the conditions of the individual site. Herbicides used included Avadex (Triallate), Treflan (Trifluralin), Roundup (Glyphosate), Goal (Oxyflourfan), Hammer (Carfentrazone-ethyl), Lontrel (Clopyralid), Tristar (Diclofop-methyl, Fenoxaprop-p-ethyl), Eclipse (Metasulam)

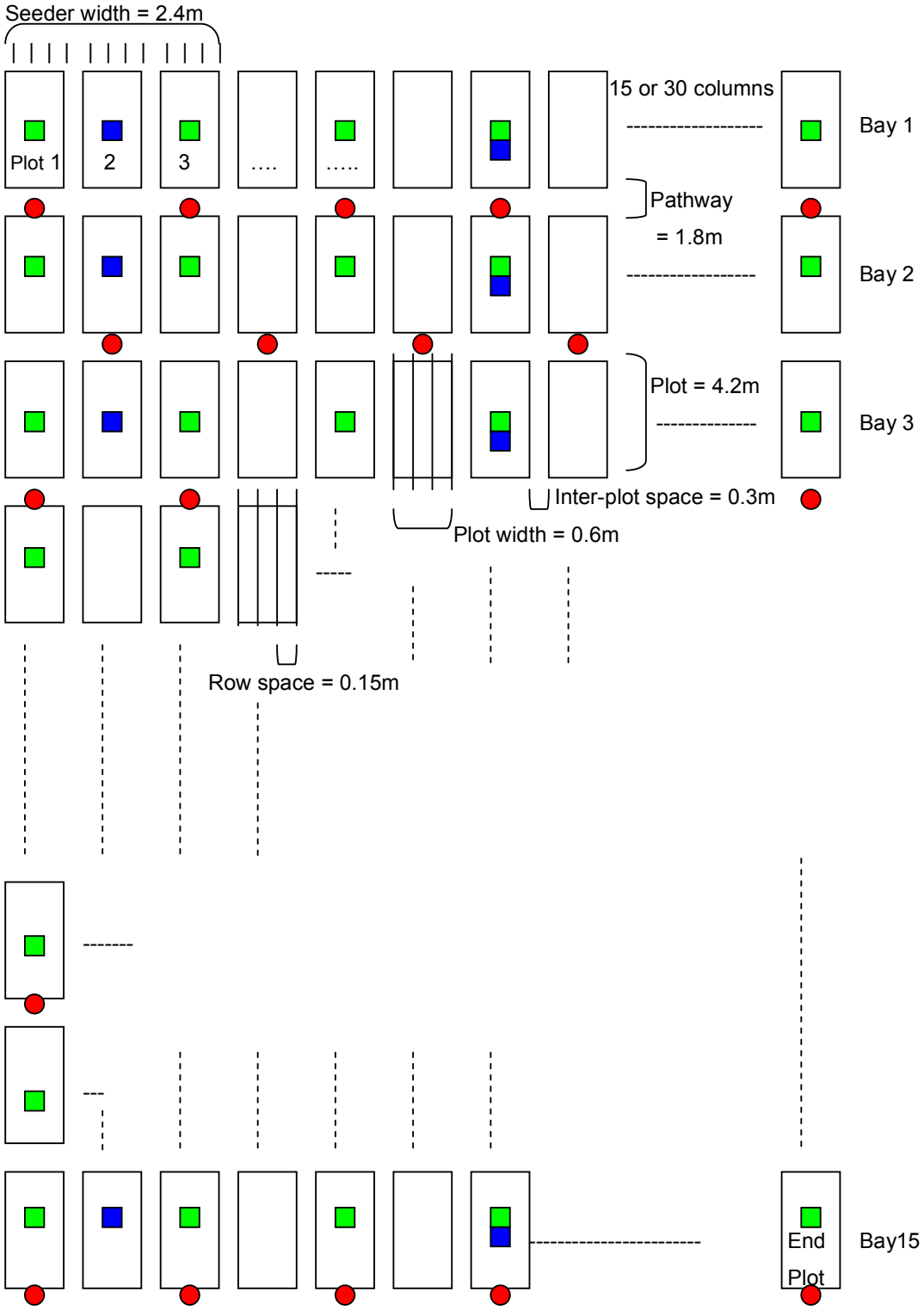


Figure 3.01: Experimental design of field plots, indicating the location of soil sampling points in 2004 (●) and 2006 (■), and Wk/TmWLYY9//WLYY9Tm in 2006, which was soil sampled every 5th plot (■).

and various other herbicides applied by the local farmers throughout the growing season for the management of weeds.

Symptoms of abiotic or biotic stress, morphological characteristics, and site conditions were recorded throughout the season for each experiment. Plots were harvested at maturity in December, using harvesters specifically designed and built for the Waite Wheat/Durum Breeding program, with a thresher or stripper front, and fan for removal of light material (chaff, small screenings and cracked grain). After harvest grain weight (g/plot) was measured for each individual plot for all experiments.

3.1.3 Tissue sampling

Twenty YEBs (Youngest fully emerged leaf blades) were collected per plot in September when plants were at Zadock's 43 (Booting). Tissue samples were placed into brown paper bags and dried for 24hrs at 70°C. Dried samples were chopped to <2mm pieces using stainless steel scissors. Tissue samples were sent to the Waite Analytical Services unit, University of Adelaide, for ICP (Inductively Coupled Plasma) spectrometry analysis.

3.1.4 Soil Sampling

A hydraulic soil core rig from the University of Adelaide was used to extract soil samples in 2004 and 2006. Soil samples in 2004 were taken in September (pre-anthesis) from the end of every second plot within an experiment at a depth of 0-10cm and 40-50cm (Figure 3.01). For the 2005 field experiments, soil samples were taken in January 2006, after harvest, from the center of plots in all bays of every second column, at the depth of 0-10cm, 30-40cm, and 50-60cm (Figure 3.01). The exception in 2006 was the Wk/TmWLYY9// WLYY9Tm population experiment, which had soil samples taken only for every 5th plot (Figure 3.01). Soil samples were limited to a maximum of only 50-60cm due to the hardness of the subsoil and low moisture at the trial sites.

A hand auger was also used for soil extraction and sampling in September 2004 and January 2006. Samples were taken at 0-10cm and 40-50cm depths in 2004 and at 0-10cm, 30-40cm, and 50-60cm depths in 2006 from 10 random plots within an experiment, where samples had not been taken with the soil core rig.

Samples of 100-400g were collected in brown paper bags, oven dried at 80°C within 24hrs, and stored in a dark, cool, dry place. Laboratory soil measurements of pH and EC were taken within 1 to 2 weeks of field collection. For each sample 40mL of de-ionised reverse osmosis (RO) water was added to 8g of soil in a 50ml plastic Eppendorf tube to give a 1:5 soil paste suspension. Tubes were mixed end over end for 1hr and left to rest for 30min before pH_w and EC electrodes were placed into the supernatant for measurement.

Individual plot pH or EC was determined by calculating the average of adjacent plots. The pH and EC of the 2004 plots (Figure 3.02) was calculated from the raw soil data (black) by firstly determining the average of the two neighbouring columns (blue), then the average of the values at each end of the plot (green).

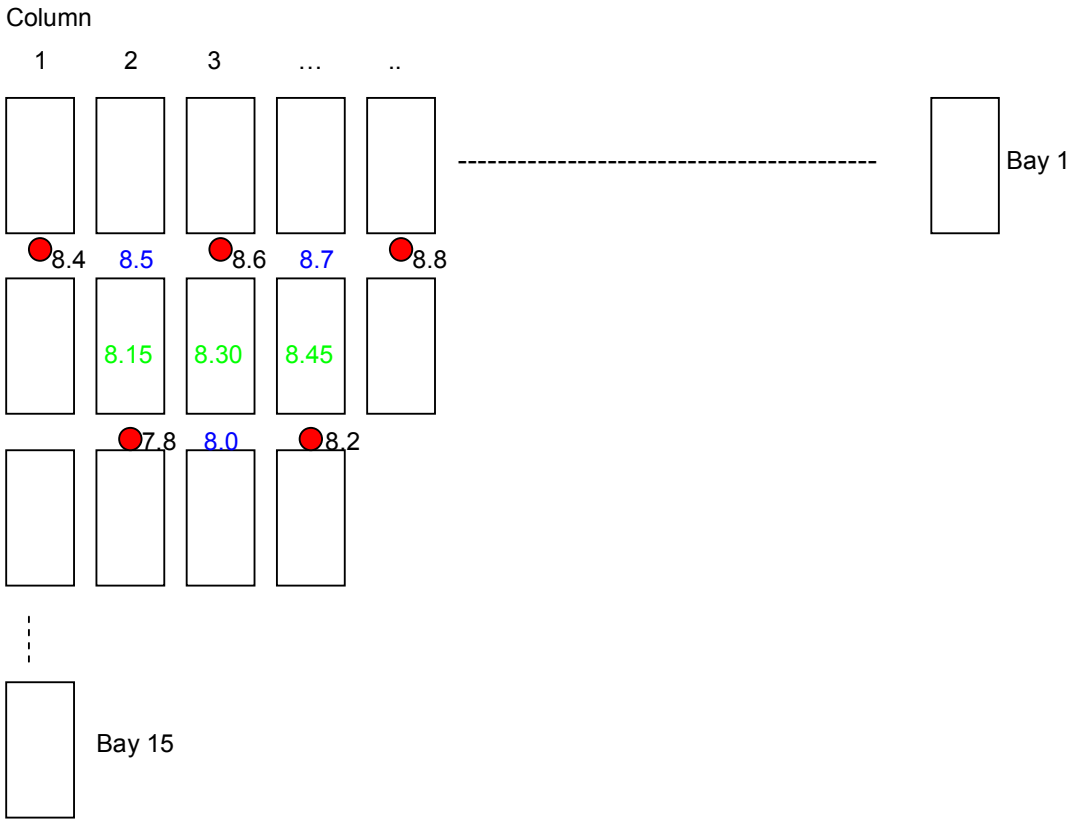


Figure 3.02: Calculating plot pH and EC from 2004 soil samples, using pH as an example.

In 2006 the plot pH and EC values were taken as the average of the neighbouring columns (green) from raw plot data (black) (Figure 3.03). For experiment Wk/TmWLYY9// WLYY9Tm, only the 90 raw soil data points were used due to the distance between sample points.

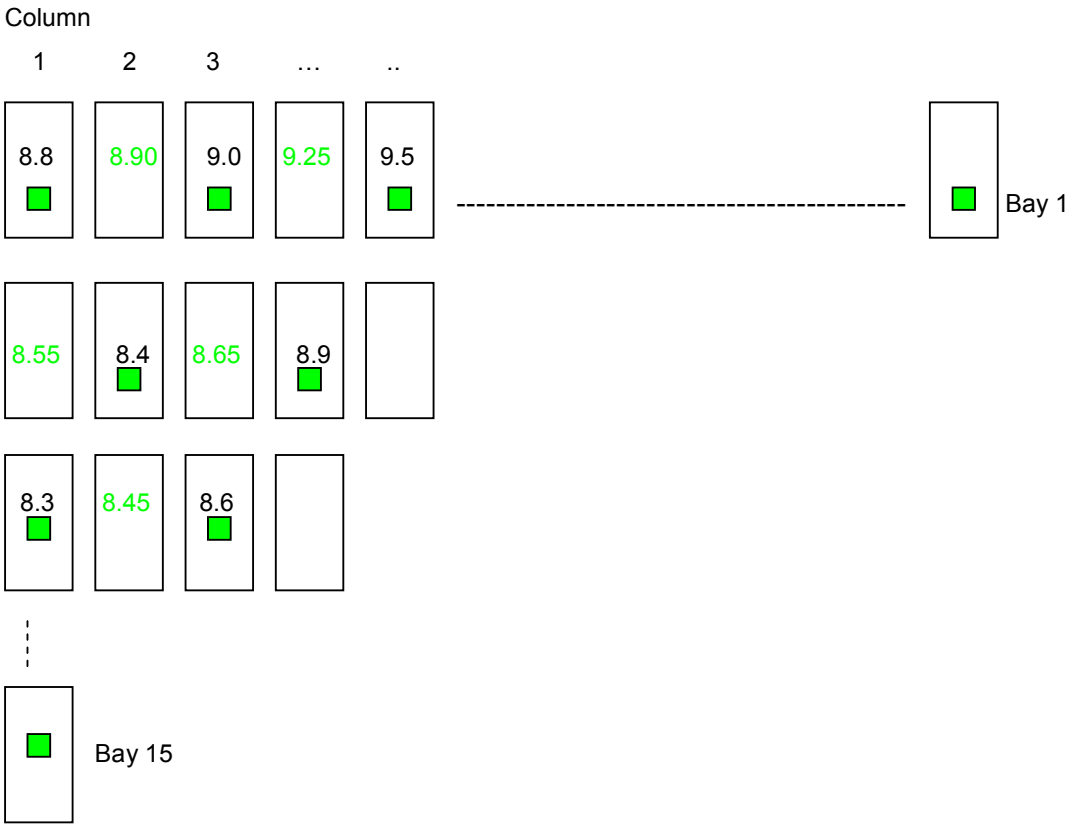


Figure 3.03: Calculating plot pH and EC from 2005 soil samples, using pH as an example.

3.1.5 Trial sites

Bread and durum wheat experiments were sown amongst or adjacent the breeding trials of the Durum Breeding group, University of Adelaide (Table 3.01).

Angas Valley (AV)

The Angas Valley site was located approximately 25km east of Mount Pleasant on the Murray Plains (Appendix 1). The site was known to have subsoil high in salinity and pH, and prone to hard-setting and poor water infiltration, under shallow, sandy, calcareous, often infertile topsoil, which had been extensively reworked through wind erosion events since the introduction of European farming.

Site	Soil Type	Rainfall (mm/annum)	Average Yield (t/ha)	Year	Experiment	Exp. Number	Section
Angas Valley(AV)	Plains and rises with mainly loamy calcareous soil. Plains and gentle slopes with mainly loamy texture contrast soil with calcareous subsoil.	380	2.5	2004	RAC875/Cascades	475	4.2, 7.2
					Landrace population	472	4.3
				2005	Frame/Yarralinka//Puglsey	576	4.2, 7.4
					Worrakatta/TmWLYY9/WLYY9Tm	571	4.2, 7.3
					RAC875/Cascades	575	7.2
Buckleboo (BR)	Rises and plains with mainly loamy texture contrast or gradational soil.	344	2.0	2004	RAC875/Cascades	475	4.2, 7.2
					Landrace population	472	4.3
				2005	Frame/Yarralinka//Puglsey	576	7.4
					Worrakatta/TmWLYY9/WLYY9Tm	571	7.3
					RAC875/Cascades	575	7.2
Claypans (CK)	Plains and rises with mainly shallow calcareous soil (or mixed calcareous and non-calcareous soil) on calcrete.	330	2.0	2004	RAC875/Cascades	475	7.2
					Landrace population	472	4.3
					Worrakatta/TmWLYY9/WLYY9Tm	571	7.3
					RAC875/Cascades	575	7.2
Coonalpyn (CS)	Dune/swale systems with mainly acid to neutral, bleached siliceous sand on dunes.	460	3.5	2004	Landrace population	472	4.3
				2005	RAC875/Cascades	574	7.2
Jamestown (JC)	Plains or gentle slopes with mainly deep calcareous soil or gradational/clayey soil with calcareous subsoil.	470	4.0	2004	Landrace population	472	4.3
				2005	Worrakatta/TmWLYY9/WLYY9Tm	571	7.3
Kapunda (KH)	Low hills and rises with mainly acid to neutral, loam to clay loam texture contrast soil.	490	4.0	2005	Frame/Yarralinka//Puglsey	576	7.4
					RAC875/Cascades	574	7.2
Redhill (RH)	Plains or gentle slopes with mainly deep calcareous soil or gradational/clayey soil with calcareous subsoil.	410	3.0	2004	RAC875/Cascades	475	7.2
					Landrace population	472	4.3
				2005	Frame/Yarralinka//Puglsey	576	7.4
					Worrakatta/TmWLYY9/WLYY9Tm	571	7.3
					RAC875/Cascades	575	7.2
Roseworthy (RAC)	Rises and plains with mainly loamy texture contrast or gradational soil.	425	3.0	2004	RAC875/Cascades	475	7.2
				2005	Frame/Yarralinka//Puglsey	576	4.2, 7.4
					Worrakatta/TmWLYY9/WLYY9Tm	571	4.2, 7.3
					RAC875/Cascades	575	7.2
Two Wells (TW)	Plains and gentle slopes with mainly loamy texture contrast soil with calcareous subsoil.	425	3.0	2005	Landrace population	472	4.3
					Worrakatta/TmWLYY9/WLYY9Tm	571	7.3
Wanderah (WJ)	Plains with mainly neutral to alkaline gradational or texture contrast soil, often marginally alkaline.	340	2.0	2004	RAC875/Cascades	475	4.2, 7.2
					Landrace population	472	4.3
				2005	Frame/Yarralinka//Puglsey	576	4.2, 7.4
					Worrakatta/TmWLYY9/WLYY9Tm	571	4.2, 7.3
					RAC875/Cascades	575	7.2
Winulta (WC)	Rises and plains with mainly calcareous soil.	330	2.0	2004	RAC875/Cascades	475	7.2

Table 3.01: Description of experiments sown at each site, including soil type, annual rainfall (mm), long-term average yield (t/ha) of bread wheat, year sown, field experiment number, and the thesis section where the experiments are analysed. Note: Average rainfall refers to the long-term average for the nearest Bureau of Meteorology site, and long-term average yields are district estimates based on the annual rainfall.

The RAC875/Cascades plots in 2004 were situated on a calcareous ridge of a Woorinen type dune system, while the landraces population was situated 200m to the South on less calcareous rubble, but with a shallow, sandy-loam overlaying Blanchtown clay (Figure 3.04).

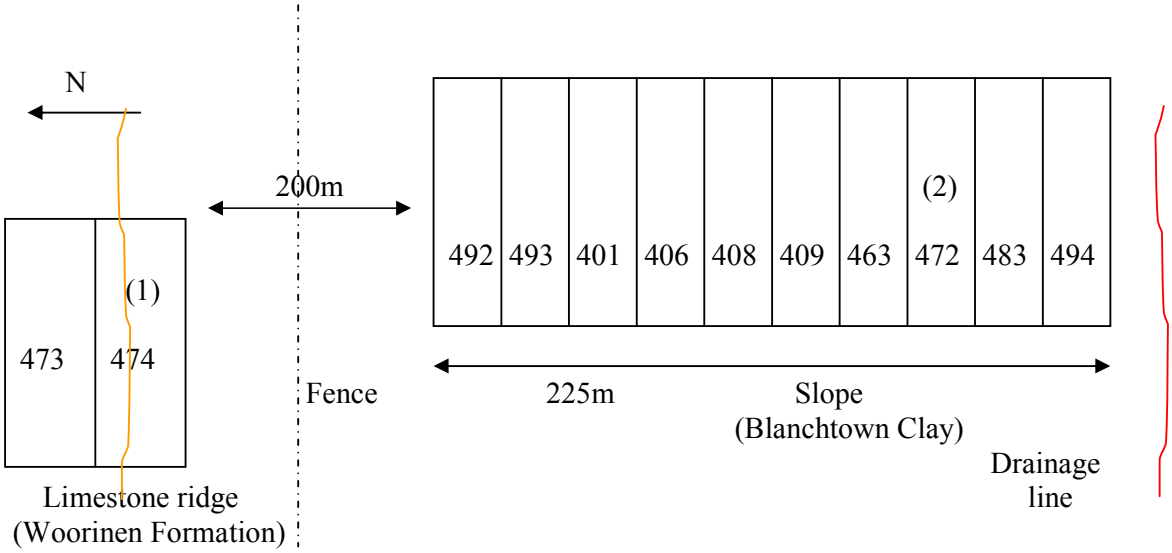


Figure 3.04: Field layout of trials at Angas Valley in 2004, including (1) RAC875/Cascades and (2) the durum landrace population.

The RAC875/Cascades population was sown late in June, but with minimal soil moisture and low rainfall, the plots had a low germination rate and were overrun with grassy weeds. No soil samples were therefore taken from the RAC875/Cascades population. Soil samples taken from the landrace population recorded a pH from 6.5 to 8.3 and $EC_{1:5}$ from 37 to 173µS/m in the topsoil (0-10cm), and a pH from 8.9 to 9.6 and $EC_{1:5}$ from 790 to 1110µS/m in the subsoil (40-50cm). Severe drought for 2004 reduced grain yield to an average of 42.5g/plot.

In 2005 the bread wheat experiment Frame/Yarralinka//Pugsley was sown twice at Angas Valley, along with the durum population Worrakatta/TmWLYY9//WLYY9Tm and bread wheat population RAC875/Cascades. One of the Frame/Yarralinka//Pugsley experiments was situated on a calcareous ridge of a Woorinen type dune system, while the other was situated 100m to the North at the junction of Molinuex and Woorinen land types, with a shallow, sandy-loam topsoil overlaying Blanchtown clay (Figure 3.05).

Soil samples were taken with the soil coring rig for the experiments Frame/Yarralinka//Pugsley and Worrakatta/TmWLYY9//WLYY9Tm. At the Angas Valley-South site for Frame/Yarralina//Pugsley, topsoil pH (0-10cm) ranged from mildly to strongly alkaline and was positively correlated with $EC_{1:5}$ in the non-toxic (<400µS) range (Section 7.4). The upper subsoil (30-40cm) pH was extremely alkaline (pH>9.2) and was positively correlated with $EC_{1:5}$ in the non-toxic range. The lower subsoil (50-60cm) pH was again extremely alkaline (pH>9.2) and positively correlated with $EC_{1:5}$, but with EC at toxic levels. The surface chart created from the mean soil values of each plot indicated minimal variation and few trends for either pH or EC across the field trial site at the three depths (Appendix 2).

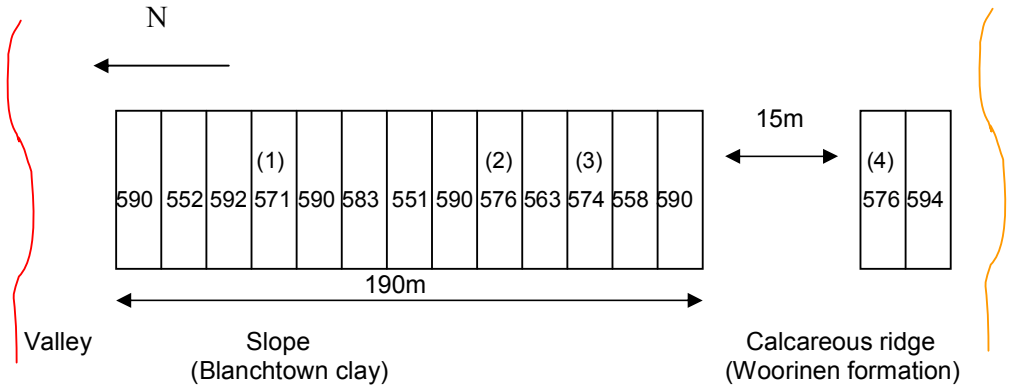


Figure 3.05: Field layout of trials at Angas Valley in 2005, including (1) Wk/TmWLYY9//WLYY9Tm, (2) and (4) Frame/Yarralinka//Puglsey, and (3) RAC875/Cascades.

At the Angas Valley-North site for Frame/Yarralina//Pugsley, topsoil pH (0-10cm) ranged from neutral to strongly alkaline and was positively correlated with $EC_{1:5}$ in the non-toxic (<400µS) range (Section 7.4). The upper subsoil (30-40cm) pH was extremely alkaline (pH>9.2) and was positively correlated with $EC_{1:5}$, although the association ceased when EC reached toxic levels. The lower subsoil (50-60cm) pH was again extremely alkaline (pH>9.2) and at toxic EC levels no correlation was identified. The surface chart indicates a more varied topsoil pH than the AV-S site, although the variation decreases with depth. No significant trends were found to occur with areas of higher pH spread irregularly across the trial site (Appendix 2). Similarly, high EC areas were spread intermittently across the site, corresponding to higher pH values, although EC increased in variation with depth.

The Worrakatta/TmWLYY9//WLYY9Tm trial site had topsoil (0-10cm) pH ranging from neutral to strongly alkaline, which was positively correlated with $EC_{1:5}$ in the non-toxic (<400µS) range (Section 7.3). The upper subsoil (30-40cm) pH was extremely alkaline (pH>9.2) and was positively correlated with $EC_{1:5,}$ which reached toxic levels. The lower subsoil (50-60cm) pH was again extremely alkaline (pH>9.2), but was not significantly correlated with $EC_{1:5}$ at toxic levels. The surface chart created from the mean soil values of each plot identified an increase in pH of the topsoil along one edge of the trial site, although $EC_{1:5}$ changed only marginally (Appendix 2). The subsoil charts had a more even distribution for pH across the site, with slight increases in an already extremely high pH corresponding to areas of higher salinity. However, at a depth of 50-60cm the pH range only varied by 0.5 units, which was likely to have prevented the detection of a significant trend with $EC_{1:5}$.

Both Frame/Yarralina//Pugsley trials were severely infected by stripe rust in 2005, which reduced yields in the bread wheat lines that did not contain the VPM gene from Pugsley. Similarly, the yield of the RAC875/Cascades population was severely reduced by stripe rust. The durum population Worrakatta/TmWLYY9//WLYY9Tm was not susceptible to the rust strain, and no yield loss was observed.

In 2005 rainfall was average to above average at Angas Valley, however, below average rainfall in 2004 and from January to May in 2005, provided no subsoil moisture and plant growth was sustained from moisture in the topsoil following rainfall events from June to November (Appendix 3).

Note: Average rainfall refers to the long-term average for the nearest Bureau of Meteorology site.

Buckleboo (BR)

The Buckleboo site was located approximately 5km north of the crossroads of the original site of the Buckleboo Primary School. The soil type at the site was an alkaline gradational soil, with a sandy-loam topsoil and loamy, calcareous subsoil. The site is prone to low soil fertility, heat stress and terminal drought (Figure 3.06).

In 2004 soil cores were collected with the hydraulic coring rig from the RAC875/Cascades and with the hand auger for the landrace experiment, at the depth of 0-10 and 40-50cm. For RAC875/Cascades, topsoil pH ranged from mildly to highly alkaline and was positively correlated with $EC_{1:5}$ in the non-toxic (<400µS) range (Section 7.2). Subsoil pH was highly alkaline and was also positively correlated with $EC_{1:5}$. The $EC_{1:5}$ was toxic (<400µS) at depth across the entire site. The surface chart shows the uniformity of the pH and $EC_{1:5}$ in the topsoil and the increasing trend in both pH and $EC_{1:5}$ across the site in the subsoil (Appendix 2).

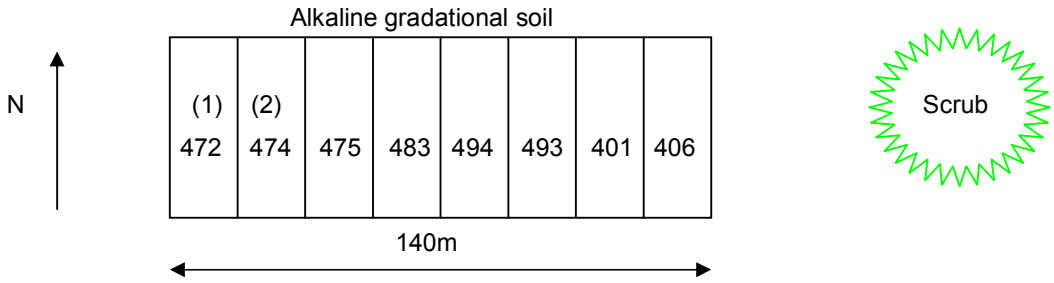


Figure 3.06: Field layout of trials at Buckleboo in 2004, including (1) the durum landrace population and (2) RAC875/Cascades.

Soil samples taken from the area where the landrace population was grown recorded a pH from 8.6 to 8.9 and $EC_{1:5}$ from 135 to 360µS/m in the topsoil (0-10cm), and a pH from 8.5 to 9.2 and $EC_{1:5}$ from 297 to 1690µS/m in the subsoil (40-50cm).

In 2004 below average rainfall in January to May provided minimal subsoil moisture at the beginning of the May sowing season. Average rainfall in June, July and August allowed for late sowing and good early growth, however, an extremely hot, and dry finish in October, extensively reduced grain yield.

In 2005 the bread wheat experiments Frame/Yarralinka//Pugsley and RAC875/Cascades, and the durum population Worrakatta/TmWLYY9//WLYY9Tm were sown at Buckleboo on a calcareous soil (Figure 3.07).

Soil samples were collected with the hand auger from the experiments Frame/Yarralinka//Pugsley and Worrakatta/TmWLYY9//WLYY9Tm at the depths 0-10cm, 30-40cm and 50-60cm. For the Frame/Yarralinka//Pugsley trial, pH averaged 8.7,

9.14 and 9.56, and $EC_{1:5}$ averaged 172.0, 866.8 and 1187.0µS/m, for each depth, respectively. Similarly, for the Worrakatta/TmWLYY9//WLYY9Tm trial, pH averaged 8.65 and 9.23 for the depths 0-10cm and 30-40cm, and $EC_{1:5}$ averaged 151.1 and 619.0µS/m for the depths 0-10cm and 30-40cm. No samples were collected at 50-60cm due to high soil strength.

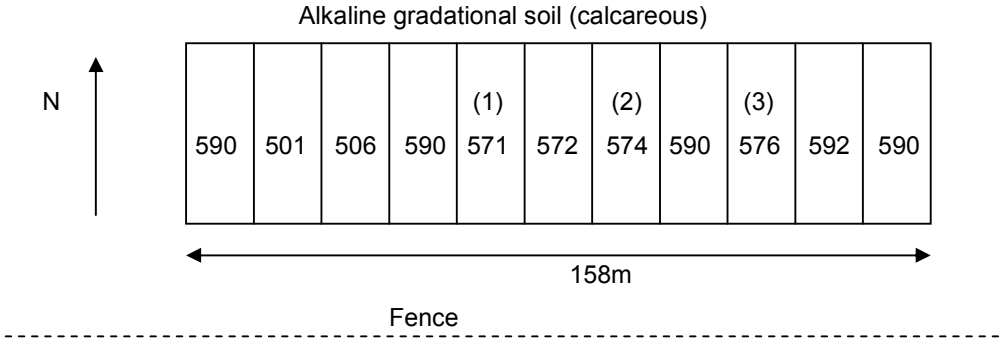


Figure 3.07: Field layout of trials at Buckleboo in 2005, including (1) Wk/TmWYLL9//WLYY9Tm, (2) RAC875/Cascades, and (3) Frame/Yarralinka//Pugsley.

In 2005 rainfall at Buckleboo was slightly below average. Similar to 2004, rainfall was well below average from January to May providing no subsoil moisture at the beginning of the May sowing season (Appendix 3). Average rainfall was again received for June, July and August, however, unlike 2004, high rainfall was also recorded for September and October, resulting in average grain yields.

Claypans (CK)

The Claypans site in 2004 was located approximately 2km south-west of the township of Claypans on a calcareous soil type, with a shallow calcareous sandy loam topsoil overlaying calcareous rubble and sheet limestone at a depth of 20-40cm. The site is prone to nutrient deficiencies, such as, Zn, P, and Fe, wind erosion and has a low water holding capacity.

In 2004 the bread wheat population RAC875/Cascades and durum landrace population was sown at Claypans on a calcareous ridge (Figure 3.08).

Soil samples were collected with the hand auger from only the durum landrace experiment. The pH of the experimental plots ranged from 8.6 to 8.8 in the topsoil and from 8.7 to 8.9

in the subsoil. The $EC_{1:5}$ ranged from 119 to 223µS/m in the topsoil and from 125 to 359µS/m in the subsoil.

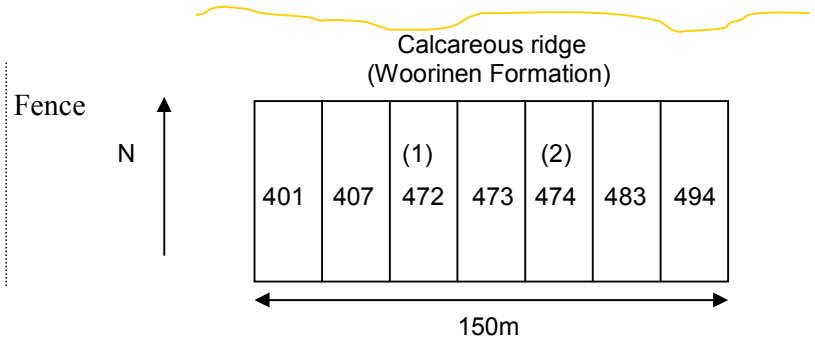


Figure 3.08: Field layout of trials at Claypans in 2004, including (1) the durum landrace population and (2) RAC875/Cascades.

In 2005 the bread wheat population RAC875/Cascades and durum wheat experiment Worrakatta/TmWLYY9//WLYY9Tm were again sown on a highly calcareous soil type (Figure 3.09).

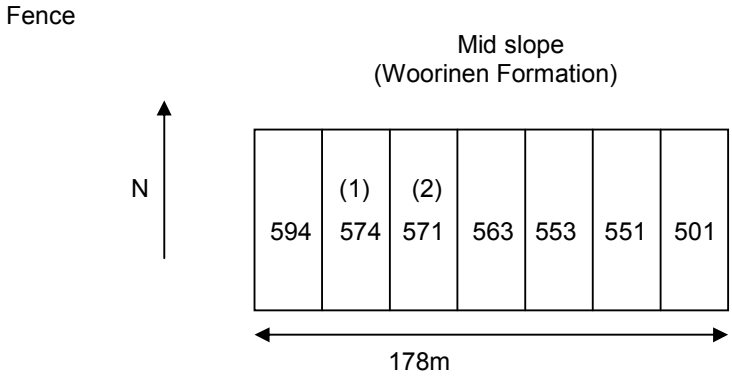


Figure 3.09: Field layout of trials at Claypans in 2005, including (1) RAC875/Cascades and (2) Wk/TmWLYY9//WLYY9Tm.

Soil samples were collected from only the Worrakatta/TmWLYY9//WLYY9Tm experiment with the hand auger. The pH averaged 8.61 and 9.03 for the depths 0-10cm and 30-40cm, and $EC_{1:5}$ averaged 169.7 and 209.2µS/m for the depths 0-10cm and 30-40cm. No samples were collected at 50-60cm due to a layer of sheet limestone at 40cm in depth.

In 2005 rainfall at Claypans was above average, with high rainfall from June to December (Appendix 3). However, moist conditions resulted in the high incidence of Stripe Rust, which reduced yield in the RAC875/Cascades bread wheat population.

Coonalpyn (CS)

The Coonalpyn site was located approximately 2km south of the township of Coonalpyn, in a gently undulating area of Woorinen and Molineaux soil types. The topsoil varied from siliceous sand to a brown calcareous sandy loam, overlaying a bleached A_2 horizon, and sodic, clay subsoil. The site is associated with trace element deficiency, high calcium carbonate concentration, moderate alkalinity, and has high sodicity.

In 2004 the durum landrace population was sown at Coonalpyn on a Molineaux type soil. The pH of the experimental plots ranged from 8.2 to 8.4 in the topsoil and from 8.7 to 9.2 in the subsoil. The $EC_{1:5}$ ranged from 116 to 163µS/m in the topsoil and from 124 to 306µS/m in the subsoil.

In 2005 the bread wheat population RAC875/Cacades was also sown on a Molineaux type soil. The pH averaged 8.33, 8.90 and 9.39 for the depths 0-10cm, 30-40cm and 50-60cm, respectively, and $EC_{1:5}$ averaged 208, 188 and 262µS/m for the depths 0-10cm, 30-40cm, and 50-60cm, respectively.

In 2005 rainfall at Coonalpyn was slightly below average, with high rainfall from June to December after a dry start to the year (Appendix 3). Stripe Rust was also observed at Coonlapyn, which reduced yield in the RAC875/Cascades bread wheat population.

Jamestown (JC)

The Jamestown site was located approximately 10km south of Jamestown on gently undulating slopes, with a slightly acid to neutral loam topsoil overlaying a red-brown clay loam, often with deep texture contrast calcareous subsoil. The site is relatively fertile with few adverse soil conditions.

In 2004 the durum landrace population was sown at Jamestown on the slope of gently undulating hill, on a deep loam-clay soil. The pH of the experimental plots ranged from 6.6 to 7.5 in the topsoil and from 7.1 to 7.7 in the subsoil. The $EC_{1:5}$ ranged from 53 to 117µS/m in the topsoil and from 81 to 187µS/m in the subsoil.

In 2005 the durum wheat population Worrakatta/TmWLYY9//WLYY9Tm was sown in the valley between gentle slopes, on a heavy clay-loam soil. The pH averaged 6.15, 7.69 and 8.63 for the depths 0-10cm, 30-40cm and 50-60cm, respectively, and $EC_{1:5}$ averaged 231.0, 188.8 and 479.7µS/m for the depths 0-10cm, 30-40cm, and 50-60cm, respectively.

Kapunda (KH)

The Kapunda site was located approximately 2km west of the township on a loam to clay-loam texture contrast soil. The site is regarded as high rainfall, has high relative fertility, but can grade into high alklalinity and salinity at depth.

In 2005 the bread wheat populations RAC875/Cascades and Frame/Yarralinka//Pugsley were sown mid-slope, on a loamy, clay texture contrast soil (Figure 3.10).

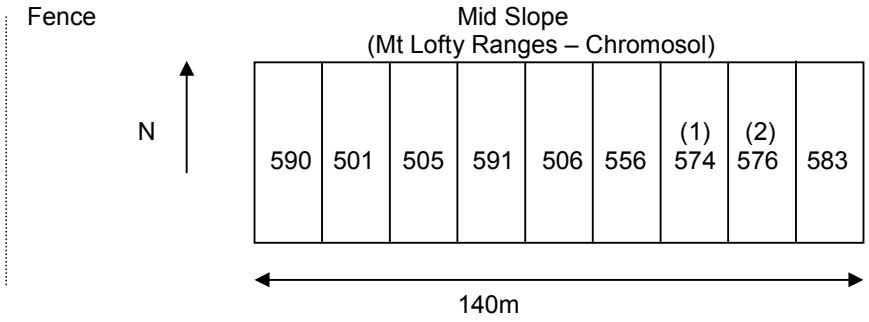


Figure 3.10: Field layout of trials at Kapunda in 2005, including (1) RAC875/Cascades and (2) Frame/Yarralink//Pugsley.

Soil samples collected with the hand auger showed that the pH averaged 8.04, 8.80 and 9.12 for the depths 0-10cm, 30-40cm and 50-60cm, respectively, and $EC_{1:5}$ averaged 165, 199 and 224µS/m for the depths 0-10cm, 30-40cm, and 50-60cm, respectively.

Rainfall at Kapunda in 2005 was above the long-term average, although most was recorded from June to December, with little subsoil moisture at the beginning of the growing season (April-May).

Redhill (RH)

The Redhill site was located approximately 8km south east of the township of Redhill on an alluvial flat, with a loamy clay topsoil and heavy, impermeable clay subsoil. The site is characterised by high levels of sodicity, salinity, high pH, boron, and prone to root diseases, such as Rhizoctonia (*Rhizoctonia solani*), and crown rot (*Fusarium pseudograminearum*).

In 2004 the bread wheat population RAC875/Cascades and durum landrace population was sown at Redhill on an alluvial flat of heavy clay-loam (Figure 3.11).

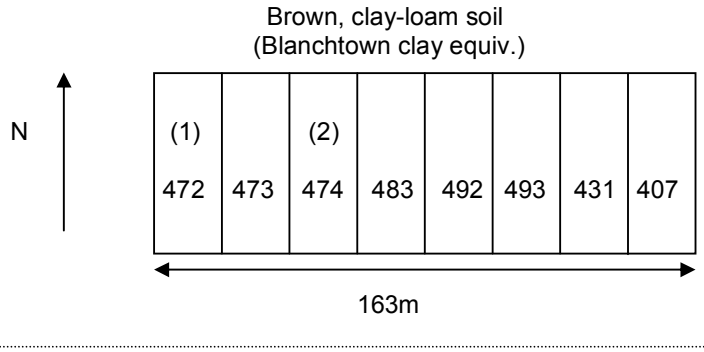


Figure 3.11: Field layout of trials at Redhill in 2004, including (1) the durum landrace population and (2) RAC875/Cascades.

The pH of the durum landrace experimental plots ranged from 6.3 to 7.2 in the topsoil and from 8.7 to 9.0 in the subsoil. The $EC_{1:5}$ ranged from 98 to 215µS/m in the topsoil and from 1510 to 2220µS/m in the subsoil.

In 2005 the bread wheat populations RAC875/Cascades and Frame/Yarralinka//Pugsley, and the durum population Worrakatta/TmWLYY9//WLYY9Tm were sown near the 2004 trial site on an heavy clay-loam soil (Figure 3.12).

Soil samples collected from the Worrakatta/TmWLYY9//WLYY9Tm experiment had pH averaging 6.41, 8.61 and 8.34 for the depths 0-10cm, 30-40cm and 50-60cm, respectively, and $EC_{1:5}$ averaging 777, 2074 and 3944µS/m for the depths 0-10cm, 30-40cm, and 50-60cm, respectively.

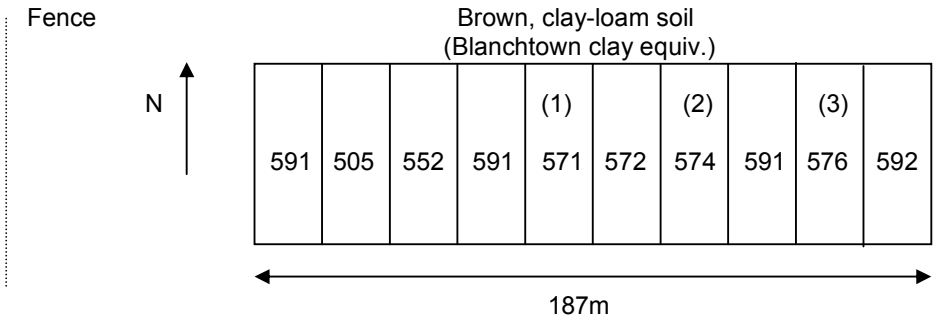


Figure 3.12: Field layout of trials at Redhill in 2005, including (1) Wk/TmWLYY9//WLYY9Tm, (2) RAC875/Cascades, and (3) Frame/Yarralink//Pugsley.

In 2005 rainfall at Redhill was near average. Seeding in the district occurred under dry conditions with no subsoil moisture, which was associated with Rhizoctonia patches. However, average rain from June to December, resulted in average yields.

Roseworthy (RAC)

The Roseworthy site in 2004 was located approximately 1km north-east of the Roseworthy Agricultural College on a red-brown earth (Blanchtown equivalent), calcareous in areas (Woorinen Formation), with lighter loam topsoil over a clay loam B horizon and alkaline at depth.

The bread wheat population RAC875/Cascades was sown on a Woorinen slope, with a calcareous loam topsoil, overlaying a loamy-clay subsoil. Topsoil (0-10cm) pH ranged from neutral to mildly alkaline and was positively correlated with $EC_{1:5}$ in the non-toxic (<400µS) range (Figure 7.2). Subsoil (40-50cm) pH ranged from mildly to highly alkaline and was positively correlated with $EC_{1:5}$, also in the non-toxic range. The surface chart created from the mean soil values of each plot identified an increase in pH of the topsoil along a trough extending across centre of the trial site, with a corresponding increase in $EC_{1:5}$ (Appendix 2) The subsoil chart had a more even distribution of both pH and $EC_{1:5}$ across the site.

Rainfall in 2004 was slightly below average, although most of the rain was recorded from June through September. Below average rainfall from January to May resulted in minimal

subsoil moisture at the beginning of sowing, and low rainfall in October and November, led to well below average grain yields.

In 2005 the trial site was located approximately 2km south of the Roseworthy Agricultural College, adjacent to an occasional drainage line, at the junction of a Woorinen dune and an alluvial clay flat (Blanchtown clay equivalent). The site was known to have a salinity and pH gradient down the slope, good fertility, but is prone to slumping and surface sealing. The soil type at the site was clay-loam topsoil over clay subsoil.

In 2005 the bread wheat experiment Frame/Yarralinka//Pugsley was sown twice at Roseworthy, along with the durum population Worrakatta/TmWLYY9//WLYY9Tm and bread wheat population RAC875/Cascades. One of the Frame/Yarralinka//Pugsley experiments was situated on a calcareous ridge of a Woorinen type dune system, while the other was situated 100m to the West at the junction of a Woorinen dune system and an alluvial clay flat (Figure 3.13).

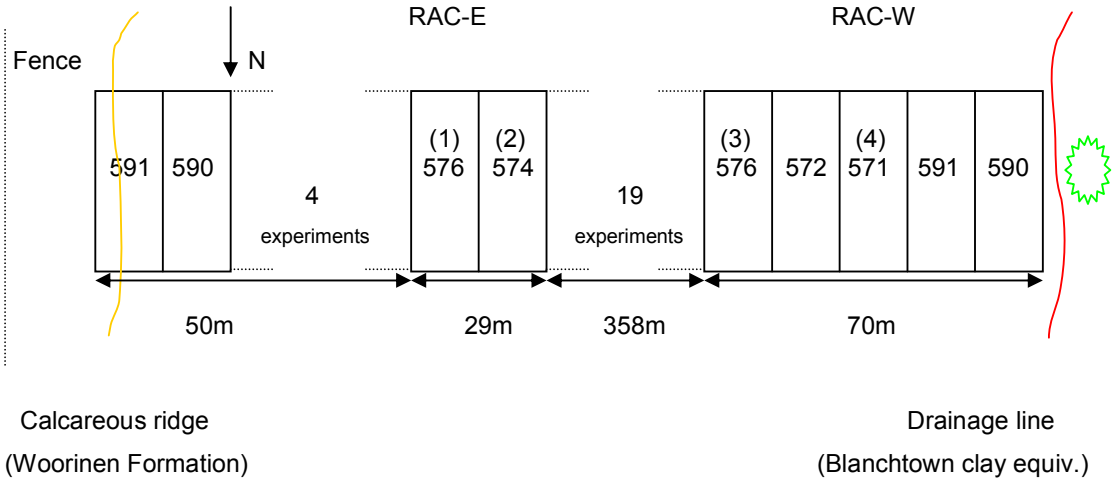


Figure 3.13: Field layout of trials at Roseworthy in 2005, including (1) and (3) Frame/Yarralinka// Pugsley, (2) RAC875/Cascades and (4) Wk/TmWLYY9//WLYYTm.

Soil samples were collected from the two Frame/Yarralina//Pugsley experiments and Worrakatta/TmWLYY9//WLYY9Tm. At the Roseworthy-East (RAC-E) site, on the calcareous ridge, topsoil pH ranged from acid to mildly alkaline and was positively correlated with $EC_{1:5}$ in the non-toxic range (Section 7.4). The upper subsoil (30-40cm) pH was mildly alkaline and was positively correlated with $EC_{1:5,}$ in the non-toxic range. The lower subsoil (50-60cm) pH ranged from mildly to extremely alkaline and again was

positively correlated with $EC_{1:5}$, but at toxic levels. The surface chart indicates a significant pH trend across the site from acid to alkaline in the topsoil, which also was observed in the subsoil, although a second band of higher pH soil stretched across the centre of the trial site (Appendix 2). Subsoil EC follows a similar trend to pH, although is less evident, and topsoil EC varies only marginally.

The Roseworthy-West (RAC-W) trial site in 2005 was located further down the ridge at the junction of the Woorinen soil and an alluvial clay flat. Topsoil pH again ranged from acid to mildly alkaline and was positively correlated with $EC_{1:5}$ in the non-toxic range (Section 7.4). The upper subsoil (30-40cm) pH was strongly alkaline and was positively correlated with toxic $EC_{1:5}$ levels. The lower subsoil (50-60cm) pH was extremely alkaline (pH>9.2), but unlike the RAC-E site, was not significantly correlated with $EC_{1:5}$ at toxic levels. The surface chart identified strips of higher pH across the site, and an area of pH in the acid range to one end of the site (Appendix 2). The trends were observed down the profile, although the variation decreased with depth. Similarly, and as with the RAC-E site, EC followed a similar trend across the site as pH, and increased with intensity with depth.

The Worrakatta/TmWLYY9//WLYY9Tm trial site in 2005 was also located at the junction of a Woorinen soil type and an alluvial clay flat, near the RAC-W site. Topsoil pH ranged from slightly acid to mildly alkaline and was positively correlated with $EC_{1:5}$ in the non-toxic range (Section 7.3). The upper subsoil (30-40cm) pH ranged from mildly alkaline to extremely alkaline (pH>9.2) and was positively correlated with $EC_{1:5,}$ which reached toxic levels. The lower subsoil (50-60cm) pH was extremely alkaline (pH>9.2), but similar to the Angas Valley site, was not significantly correlated with $EC_{1:5}$ at toxic levels. The surface chart indicates a trend towards greater topsoil pH to one corner of the trial site, although the trend was not evident with depth (Appendix 2). In contrast, $EC_{1:5}$ varies more with depth, but again is higher in the same corner of the trial site as the pH trend.

Rainfall at Roseworthy in 2005 was well above average, following a dry 2004 and beginning of 2005. Sowing started in dry conditions with no subsoil moisture, however, above average rain for the remainder of the year resulted in good grain yields. Moist conditions provided favourable conditions for Stripe Rust infection, although fungicide application prevented significant yield loss in the Frame/Yarralinka//Pugsley bread wheat population, which had variable levels of susceptibility in the individual lines.

Two Wells (TW)

The Two Wells site was located approximately 2km north of the township of Two Wells on a soil derived from alluvium (Blanchtown clay equivalent), with a loamy clay topsoil and heavy, red-brown clay subsoil. The site has high levels of sodicity, salinity, high pH, boron, and is prone to waterlogging, and hard-setting.

The pH of durum landrace experimental plots ranged from 6.4 to 7.8 in the topsoil and from 8.6 to 9.3 in the subsoil. The $EC_{1:5}$ ranged from 84 to 200µS/m in the topsoil and from 270 to 1440µS/m in the subsoil.

In 2005 the durum population Worrakatta/TmWLYY9//WLYY9Tm was sown on a similar alluvial soil as in 2004. The pH averaged 7.48, 9.39 and 9.34 for the depths 0-10cm, 30-40cm and 50-60cm, respectively, and $EC_{1:5}$ averaged 301, 791 and 1204µS/m for the depths 0-10cm, 30-40cm, and 50-60cm, respectively.

Similar weather conditions were observed at Two Wells as recorded for Roseworthy.

Wanderah (WJ)

The Wanderah site in 2004 was located approximately 10km south of Port Pirie on an alluvial clay flat with a shallow loam topsoil, which was absent in sections across the site. The site was chosen as an experimental location, since the area was prone to magnesia patches (surface salinity), sodicity, salinity, high pH and surface crusting.

In 2004 the experiment RAC875/Cascases population was sown on an alluvial clay flat, while the durum landrace population was sown separately at a site 500m to the North on a similar alluvial clay soil. Topsoil pH of the RAC875/Cascades site ranged from slightly acid to mildly alkaline and was not correlated with $EC_{1:5.}$ The $EC_{1:5}$ reached toxic (<400µS) levels across parts of the site, consistent with the sites prior history of magnesia patches (Section 7.2). Subsoil pH was mildly alkaline and was negatively correlated with $EC_{1:5}$ in the highly toxic range. The surface chart identified a slight topsoil pH trend across the site, which was weakly associated with variable areas of surface salinity (Appendix 2).

The subsoil chart showed an area of slightly higher pH to one end of the site, and a highly variable $EC_{1:5}$ distribution across the site.

In 2004 total rainfall at Wanderah was near average, however, below average rainfall from February to May, and in September and October, severely reduced grain yield.

In 2005 the Wanderah site was located approximately 15km south of Port Pirie on the side of a low relief dune, grading from calcareous to an alluvial clay flat with a shallow loam topsoil, which was absent in sections across the site. The bread wheat populations Frame/Yarralinka//Pugsley and RAC875/Cascades, and durum wheat population Worrakatta/TmWLYY9// WLYY9Tm were sown at the site (Figure 3.14).

For the Frame/Yarralinka//Pugsley experiment topsoil pH ranged from acid to mildly alkaline and was positively correlated with $EC_{1:5}$, which reached toxic levels for several plots (Section 7.4). The upper subsoil (30-40cm) pH ranged from mildly alkaline to strongly alkaline, but contrary to the other sites, was negatively correlated with $EC_{1:5,}$ at highly toxic levels. Similarly, the lower subsoil (50-60cm) pH was mildly to strongly alkaline and was negatively correlated with $EC_{1:5}$ at highly toxic levels. The surface chart indicates that the topsoil grades from slightly acidic to moderately alkaline across the site, with significantly higher EC patches in the areas of high pH (Appendix 2). The subsoil pH, however, is greatest at the slightly acid topsoil end (North) and declines slightly towards the Southern end of the trial. Subsoil EC increased in intensity at either end of the trial site with depth.

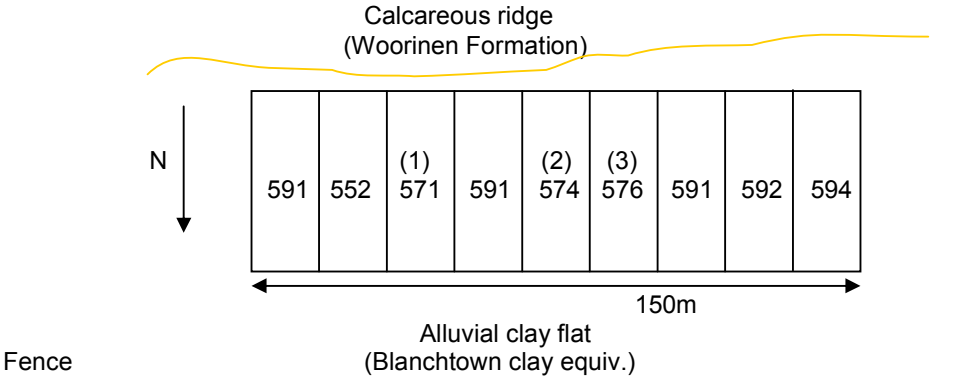


Figure 3.14: Field layout of trials at Wanderah in 2005, including (1) Wk/TmWLYY9//WLYY9Tm, (2) RAC875/Cascades and (3) Frame/Yarralinka//Pugsley.

For the Worrakatta/TmWLYY9//WLYY9Tm trial, soil pH and EC values were in a similar range as the Frame/Yarralinka//Pugsley trial, however, topsoil pH was not correlated with $EC_{1:5}$ (Section 7.2) and the lower subsoil pH was not significantly correlated with $EC_{1:5}$. The surface chart also had a similar trend across the site as the Frame/Yarralinka//Pugsley site.

Rainfall in 2005 was near average for Wanderah and similar to the other sites, following drought conditions in 2004, the beginning of 2005 was also dry, with no subsoil moisture at the start of the growing season. Crop growth relied on average rain received from June to December to produce average grain yield.

Winulta (WC)

The Winulta site was located approximately 12km west of Port Clinton on a calcareous rise (Woorinen Formation), with a loamy to sandy topsoil and heavier loamy clay subsoil. The site has medium rainfall, relatively good fertility, with moderate soil pH and transient salinity on the slopes of Woorinen type dune systems.

In 2004 the experiment RAC875/Cascases population was sown. The pH ranged from 7.1 to 7.6 in the topsoil (0-10cm) and 7.8 to 8.4 in the subsoil (40-50cm), while the $EC_{1:5}$ was non-toxic in the topsoil and subsoil.

3.2 Seed Source for field trials

The seed for the durum landrace population grown in 2004 was sourced from 2003 field trials of the Durum Breeding group, University of Adelaide. The population had previously been grown for two years, with some selection for uniform types in the landrace lines. The landrace lines originated for the west Asia region of Iran, Iraq, and Afganistan, and were supplied from the Winter Cereals Collection in Tamworth.

The RAC875/Cascades bread wheat population had been grown in field trials by the Durum Breeding group for several years. The double haploid population was developed by Hugh Wallwork, Molecular Plant Breeding CRC. Trials were re-sown each year using seed harvested from the previous years field trials, mostly from the Roseworthy site.

In 2005 and 2006 the durum population Worrakatta/TmWLYY9//WLYY9Tm was sown using seed from 2003 and 2005 field trials, respectively. The population had been developed by the Durum Breeding group by crossing the F_1 of the inbred parents Worrakatta and TmWLYY9 by the inbred line WLYY9Tm. The population was created by four generations of single-seed decent from the F_2, and multiplication in the field.

The bread wheat population Frame/Yarralinka//Pugsley grown in 2006 was sourced from a single trial site at Roseworthy in 2005. The population was developed by the Durum Breeding group by crossing the inbred parents Frame/Yarralink/23/1 and Pugsley. Seed from the F_2 were selected for single-seed descent, undergoing three generations of single seed selection, before multiplication in the field in 2005.

3.3 Hydroponic bicarbonate screening

Bread or durum wheat seeds were sourced from previous field trials or collections of the Durum Breeding group, Waite Campus, University of Adelaide. Seeds of uniform size were selected and placed onto plastic and mesh racks. The plastic racks were 330mm long and 12mm wide, on 3cm legs, consisting of 120 holes (wells) that were 30mm x 10mm and 6mm deep, with 2mm mesh on the underside of the wells (Figure 3.15). Four seeds were placed into each well on the mesh and the entire tray soaked in 2% hypochloride for 10 minutes. Trays and seed were then rinsed under de-ionised reverse osmosis (RO) water for 2 minutes, before being soaked in RO water for a further 10 minutes. Trays were placed into plastic containers over 1cm of RO water, sealed and placed in the dark at 4°C. After 48 hrs the containers were opened, seeds turned crease down and one seed removed from each well, leaving three seeds per well. Containers were re-sealed and placed in the dark at 25°C for a further 48hrs or until roots were 20mm long.

The treatment solution tanks were set up 24hrs prior to use, with the addition of carbonates, nutrients and/or RO water, depending on the test (Chapter 5 and 6). The tanks were 25L plastic tubs that were filled to 22L, allowing the plastic germination trays to be suspended over the solution by stainless steel rods, with the bottom of the tray in contact with the solution. The plastic trays were placed over the treatment solution when the germinated seeds had 20mm long roots. The tanks were placed on laboratory benches, and

therefore varied in temperature (18-26°C) and light (10-16hrs/day). Air was supplied to the treatment tanks via 10" x ½" airstones, run by a small aqua pump, through 4mm air hose. To maintain a constant pH the air was bubbled through a concentrated (10M) KOH solution, to remove CO_2. The pH was measured daily and adjusted accordingly with $NaHCO_3$, Na_2CO_3 or NaOH. If fungal problems occurred the trays were sprayed with the fungicide Bayleton® (Triadimefon), but only after shoot elongation was >30mm. The root length of each seedling was measured after 9-10 days and the average root length of the well (three seeds) recorded.

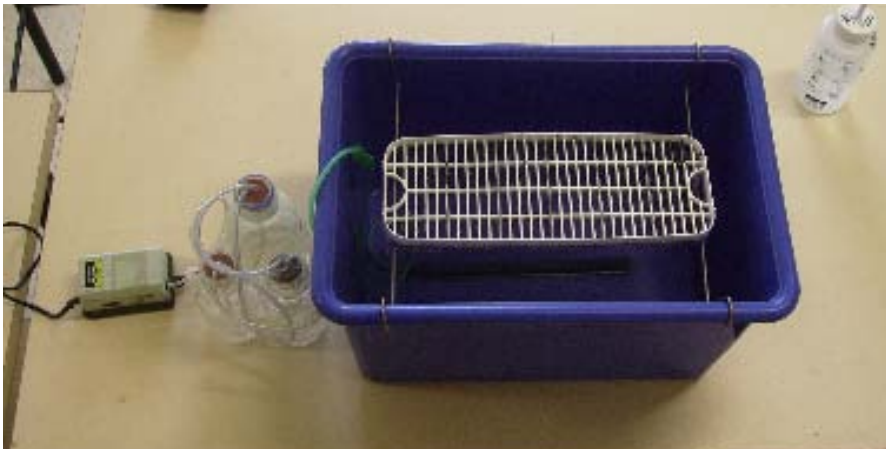


Figure 3:15: Hydroponic screening equipment, including aqua pump, KOH solution bottles, 25L tank, stainless steel rods, airstone and plastic seed tray with mesh.

3.4 Statistical analysis

Basic statistical analysis, such as, mean, standard deviation, and r^2 correlations, were performed using Microsoft Excel 2000®. Multiple linear regression analysis was performed using Genstat 6®.

Chapter 4

Field evaluation of high pH and bicarbonate toxicity on bread and durum wheat growth.

4.1 Introduction

High pH soils contain numerous physical and chemical properties, which can adversely affect plant growth. Physically, calcareous soils tend to drain easily and have a low water holding capacity, and sodic-alkaline soils tend to be impermeable with low porosity, high soil strength, poor water-soil relations and have dispersive properties (Rengasamy 2002). Few economically feasible methods for correction of high pH soils exist, due to the presence of high concentrations of calcium carbonates and increased severity of pH and sodicity with depth. Improving the adaptation of crop varieties to high pH soils provides the most financially viable option for increasing productivity.

Alkaline soils are widespread in semi-arid areas around the world, yet only a limited number of reports exist on the effects of high pH soils on crop performance. Plant growth on high pH soils may be directly affected by high OH^- concentrations and HCO_3^-/CO_3^{2-} toxicity, or indirectly by several nutritional disorders. High OH^- concentrations were found to limit root growth, although the direct effects of OH^- are relatively unknown (Kopittke and Menzies 2004). The toxicity of HCO_3^- in soils has been studied in more detail, but mainly in relation to the irrigation of horticultural crops or rice paddies, particularly the influence of HCO_3^- on Fe and Zn nutrition (Romheld 1985, Ao *et. al.* 1987, Shi *et. al.* 1993, Yang *et. al.* 1993). The most extensively studied aspect of high pH has been the soil chemistry associated with the solubility of ions. In high pH soils the solubility of phosphorus, calcium, magnesium, iron, zinc, manganese and copper declines, corresponding with numerous reports of nutrient disorders amongst different species (Marschner 1995, Baar and Roelofs 2002).

The complex nature of high pH soils, particularly in the field where multiple stresses both chemical and physical interact, has made any quantification of the field affects of pH on crop yield difficult. High pH soils in South Australia can be divided into two main types,

calcareous (pH 7.5-8.5) and sodic-alkaline (pH>8.5), although almost inevitably a combination of both exist spatially and at varying depths at any one site. Poor crop growth has commonly been associated with both calcareous and sodic-alkaline soils (Lee and Woolhouse 1969, Marschner 1997), although the relationship between measurement of field pH and crop growth have varied significantly between studies and for different species. Lupins are noted for being highly sensitive to high pH soils (Fe deficiency). Cereals species are less sensitive to nutrient disorders associated with high pH soils, although triticale and barley are more tolerant than bread wheat, which is more tolerant than durum wheat and oats (Lee and Woolhouse 1969, Marschner 1997, Alhendawi *et. al.* 1997, Lui and Rathjen 1998, Zubaidi *et. al.* 1999, Maschmedt 2002).

The aims were (i) to investigate the effect of soil pH on both bread and durum wheats at a range of field sites, (ii) to determine the effects of increasing soil pH on the ability of durum wheats to acquire adequate nutrition, (iii) to assess HCO_3^- tolerant durum lines for improved uptake of elements, such as Zn and Fe, and (iv) to verify the use of soil pH measurements as a suitable indicator for predicting the concentration of HCO_3^- and CO_3^{2-} for the occurrence of toxicity.

4.2 High pH effect on Bread and Durum wheats grown in the field.

4.2.1 Introduction

The distribution of both bread and durum wheat varieties in South Australia is often associated with soil type. Bread wheat varieties, such as, Krichauff, Yitpi and Spear derivatives, and to a lesser extent the durum wheat variety Kalka, have been dominant in the semi-arid areas of South Australia, such as the Murray Mallee and Eyre Peninsula, which are commonly calcareous and sodic-alkaline. The production of bread and durum wheat varieties, such as, Janz and Yallaroi, however, has been more restricted to the higher rainfall, texture contrast soils of the northern Mt Lofty Ranges. The superior performance of varieties, such as Krichauff, in alkaline soils has generally been associated with greater tolerance to salinity, boron and bicarbonate (Rathjen *et. al.* 1999), which are more prevalent and often at toxic levels in calcareous and sodic-alkaline soil types. Both boron and salinity have been identified in the field as major yield limiting factors to grain yield (Paull 1990, Cooper 2004), while the effects of bicarbonate are less clear.

In field studies Cooper (2004) was able to demonstrate the significance of growth limiting factors at various sites across South Australia. Various combinations of toxic levels of soil salinity (>4ds/m), pH (>8.5) and boron (>3mg/kg) were found at each of the sites and correlations identified with grain yield. At Angas Valley increasing topsoil pH (0-10cm) was associated with reduced grain yield of Tamaroi by 43% over the pH range 7 to 9. At Redhill subsoil pH (50-60cm) reduced grain yield of Tamaroi by 36% (1.3t/ha) over the pH range 8.9 to 9.5. A second site at Redhill topsoil pH (0-10cm) reduced grain yield of Tamaroi by 28% (1.2t/ha) over the pH range 7.7 to 8.7. At Roseworthy subsoil pH (30-40cm) reduced grain yield of Tamaroi by 27% over the pH range 8.0 to 9.8. Soil salinity and boron were also found correlated with grain yield at many of the sites, although, similar to pH, significant correlations were only identified in the durum wheat Tamaroi and not the bread wheat varieties tested.

Cooper's (2004) study used only one durum variety, few bread wheat varieties, limited replication, and only one year of results. The following experiments therefore aim to further investigate the effect of soil pH on both bread and durum wheat using a range of sites and varieties, with greater replication and soil sampling.

4.2.2 Materials and Methods

Three durum wheat varieties, Tamaroi, Yallaroi and Kalka, and four bread wheat varieties, RAC875, Cascades, Frame/Yarralinka, and Pugsley, known to differ in bicarbonate response (Table 4.01), were grown at multiple sites (Table 3.01). The selection of test varieties was based on their use as parental lines in mapping populations. Seed for field trials was sourced from high yielding field sites grown in 2004 or 2003.

Table 4.01: Three durum and four bread wheat varieties, pedigrees, state of origin and their root length (mm) in bicarbonate treatment.

Genotype	Pedigree	Origin	Bicarbonate Response
Tamaroi	Altar 84/4/Tam1B-17/Kamillaroi/3/Wells/56111//Guillemont	SA	49.33
Yallaroi	Guillmont 3/Kamillaroi sib	NSW	44.25
Kalka	Wollaroi*/(Linghzi*Yallaroi#)*RH880009	SA	79.00
RAC875	RAC655/(Sr21/4*Lance//4*Bayonet)	SA	49.33
Cascades	Aroona*3/(AusenVII-95)Tadorna.Inia66	WA	83.33
(Frame/Yarralinka)/23/1	(Molinuex/Dagger#3)*(MSMS//Crim/4/CombIII/2*Warigal)	SA	66.77
Pugsley	Frame/Corrigin//Frame/3/Trident	SA	49.50

The varieties were sown in field experiments as check plots within their corresponding population (Chapter 7), such that, three separate experiments were conducted.

Experiment 1: Tamaroi, Yallaroi and Kalka

The durum varieties were grown with the single-seed decent (SSD) population Wk/TmWLYY9//WLYY9Tm in 2005, at the field sites AV, BR, CK, JC, RAC, RH, TW, and WJ. Thirty plots each of Tamaroi, Yallaroi and Kalka were sown consecutively every 5th plot with 350 Wk/TmWLYY9//WLYY9Tm progeny lines and 10 standard varieties, giving a total experiment size of 450 plots.

Experiment 2: RAC875 and Cascades

The bread wheats Cascades and RAC875 were grown with the double haploid (DH) population RAC875/Cascades in 2004, at the field sites AV, BR, CS, RAC, KH, RH, WC, and WJ. Twenty plots of RAC875 and twenty-five plots of Cascades were sown alternatively every 10th plot across the experiment along with 81 RAC875/Cascades progeny lines in 2 replications (total 162), and l8 standard varieties, giving a total experiment size of 225 plots.

Experiment 3: Frame/Yarralinka and Pugsley

Frame/Yarralinka and Pugsley were grown with the SSD population Frame/Yarralinka//Pugsley in 2005, at the field sites AV-North, AV-South, BR, KH, RAC-East, and RAC-West, RH, WJ. Twenty-three plots of Frame/Yarralinka and twenty-two plots of Pugsley were sown every 10th plot across the experiment with 144 Frame/Yarralinka//Pugsley progeny lines, and 36 standard varieties, giving a total experiment size of 225 plots.

The experiments were sown and managed according to the methods in Section 3.1. The hydraulic soil core rig was used to take soil samples for Experiment 1 at AV, RAC and WJ, Experiment 2 at BR, RAC and WJ, and experiment 3 at AV-N, AV-S, RAC-E, RAC-W and WJ. At all other sites a hand auger was used to take several random soil samples per experiment. Soil samples were collected and measured for pH and EC according to the methods in Section 3.1.4.

Mean, standard deviation, and ranges were calculated for site pH and $EC_{1:5}$ values at 2 or 3 soil sample depths. Plot yield (g/plot) was correlated with plot pH or $EC_{1:5}$ and significance determined. When more than one soil covariate was significantly correlated with yield, a multiple linear regression analysis was performed to identify the covariate that explained the greatest proportion of the variation. Soil covariates of significance were plotted against yield to determine the extent of the effects on yield due to changes in soil pH or $EC_{1:5}$ at varying depths.

4.2.3 Results

Experiment 1 - Tamaroi, Yallaroi and Kalka

Trials were harvested in December 2005 and soil samples collected from three sites, Wanderah, Angas Valley, and Roseworthy, in 2006, to determine primarily the effects of pH, but also EC on the grain yield of durums, using the varieties Tamaroi, Yallaroi, and Kalka. The pH, EC and yield results are summarised in Table 4.02.

Table 4.02: The mean (M), standard deviation (SD) and soil pH (pH>8.5) and $EC_{1:5}$µS/m (EC>400) range at the depths 0-10cm, 30-40cm and 50-60cm, and grain yield (g/plot) (highest) for the durum varieties Kalka, Tamaroi and Yallaroi, at the three sites Wanderah, Angas Valley and Roseworthy.

		Wanderah		Angas Valley		Roseworthy	
	Depth (cm)	M±SD	Range	M±SD	Range	M±SD	Range
pH	0-10	7.24±0.35	6.3-8.1	8.05±0.79	6.5-9.7	7.51±0.73	6.3-8.9
	30-40	8.35±0.28	7.9-9.1	10.00±0.17	9.5-10.3	9.44±0.29	8.4-9.9
	50-60	8.59±0.26	8.1-9.1	10.14±0.10	9.8-10.3	9.69±0.21	8.9-10.1
$EC_{1:5}$ µS/m	0-10	264.9±91.1	95-610	83.6±35.2	35-180	130.9±48.6	55-295
	30-40	1139.1±445.2	310-2000	486.3±218.8	200-1000	744.9±305.2	180-1450
	50-60	1528.8±338.9	695-2340	615.5±204.9	250-1200	1189.8±356.3	230-2000
Grain Yield	Kalka	177.5±65.7	73-305	405.6±89.5	226-616	820.4±143.0	463-1093
g/plot	Tamaroi	228.9±76.4	99-382	437.7±112.9	221-705	815.7±155.1	461-1084
	Yallaroi	187.9±71.9	77-343	383.5±96.3	228-577	751.4±186.7	363-1102
	Combined	197.8±71.9	73-382	408.9±101.5	221-705	796.4±163.5	363-1102

Wanderah

Correlations between grain yield (g/plot) and soil pH and EC for each durum variety and the varieties combined were calculated (Table 4.03). The relationship of the yield to pH or EC, for the significant correlations identified, are described in Table 4.06.

Table 4.03: Correlations between grain yield (g/plot) of the durum varieties Kalka, Tamaroi and Yallaroi, and soil pH or $EC_{1:5}$µS/m at the depths 0-10cm, 30-40cm and 50-60cm for the Wanderah site.

		Grain yield (g/plot)			
	Depth (cm)	Kalka	Tamaroi	Yallaroi	Combined
pH	0-10	0.472**	0.458**	0.498**	0.481***
	30-40	-0.151^{ns}	-0.003^{ns}	0.017^{ns}	0.046^{ns}
	50-60	-0.171^{ns}	-0.164^{ns}	-0.085^{ns}	-0.111^{ns}
$EC_{1:5}$ µS/m	0-10	-0.356^{ns}	-0.351^{ns}	-0.054^{ns}	-0.202^{ns}
	30-40	-0.295^{ns}	-0.430*	-0.528**	-0.349**
	50-60	-0.438*	-0.299^{ns}	-0.422*	-0.466***

ns not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

Multiple linear regression analysis found that pH (0-10cm) and EC (50-60cm) combined, accounted for 41.6, 48.9, 34.4 and 32.9 percent of the variation in grain yield for Kalka, Tamaroi, Yallaroi and varieties combined, respectively. The combined results of the three varieties, show that the general response of grain yield at the Wanderah site to soil pH and EC, is an increase in grain yield with increasing topsoil pH from the slightly acid to slightly alkaline range, and a decrease in grain yield with increasing subsoil EC in the highly toxic range (Figure 4.01 and 4.02).

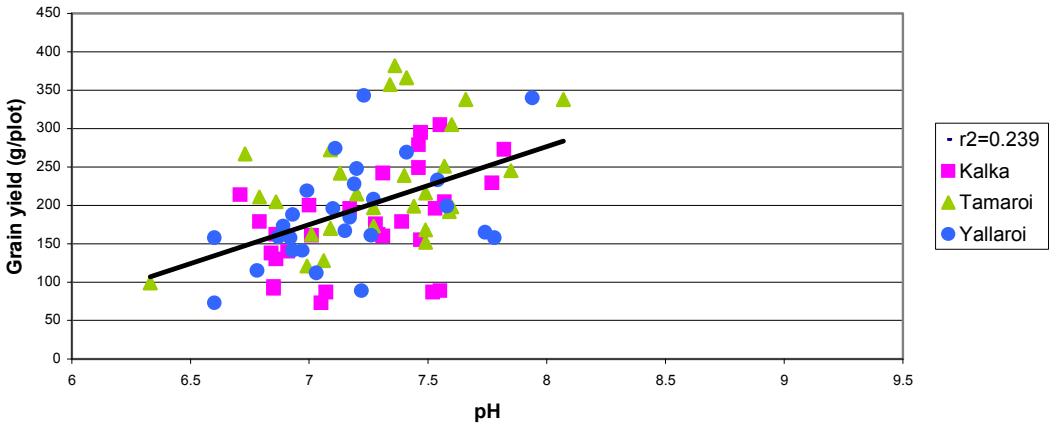
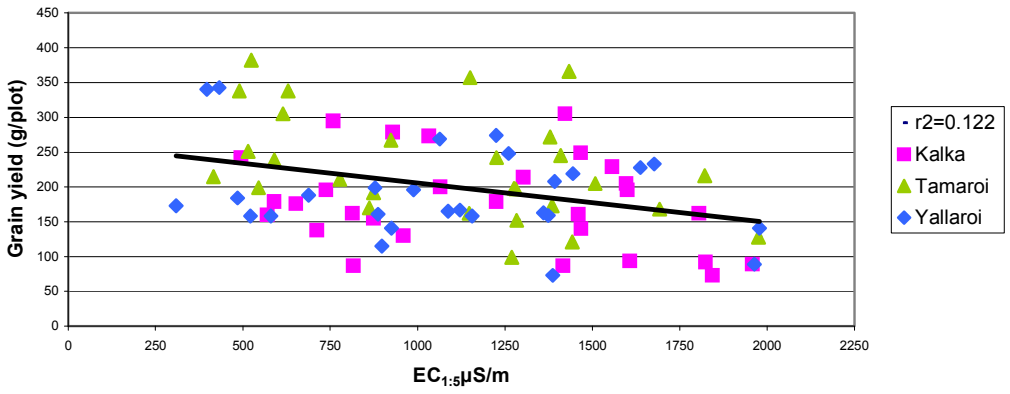


Figure 4.01: Relationship between grain yield (g/plot) at Wanderah for Kalka, Tamaroi, and Yallaroi, and soil pH at the depth 0-10cm.

(a) Wanderah, EC (30-40cm)



(b) Wanderah, EC (50-60cm)

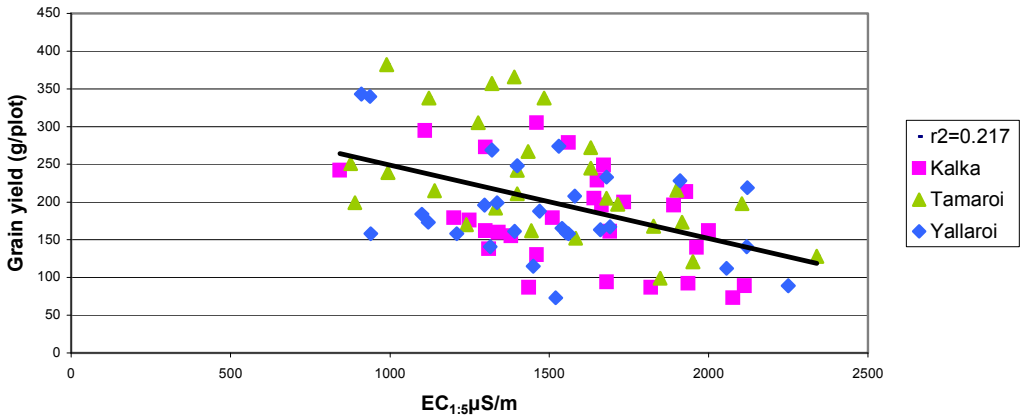


Figure 4.02: Relationship between grain yield (g/plot) at Wanderah for Kalka, Tamaroi, and Yallaroi, and soil $EC_{1:5}$µS/m at the depths (a) 30-40cm and (b) 50-60cm.

Angas Valley

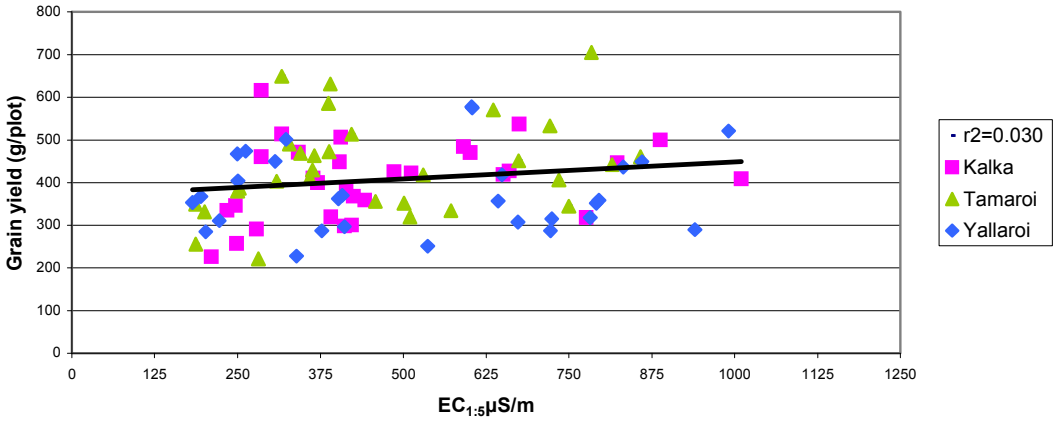
Correlations between grain yield (g/plot) and soil pH and EC for each durum variety and the varieties combined were calculated (Table 4.04). No significant correlations were identified between soil pH at any of the three depths and grain yield for Kalka, Tamaroi or Yallaroi. Kalka showed a significant correlation between and EC ($P<0.05$) at a depth of 0-10cm and Tamaroi at a depth of 50-60cm (Table 4.06, Figure 4.03). The combined results of the three varieties show that there is no significant response of grain yield at the Angas Valley site to soil pH and EC, and that the variation observed in grain yield was due to other unexplained factors.

Table 4.04: Correlations between grain yield (g/plot) of the durum varieties Kalka, Tamaroi and Yallaroi, and soil pH or $EC_{1:5}$µS/m at the depths 0-10cm, 30-40cm and 50-60cm for the Angas Valley site.

		Yield (g/plot)			
	Depth (cm)	Kalka	Tamaroi	Yallaroi	Combined
pH	0-10	0.270^{ns}	0.032^{ns}	0.098^{ns}	0.111^{ns}
	30-40	0.145^{ns}	0.320^{ns}	-0.024^{ns}	0.144^{ns}
	50-60	0.003^{ns}	-0.040^{ns}	0.061^{ns}	0.044^{ns}
$EC_{1:5}$ µS/m	0-10	0.373*	-0.017^{ns}	0.279^{ns}	0.181^{ns}
	30-40	0.283^{ns}	0.287^{ns}	0.082^{ns}	0.174^{ns}
	50-60	0.333^{ns}	0.401*	0.046^{ns}	0.214^{ns}

ns not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

(a) Angas Valley, EC (30-40cm)



(b) Angas Valley, EC (50-60cm)

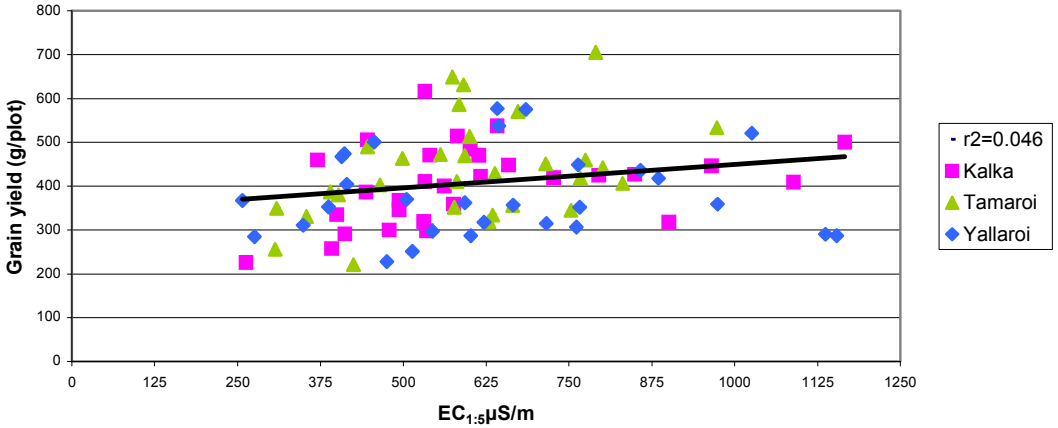


Figure 4.03: Relationship between grain yield (g/plot) at Angas Valley for Kalka, Tamaroi, and Yallaroi, and soil $EC_{1:5}$µS/m at the depths (a) 30-40cm and (b) 50-60cm.

Roseworthy

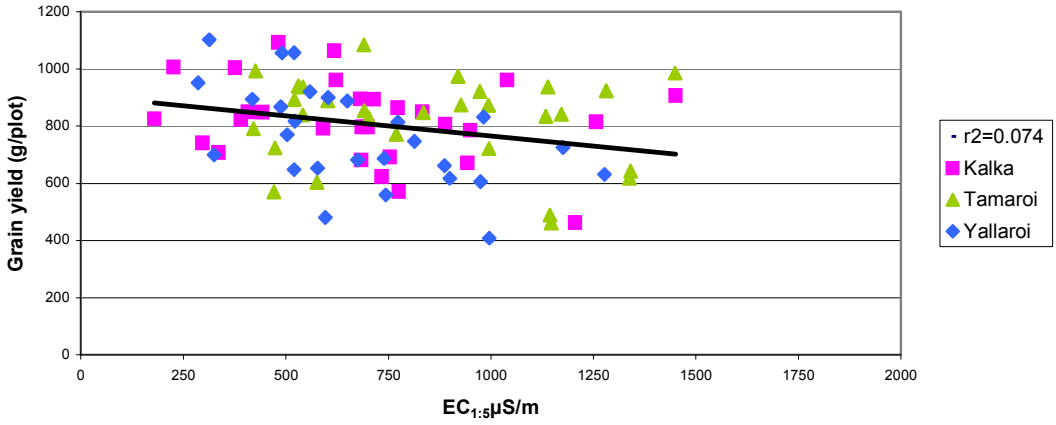
Grain yield (g/plot) and soil pH and EC were correlated for each durum variety and the varieties combined (Table 4.05). Kalka, Tamaroi and Yallaroi showed no significant yield responses to pH at any depth, and Kalka and Tamaroi showed no significant yield responses to EC at any depth. Yallaroi showed a significant yield response at the 0.01% level to EC at a depth of 30-40cm and 50-60cm (Table 4.06, Figure 4.04). Multiple linear regression analysis found that $EC_{1:5}$(50-60cm) alone, accounted for 28.5 percent of the variation in grain yield.

Table 4.05: Correlations between grain yield (g/plot) of the durum varieties Kalka, Tamaroi and Yallaroi, and soil pH or $EC_{1:5}$µS/m at the depths 0-10cm, 30-40cm and 50-60cm for the Roseworthy site.

		Yield (g/plot)			
	Depth (cm)	Kalka	Tamaroi	Yallaroi	Combined
pH	0-10	-0.139^{ns}	-0.042^{ns}	0.315^{ns}	0.082^{ns}
	30-40	-0.119^{ns}	0.159^{ns}	-0.258^{ns}	-0.069^{ns}
	50-60	0.003^{ns}	0.014^{ns}	-0.076^{ns}	-0.017^{ns}
$EC_{1:5}$ µS/m	0-10	-0.241^{ns}	-0.107^{ns}	-0.001^{ns}	-0.095^{ns}
	30-40	-0.262^{ns}	-0.159^{ns}	-0.581***	-0.288*
	50-60	-0.317^{ns}	-0.009^{ns}	-0.588***	-0.288*

ns not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

(a) Roseworthy, EC (30-40cm)



(b) Roseworthy, EC (50-60cm)

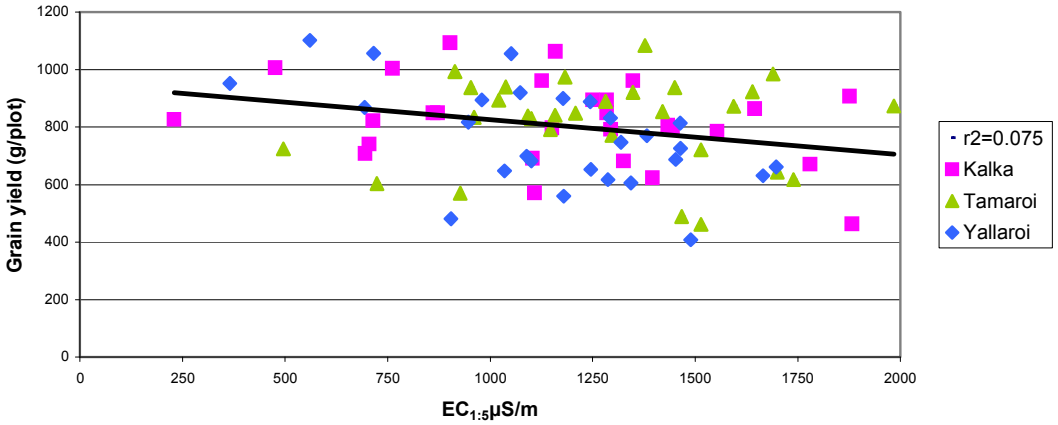


Figure 4.04: Relationship between grain yield (g/plot) at Roseworthy for Kalka, Tamaroi, and Yallaroi, and soil $EC_{1:5}$µS/m at the depths (a) 30-40cm and (b) 50-60cm.

Table 4.06: Yield response for significant correlations identified between grain yield (g/plot) and soil pH and $EC_{1:5}$µS/m for Kalka, Tamaroi, Yallaroi and varieties combined, at the sites Wanderah, Angas Valley and Roseworthy.

Site	Variety	pH/EC	Depth	Range	Yield Response
Wanderah	Kalka	pH	0-10	6.7 – 8.1	100% increase
		EC	50-60	800 – 2100	50% decrease
	Tamaroi	pH	0-10	6.3 – 8.1	140% increase
		EC	30-40	400 – 2000	36% decrease
	Yallaroi	pH	0-10	6.3 – 8.1	67% increase
		EC	30-40	400 – 2000	33% decrease
		EC	50-60	900 – 2300	89% decrease
	Combined	pH	0-10	6.3 – 8.1	175% increase
		EC	30-40	400 – 2000	40% decrease
		EC	50-60	900 – 2300	55% decrease
Angas Valley	Kalka	EC	0-10	30 – 180	43% increase
	Tamaroi	EC	50-60	300 – 1000	57% increase
Roseworthy	Yallaroi	EC	30-40	300 – 1300	44% increase
		EC	50-60	350 – 1700	48% increase
	Combined	EC	30-40	200 – 1500	22% increase
		EC	50-60	250 – 2000	22% increase

Experiment 2 – RAC875 and Cascades

Trials were harvested in December 2004 and soil samples collected from three sites, Wanderah, Buckleboo, and Roseworthy, in September 2004, to determine the effects of pH and EC on the grain yield of the bread wheat varieties RAC875 and Cascades. The pH, EC and yield results are summarised in Table 4.07.

Table 4.07: The mean (M), standard deviation (SD) and range of soil pH (pH>8.5) and $EC_{1:5}$µS/m (EC>400) values at the depths 0-10cm and 40-50cm, and grain yield (g/plot) (highest) for the bread varieties RAC875 and Cascades, at the three sites Wanderah, Roseworthy and Buckleboo.

		Wanderah		Roseworthy		Buckleboo	
	Depth (cm)	M±SD	Range	M±SD	Range	M±SD	Range
pH	0-10	7.23±0.25	6.8-8.0	7.18±0.45	6.4-7.9	8.70±0.06	8.6-8.9
	40-50	8.34±0.07	8.2-8.5	8.80±0.10	8.6-9.1	9.07±0.14	8.8-9.3
$EC_{1:5}$ µS/m	0-10	203.8±53.9	112-413	92.8±36.4	32-181	139.4±27.3	114-262
	40-50	1848.4±178.9	1350-2172	139.7±23.9	100-202	996.8±220.3	660-1487
Grain Yield	RAC875	425.1±71.1	269-549	531.4±144.6	261-846	107.1±24.7	35-135
g/plot	Cascades	307.7±90.3	147-511	516.8±147.0	173-725	106.2±14.3	70-131
	Combined	362.7±101.2	147-549	523.6±146.1	221-705	106.6±19.7	35-135

Wanderah

Correlations between grain yield (g/plot) and soil pH and EC for each bread wheat variety and the varieties combined (Table 4.08) showed no significant yield responses to pH or EC at any depth. Subsoil pH in the mildly alkaline range and subsoil EC in the highly toxic range had no effect on the variation observed in grain yield.

Table 4.08: Correlations between grain yield (g/plot) of the bread wheat varieties RAC875 and Cascades, and soil pH or $EC_{1:5}$µS/m at the depths 0-10cm and 40-50cm for the Wanderah site.

		Yield (g/plot)		
	Depth (cm)	RAC875	Cascades	Combined
pH	0-10	-0.315^{ns}	-0.218^{ns}	0.000^{ns}
	40-50	-0.244^{ns}	0.114^{ns}	-0.126^{ns}
$EC_{1:5}$ µS/m	0-10	-0.219^{ns}	-0.210^{ns}	-0.195^{ns}
	40-50	0.190^{ns}	0.035^{ns}	0.118^{ns}

ns not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

Roseworthy

Grain yield (g/plot) and soil pH and EC correlations for each bread wheat variety and the varieties combined (Table 4.09) also showed no significant yield responses to pH or EC at any depth. Subsoil pH in the moderately-highly alkaline range and subsoil EC at a low level had no effect on the variation observed in grain yield.

Table 4.09: Correlations between grain yield (g/plot) of the bread wheat varieties RAC875 and Cascades, and soil pH or $EC_{1:5}$µS/m at the depths 0-10cm and 40-50cm for the Roseworthy site.

		Yield (g/plot)		
	Depth (cm)	RAC875	Cascades	Combined
pH	0-10	0.014^{ns}	-0.229^{ns}	-0.134^{ns}
	40-50	0.097^{ns}	0.334^{ns}	0.239^{ns}
$EC_{1:5}$ µS/m	0-10	-0.119^{ns}	-0.191^{ns}	-0.148^{ns}
	40-50	-0.134^{ns}	0.089^{ns}	0.077^{ns}

ns not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

Buckleboo

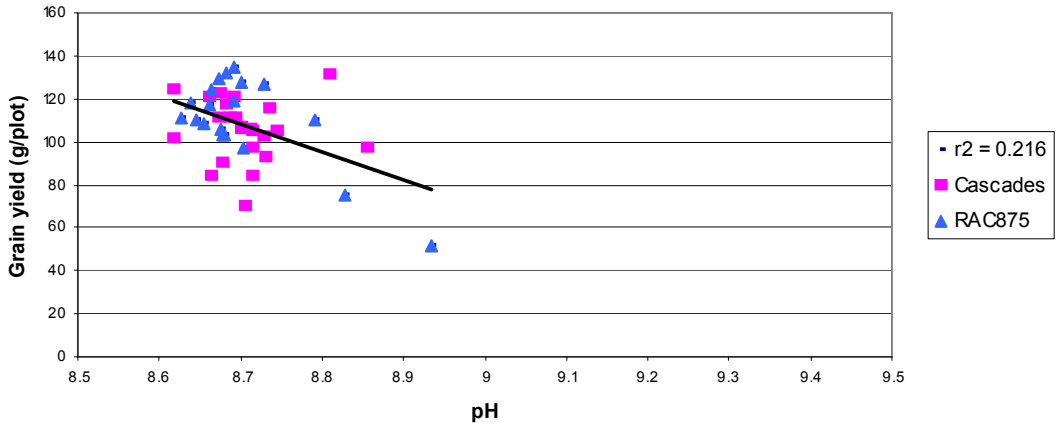
Cascades showed no significant yield responses to pH or EC at any depth, but RAC875 showed a significant yield response at the 0.01% level to pH at a depth of 0-10cm and EC at a depth of 40-50cm (Table 4.10). An increase in pH (0-10cm) from 8.63 to 8.94 led to a 60g/plot decrease in grain yield (Figure 4.05), and an increase in $EC_{1:5}$(40-50cm) from 700µS/m to 1400µS/m led to a 40g/plot decrease in grain yield (Figure 4.06). Multiple linear regression analysis found that pH(0-10cm) and pH(40-50cm), accounted for 73.1 percent of the variation in grain yield. The main response of grain yield at the Buckleboo site to soil pH appears to be a decrease in grain yield with increasing pH in the moderate to highly alkaline range, even though subsoil EC is in the highly toxic range.

Table 4.10: Correlations between grain yield (g/plot) of the bread wheat varieties RAC875 and Cascades, and soil pH or $EC_{1:5}$µS/m at the depths 0-10cm and 40-50cm for the Buckleboo site.

		Yield (g/plot)		
	Depth (cm)	RAC875	Cascades	Combined
pH	0-10	-0.727***	-0.054^{ns}	-0.465**
	40-50	-0.362^{ns}	-0.253^{ns}	-0.105^{ns}
$EC_{1:5}$ µS/m	0-10	-0.302^{ns}	0.288^{ns}	0.032^{ns}
	40-50	-0.657***	0.248^{ns}	-0.195^{ns}

ns not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

(a) Buckleboo, pH (0-10cm)



(b) Buckleboo, pH (40-50cm)

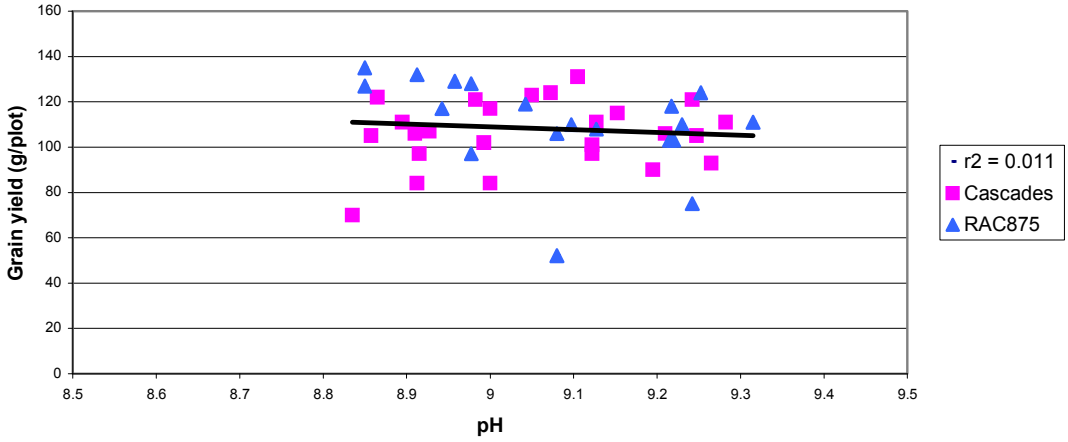


Figure 4.05: Relationship between grain yield (g/plot) at Buckleboo for RAC875 and Cascades, and soil pH at the depths (a) 0-10cm and (b) 40-50cm.

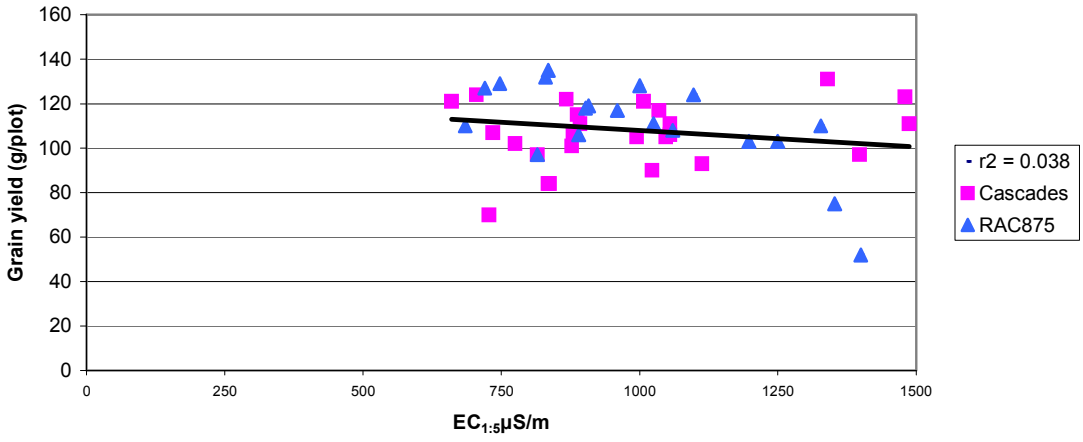


Figure 4.06: Relationship between grain yield (g/plot) at Buckleboo for RAC875 and Cascades, and soil $EC_{1:5}$µS/m at the depth 40-50cm.

Table 4.11: The mean (M), standard deviation (SD) and range of soil pH (pH>8.5) and $EC_{1:5}$µS/m (EC>400) values at the depths 0-10cm , 30-40cm and 50-60cm, and grain yield (g/plot) for the bread varieties Frame/Yarralinka and Pugsley (highest yielding), at the three sites Angas Valley-North, Angas Valley-South, Roseworthy-East, Roseworthy-West and Wanderah.

		Angas Valley-North		Angas Valley-South		Roseworthy-East		Roseworthy-West		Wanderah	
	Depth (cm)	M±SD	Range	M±SD	Range	M±SD	Range	M±SD	Range	M±SD	Range
pH	0-10	8.46±0.58	7.1-9.7	8.79±0.19	8.4-9.0	7.29±1.00	6.0-8.6	7.79±0.64	6.6-8.7	7.18±0.62	6.0-8.5
	30-40	9.98±0.18	9.4-10.4	9.76±0.20	9.3-10.2	8.47±0.31	7.8-9.4	9.41±0.26	8.6-9.8	8.24±0.31	7.8-9.0
	50-60	10.01±0.24	8.8-10.4	10.21±0.06	10.0-10.3	8.93±0.31	8.4-9.8	9.61±0.13	9.2-9.8	8.51±0.27	8.1-9.2
$EC_{1:5}$ µS/m	0-10	145.7±74.8	54-402	106.9±13.0	78-161	123.3±35.2	73-195	155.0±47.3	91-266	275.2±211.3	99-976
	30-40	675.3±293.9	175-1158	272.7±56.8	173-405	240.8±37.0	143-438	749.1±264.0	277-1329	1159.1±396.1	360-1884
	50-60	685.3±209.3	285-1062	516.1±80.9	241-683	319.5±134.2	180-743	1238.2±277.0	801-1756	1461.6±279.1	760-2010
Grain Yield	Fm/Yk	463.6±94.5	262-675	471.8±81.4	335-638	1018.3±116.0	803-1184	959.1±108.5	797-1155	378.7±58.5	284-474
g/plot	Pugsley	630.6±113.6	339-757	617.9±61.9	514-774	1066.2±177.0	671-1379	878.7±163.0	629-1154	420.4±81.0	265-647
	Combined	545.1±133.2	262-757	541.5±103.1	335-774	1041.7±149.2	671-1379	915.5±144.6	629-1155	399.1±72.7	265-647

Experiment 3 - Frame/Yarralinka and Pugsley

Trials were harvested in December 2005 and soil samples collected from five sites, Wanderah, Angas Valley – North, Angas Valley - South, Roseworthy – East, and Roseworthy-West, in January 2006, to determine the effects of pH and EC on the grain yield on the bread wheat varieties Frame/Yarralinka and Pugsley. The pH, EC and yield results are summarised in Table 4.11. Correlations were calculated between grain yield (g/plot) and soil pH and EC for each bread wheat varieties. The yield responses to pH or EC for the significant correlations identified are described in Table 4.16.

Wanderah

Frame/Yarralinka showed a significant yield response to pH at a depth of 30-40cm and 50-60cm, and EC at a depth of 30-40cm (Table 4.12). Multiple linear regression analysis found that pH (30-40cm) alone, accounted for 54.0 percent of the variation in grain yield. Pugsley showed similar responses to Frame/Yarralinka, except a significant yield response was also found for EC at a depth of 0-10cm and 50-60cm. Multiple linear regression analysis found that pH (50-60cm) alone, accounted for 47.1 percent of the variation in grain yield. While similar results were observed between the two varieties, Frame/Yarralinka and Pugsley, the yield response was greater in Pugsley than Frame/Yarralinka. At Wanderah grain yield increased with increasing subsoil pH from the slightly alkaline to highly alkaline range, despite pH being within a range expected to negatively affect plant growth (Figure 4.07). Moderately to highly toxic subsoil $EC_{1:5}$µS/m was, however, found to decrease grain yield with increasing EC levels (Figure 4.08).

Table 4.12: Correlations between grain yield (g/plot) of the bread wheat varieties Frame/Yarralinka and Pugsley, and soil pH or $EC_{1:5}$µS/m at the depths 0-10cm, 30-40cm and 50-60cm for the Wanderah site.

		Yield (g/plot)		
	Depth (cm)	Frame/Yarralinka	Pugsley	Combined
pH	0-10	-0.136^{ns}	-0.215^{ns}	-0.160^{ns}
	30-40	0.642***	0.588***	0.539^{ns}
	50-60	0.640***	0.704***	0.606***
$EC_{1:5}$ µS/m	0-10	-0.323^{ns}	-0.471*	-0.375*
	30-40	-0.438*	-0.556***	-0.464**
	50-60	-0.358^{ns}	-0.557***	-0.424**

ns not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

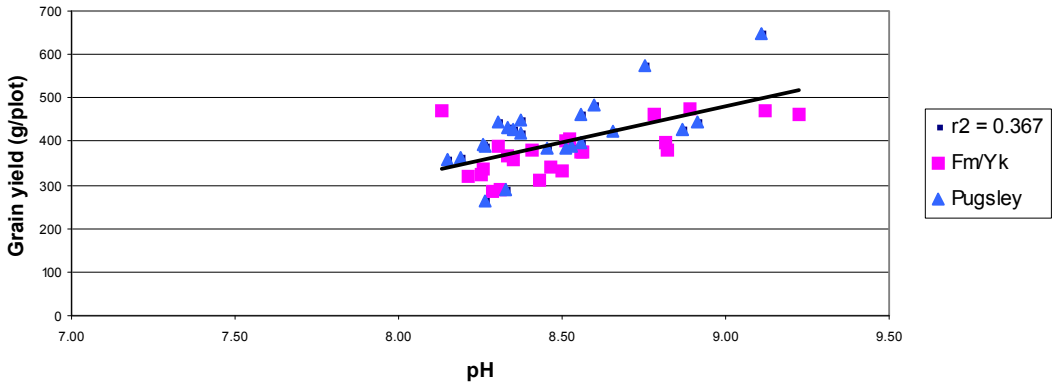
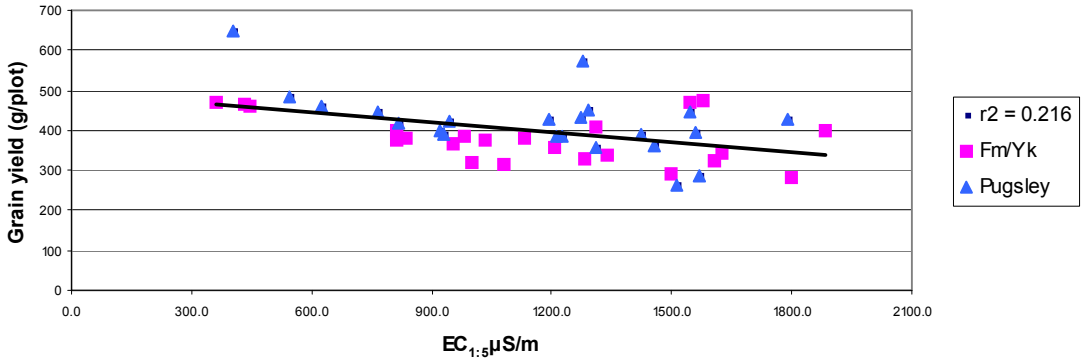


Figure 4.07: Relationship between grain yield (g/plot) at Wanderah for Frame/Yarralinka and Pugsley, and soil pH at the depth 50-60cm.

(a) Wanderah, EC (30-40cm)



(b) Wanderah, EC (50-60cm)

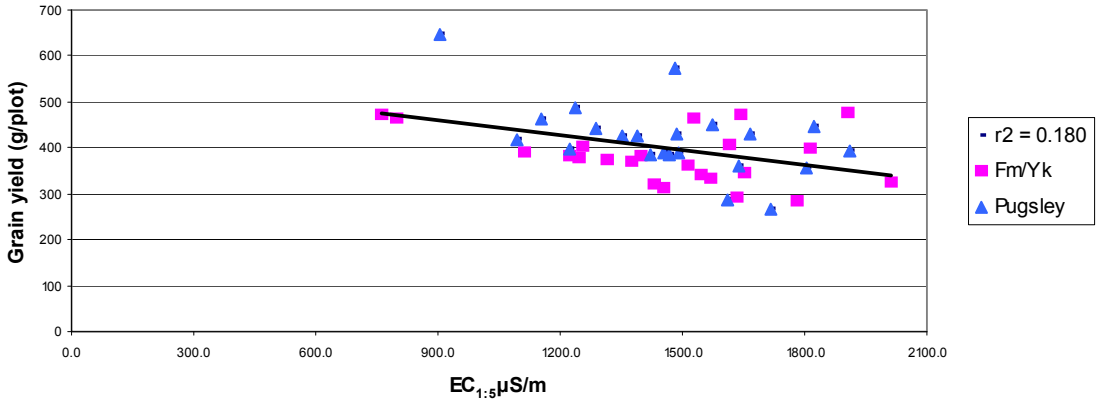


Figure 4.08: Relationship between grain yield (g/plot) at Wanderah for Frame/Yarralinka and Pugsley, and soil $EC_{1:5}$µS/m at the depths (a) 30-40cm and (b) 50-60cm.

Angas Valley – South

Pugsley showed a significant yield response to pH at a depth of 30-40cm and 50-60cm, and EC at a depth of 30-40cm (Table 4.13), although pH (50-60cm) alone, accounted for 29.6 percent of the variation in grain yield. However, Frame/Yarralinka had no significant relationship between grain yield and pH or EC at any depth.

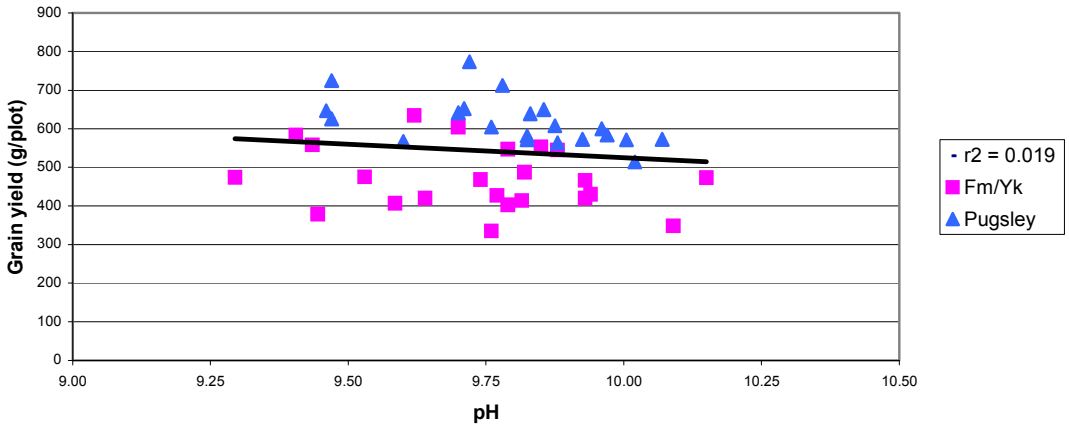
At Angas Valley – South, grain yield increased with increasing subsoil pH in the highly alkaline range, although only for the bread wheat variety Pugsley (Figure 4.09). Yield was also found to be affected by moderately toxic subsoil $EC_{1:5}$µS/m (Figure 4.10), but did not account for a significant proportion of the observed variation in grain yield.

Table 4.13: Correlations between grain yield (g/plot) of the bread wheat varieties Frame/Yarralinka and Pugsley, and soil pH or $EC_{1:5}$µS/m at the depths 0-10cm, 30-40cm and 50-60cm for the Angas Valley-South site.

		Yield (g/plot)		
	Depth (cm)	Frame/Yarralinka	Pugsley	Combined
pH	0-10	-0.180^{ns}	-0.176^{ns}	-0.216^{ns}
	30-40	-0.251^{ns}	-0.540*	-0.136^{ns}
	50-60	-0.072^{ns}	-0.602**	-0.125^{ns}
$EC_{1:5}$ µS/m	0-10	-0.320^{ns}	-0.259^{ns}	-0.209^{ns}
	30-40	-0.213^{ns}	-0.582**	-0.192^{ns}
	50-60	-0.107^{ns}	-0.333^{ns}	-0.166^{ns}

ns not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

(a) Angas Valley – South, pH (30-40cm)



(b) Angas Valley – South, pH (50-60cm)

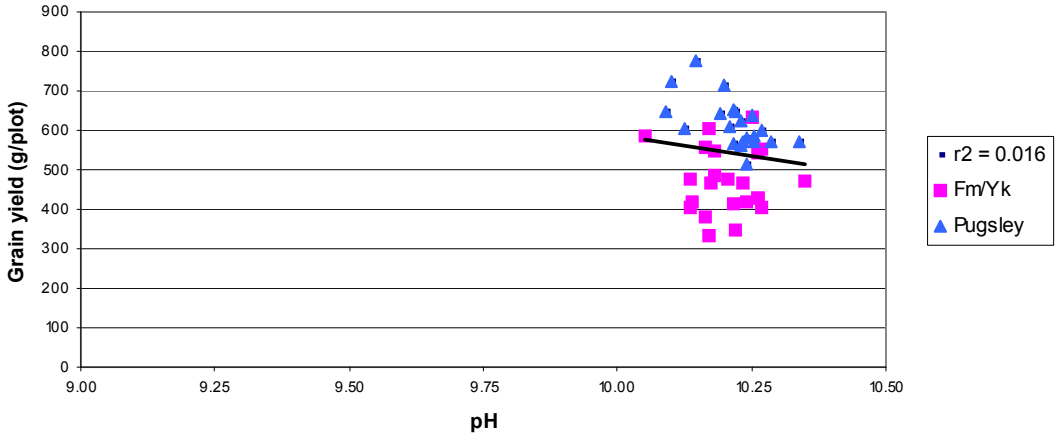


Figure 4.09: Relationship between grain yield (g/plot) at Angas Valley - South for Frame/Yarralinka and Pugsley, and soil pH at the depths (a) 30-40cm and (b) 50-60cm.

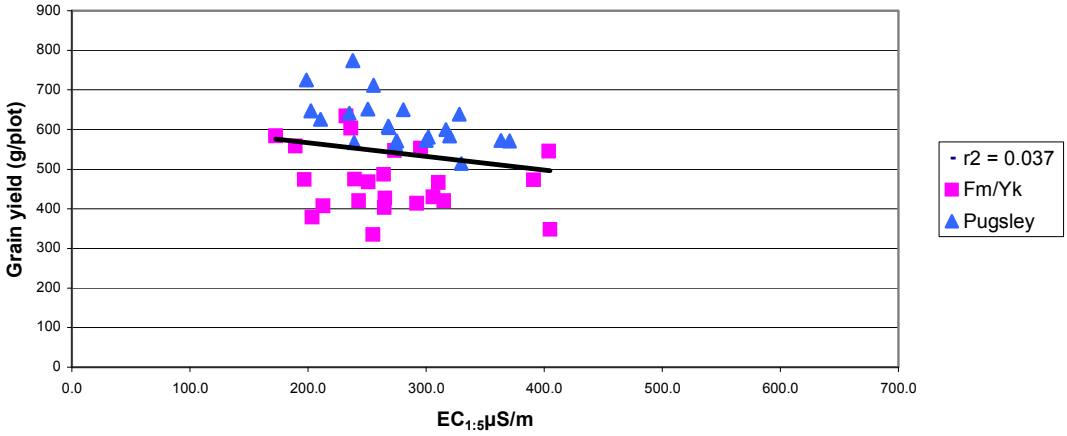


Figure 4.10: Relationship between grain yield (g/plot) at Angas Valley - South for Frame/Yarralinka and Pugsley, and soil $EC_{1:5}$µS/m at the depth 30-40cm.

Angas Valley - North

The bread wheat varieties Frame/Yarralinka and Pugsley showed no significant yield responses to pH or EC at any depth at the Angas Valley - North site (Table 4.14). Topsoil pH in the mildly to highly alkaline range, subsoil pH in the high to extreme alkaline range, and subsoil EC in the mildly to highly toxic range had no effect on the variation observed in grain yield.

Table 4.13: Correlations between grain yield (g/plot) of the bread wheat varieties Frame/Yarralinka and Pugsley, and soil pH or $EC_{1:5}$µS/m at the depths 0-10cm, 30-40cm and 50-60cm for the Angas Valley-North site.

		Yield (g/plot)		
	Depth (cm)	Frame/Yarralinka	Pugsley	Combined
pH	0-10	-0.217^{ns}	0.185^{ns}	0.042^{ns}
	30-40	-0.044^{ns}	-0.084^{ns}	0.066^{ns}
	50-60	0.077^{ns}	-0.146^{ns}	0.290^{ns}
$EC_{1:5}$ µS/m	0-10	-0.274^{ns}	0.202^{ns}	-0.066^{ns}
	30-40	-0.051^{ns}	0.283^{ns}	0.046^{ns}
	50-60	-0.094^{ns}	0.116^{ns}	-0.056^{ns}

ns not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

Roseworthy - West

No significant grain yield response was identified for pH at any depth, even though subsoil pH was in the highly alkaline range (Table 4.14). However, Frame/Yarralinka showed a significant yield response to EC at a depth of 50-60cm, with an increase in $EC_{1:5}$(50-60cm) from 850µS/m to 1800µS/m leading to a 225g/plot decrease in grain yield (Figure 4.11).

Table 4.14: Correlations between grain yield (g/plot) of the bread wheat varieties Frame/Yarralinka and Pugsley, and soil pH or $EC_{1:5}$µS/m at the depths 0-10cm, 30-40cm and 50-60cm for the Roseworthy-West site.

		Yield (g/plot)		
	Depth (cm)	Frame/Yarralinka	Pugsley	Combined
pH	0-10	-0.380^{ns}	-0.319^{ns}	-0.318^{ns}
	30-40	-0.135^{ns}	-0.162^{ns}	-0.164^{ns}
	50-60	0.028^{ns}	0.112^{ns}	0.131^{ns}
$EC_{1:5}$ µS/m	0-10	-0.277^{ns}	0.186^{ns}	0.014^{ns}
	30-40	-0.237^{ns}	-0.370^{ns}	-0.264^{ns}
	50-60	-0.498*	-0.311^{ns}	-0.348*

ns not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

Roseworthy - East

Frame/Yarralinka showed a significant yield response to pH at a depth of 0-10cm and EC at a depth of 0-10cm (Table 4.15), with $EC_{1:5}$(0-10cm) alone accounting for 34.5 percent of the variation in grain yield. Similarly, Pugsley showed a significant yield response,

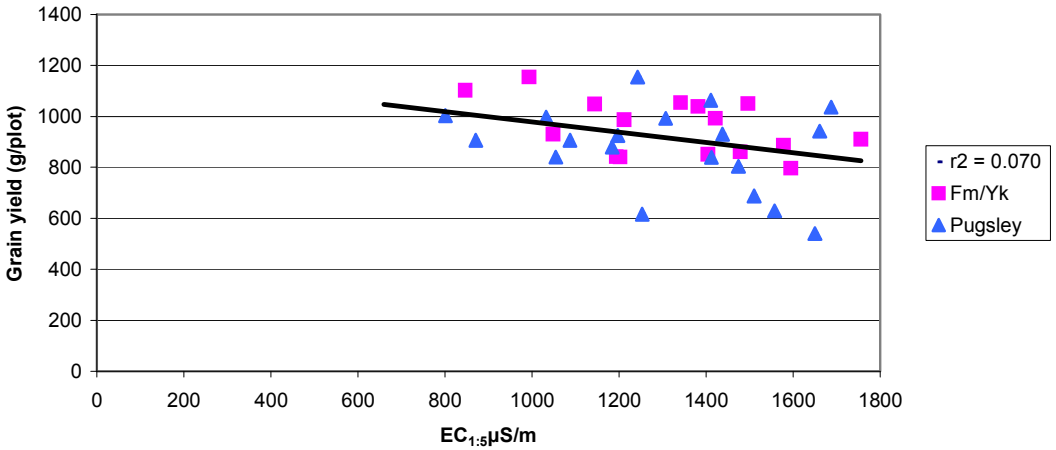


Figure 4.11: Relationship between grain yield (g/plot) at Roseworthy - West for Frame/Yarralinka and Pugsley, and soil $EC_{1:5}$µS/m at the depth 50-60cm.

although greater than the yield response for Frame/Yarralinka, to pH at a depth of 0-10cm and EC at a depth of 0-10cm, with pH (0-10cm) alone accounting for 27.1 percent of the variation in grain yield. However, multiple linear regression analysis for the Frame/Yarralinka and Pugsley combined found that pH (0-10cm) and EC (30-40cm), accounted for 59.7 percent of the variation in grain yield.

Table 4.15: Correlations between grain yield (g/plot) of the bread wheat varieties Frame/Yarralinka and Pugsley, and soil pH or $EC_{1:5}$µS/m at the depths 0-10cm, 30-40cm and 50-60cm for the Roseworthy-East site.

		Yield (g/plot)		
	Depth (cm)	Frame/Yarralinka	Pugsley	Combined
pH	0-10	0.551*	0.679***	0.609***
	30-40	0.146^{ns}	0.325^{ns}	0.240^{ns}
	50-60	-0.134^{ns}	0.030^{ns}	-0.047^{ns}
$EC_{1:5}$ µS/m	0-10	0.612**	0.588*	0.592***
	30-40	0.014^{ns}	-0.316^{ns}	-0.170^{ns}
	50-60	0.119^{ns}	-0.095^{ns}	-0.009^{ns}

ns not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

The topsoil pH in the slightly acidic to mildly alkaline range and topsoil EC at low levels was positively influencing grain yield at the Roseworthy – East site (Figure 4.12). Subsoil pH had no significant effect even through subsoil pH was in the moderately to highly alkaline range. Increasing subsoil EC appeared to have a minor effect of decreasing grain yield, particularly for Pugsley.

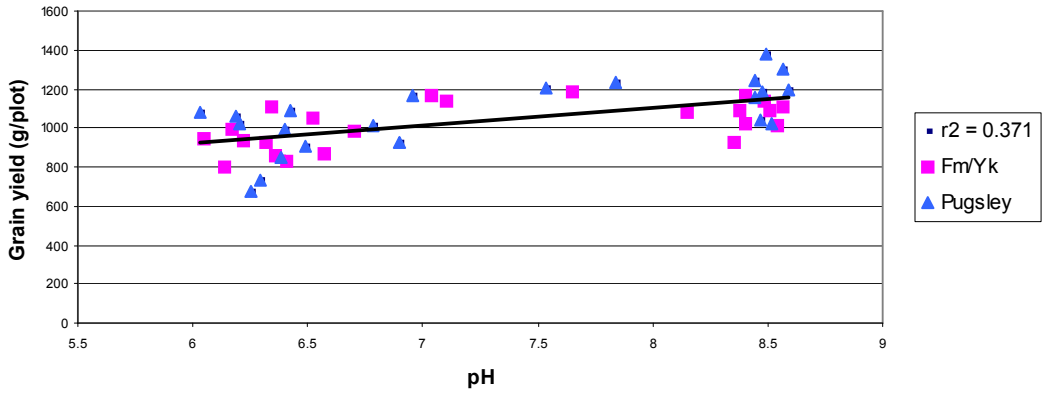
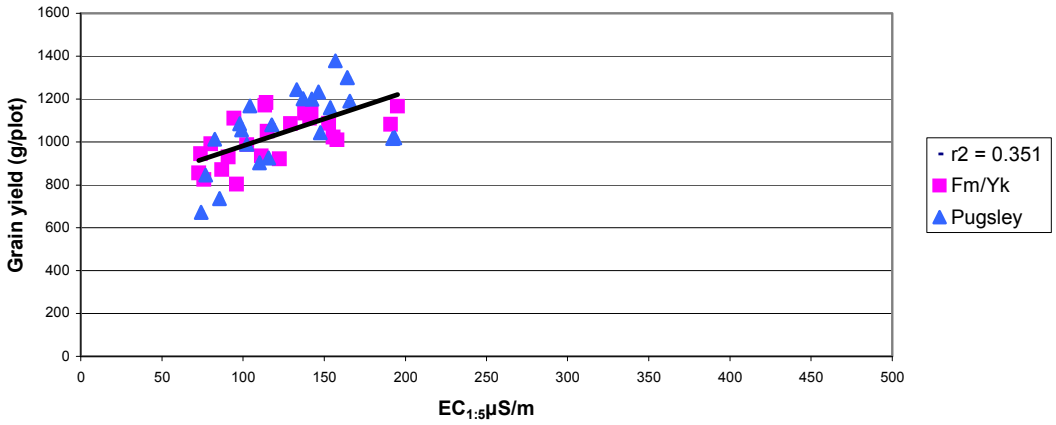


Figure 4.12: Relationship between grain yield (g/plot) at Roseworthy - East for Frame/Yarralinka and Pugsley, and soil pH at the depth 0-10cm.

(a) Roseworthy – East, EC (0-10cm)



(b) Roseworthy – East, EC (30-40cm)

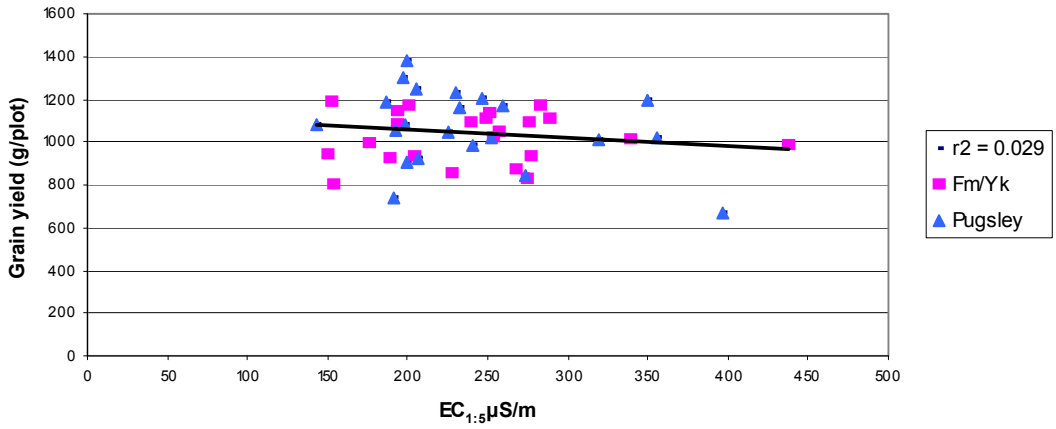


Figure 4.13: Relationship between grain yield (g/plot) at Roseworthy - East for Frame/Yarralinka and Pugsley, and soil $EC_{1:5}$µS/m at the depths (a) 30-40cm and (b) 50-60cm.

Table 4.16: Yield response for significant correlations identified between grain yield (g/plot) and soil pH and $EC_{1:5}$µS/m for Frame/Yarralinka, Pugsley and varieties combined, at the sites Wanderah, Angas Valley – South, Angas Valley – North, Roseworthy - West and Roseworthy - East.

Site	Variety	pH/EC	Depth	Range	Yield Response
Wanderah	Frame/Yarralinka	pH	30-40	7.8 – 9.0	46% increase
		pH	50-60	8.1 – 9.2	46% increase
		EC	30-40	380 – 1900	26% decrease
	Pugsley	pH	30-40	7.8 – 9.0	57% increase
		pH	50-60	8.1 – 9.1	57% increase
		EC	0-10	100 – 1000	33% decrease
		EC	30-40	400 – 1800	30% decrease
		EC	50-60	900 – 1900	34% decrease
	Combined	pH	50-60	8.1 – 9.2	49% increase
		EC	0-10	100 – 1000	28% decrease
		EC	30-40	360 – 1900	29% decrease
		EC	50-60	760 – 2000	29% decrease
Angas Valley	Puglsey	pH	30-40	9.4 – 10.1	15% decrease
(South)		pH	50-60	10.1 – 10.3	21% decrease
		EC	30-40	200 – 375	19% decrease
Roseworthy	Frame/Yarralinka	EC	50-60	850 – 1800	20% decrease
(West)	Combined	EC	50-60	800 – 1800	21% decrease
Roseworthy	Frame/Yarralinka	pH	0-10	6.0 – 8.6	16% increase
(East)		EC	0-10	75 – 200	26% decrease
	Pugsley	pH	0-10	6.0 – 8.6	33% increase
		EC	0-10	75 – 200	38% decrease
	Combined	pH	0-10	6.0 – 8.6	20% increase
		EC	0-10	75 – 200	32% decrease

4.2.4 Discussion

The response of durum wheats to high soil pH and EC in 2005 (Experiment 1).

Increasing soil pH in the alkaline range was not identified as a yield limiting factor for the durum varieties, Kalka, Tamaroi and Yallaroi, at the field sites, Wanderah, Angas Valley and Roseworthy, in 2005. The failure to identify a significant correlation between increasing pH in the alkaline range and a decline in grain yield is an indication of the complexity of the plant-soil-environment relationship, where multiple interactive factors

are responsible for the final grain yield of a field grown crop. At all three sites, subsoil pH (30-40cm and 50-60cm) reached values >pH 8.5, where pH is considered to influence yield. However, subsoil EC at the three site also reached values >400µS/m and was identified as a significant factor in decreasing grain yield at both Wanderah and Roseworthy.

Wanderah

At Wanderah increasing topsoil pH from slightly acid to moderately alkaline was found to significantly increase grain yield and increasing subsoil EC from moderate to high significantly decreased grain yield, accounting for an average of 40% of the variation in grain yield. Soil pH in the slightly acid to moderately alkaline range is considered to be optimal for wheat growth (Maschmedt 2002) and is unlikely to be responsible for the observed positive response. Instead, the relationship between yield and soil pH is almost certainly related to other factors. The Wanderah site was sown on the side of a calcareous rise (pH 8.1) grading down to an alluvial clay flat (pH 6.3), which provided a pH gradient in the topsoil. At the bottom of the rise on the alluvial clay, the plots were thin and patchy due to high sodicity and magnesia patches, which caused surface sealing, poor germination and the proliferation of weeds from a lack of crop competition. The reduction of plot yield on the more acid clay loam, as compared to that on the less sodic and more friable, alkaline calcareous soil further up the slope undoubtedly led to the correlation between increased yield and increasing topsoil pH.

Angas Valley

At Angas Valley increasing topsoil EC in the non-toxic range was found to significantly increase grain yield in the durum variety Kalka, and increasing subsoil EC from levels not considered to be toxic to highly toxic, significantly increased grain yield in Tamaroi. The increase in grain yield in response to soil EC also occurred across the other varieties at all three depths, although not at the 5% significance level. The EC of the subsoil (30-40cm and 50-60cm) reached potentially toxic levels and contrary to the results, was expected to negatively impact on yield. The Angas Valley site was sown on shallow, sandy, calcareous topsoil, overlaying an impermeable, sodic, calcareous, red-brown clay. No root growth was observed below 30cm due to both high sodicity and very high pH. Furthermore, no subsoil

moisture and below average rainfall, probably resulted in moisture being a major limiting factor at the Angas Valley site in 2005. The EC value may have related to the soil type, such as, amount of clay, type of clay, friability, or organic level, and consequently, the amount of plant available moisture present, resulting in a positive relationship with yield.

Roseworthy

At Roseworthy, increasing subsoil EC from levels considered to be non-toxic to highly toxic correlated with significantly decreased grain yield of Yallaroi. High subsoil EC also negatively correlated with the yield of other varieties, although not a significant level. Subsoil pH ranged from moderately to highly alkaline, yet had no significant impact on grain yield. Despite both toxic EC levels and highly alkaline pH, subsoil EC only accounted for 28.5% of the variation in grain yield for Yallaroi, implying that other factors were largely responsible for the variations observed in grain yield at the Roseworthy site in 2005.

The differences in grain yield response of the three durum varieties may indicate factors influencing yield at the field sites. At the Roseworthy site, Kalka was slightly higher yielding than Tamaroi, and significantly better than Yallaroi. Yallaroi was initially developed in NSW on more fertile, higher rainfall sites, and is poorly adapted to South Australia's drier, less fertile, and often toxic, soil conditions. Yallaroi has low tolerance to high B, NaCl, and HCO_3^-, which were at toxic concentrations at the Roseworthy site. Tamaroi was also developed in NSW, but was selected for grain yield in advanced trials under South Australian conditions. Tamaroi has poor tolerance to B, NaCl, and HCO_3^-, but has reduced yield loss under hot, dry conditions at anthesis, and has some tolerance to Zn deficiency. Kalka was bred and selected for under South Australian conditions, has moderate B and HCO_3^- tolerance, is similar to Yallaroi and Tamaroi for NaCl tolerance, but due to Kalka's later maturity, it often suffers a yield penalty under hot, dry conditions at anthesis.

At the Wanderah site Tamaroi averaged a significantly higher grain yield than Kalka or Yallaroi. The poor performance of Kalka in comparison to Tamaroi may be partly due to the below average rainfall and hot climatic conditions at the end of the growing season, with Kalka's later maturity exposing it to a higher degree of stress. At Angas Valley,

Tamaroi yielded significantly better than Kalka, which in turn yielded significantly better than Yallaroi. The poor yield of Yallaroi could be associated with the variety's lack of tolerance to various soil conditions, such as, B, NaCl, and HCO_3^-, which are highly prevalent at the Angas Valley site, and Tamaroi's better ability to withstand hotter, drier conditions than Kalka, and greater tolerance to Zn and other unidentified soil conditions.

The response of bread wheats to high soil pH in 2004 and 2005 (Experiment 2 and 3).

Increasing soil pH in the alkaline range was identified as a yield limiting factor for only the bread wheat variety Pugsley at the Angas Valley – South site in 2005. From the eight field sites in 2004 and 2005, seven had subsoil pH values higher than the critical value of pH 8.5, with only the subsoil at the site Wanderah 2004 having pH values below the critical level. The negative correlation between grain yield of the bread wheat variety Puglsey at the Angas Valley - Sth site suggests pH may have a significant impact on grain yield of some bread wheats when alkaline pH is a dominant factor. However, similar to the results for durum wheat, a number of interactive factors are likely responsible for the final grain yield, which may mask the negative effects of high pH on other bread wheats.

At all sites except Roseworthy 2004, subsoil EC (40-50cm or 30-40cm and 50-60cm) reached values >400µS/m and was identified as a significant factor in decreasing grain yield at Wanderah 2005, Angas Valley – Sth 2005, and Roseworthy – W 2005.

<u>Wanderah 2005</u>

At Wanderah 2005 increasing soil EC from non-toxic to moderately toxic in the topsoil and moderately to highly toxic in the subsoil was found to significantly decrease grain yield in both Pugsley and Frame/Yarralinka. Soil EC reached toxic levels at all three depths, although multiple linear regression analysis found that subsoil pH alone accounted for a greater percentage (≈50%) of variation in grain yield. Contrary to expectations, increasing subsoil pH from slightly alkaline to highly alkaline was found to significantly increase yield in both Frame/Yarralinka and Pugsley. Similar results were obtained for the durum experiment, discussed above, for the Wanderah site. Since the bread wheat experiment was sown adjacent the durum experiment, similar factors were likely to be involved between the two experiments. The more acid clay soil recorded at the bottom of

the slope was associated with poor yields due to high sodicity and magnesia patches (ie. topsoil $EC_{1:5}$>400 μS/m), which reduced germination and growth compared to the higher yields on the less sodic and more friable, alkaline calcareous soil further up the slope. Even though pH and EC were above their critical levels, the changing soil type up the slope, associated with an increase in pH and decrease in EC, was likely the more dominant factor in explaining grain yield.

Angas Valley – South 2005

At Angas Valley – Sth increasing subsoil pH from highly alkaline to extremely alkaline was found to significantly decrease grain yield in the bread wheat variety Pugsley. A decrease in grain yield in response to increasing subsoil pH was also identified for Frame/Yarralinka, although only at P=0.1 or 0.2. Increasing subsoil EC in a non-toxic range was also found to significantly decrease grain yield in Pugsley. The significant correlation is likely due to subsoil EC being strongly positive correlated with subsoil pH. This conclusion is supported by the multiple linear regression analysis, which identified pH(50-60cm) as accounting for 29.6% of the variation in grain yield. The low variation in grain yield may have been associated with minimal root growth past 40cm due to extreme pH levels and low subsoil moisture. In 2005 the Angas Valley site was also severely infected with stripe rust (*Puccinia striiformis*), which significantly reduced grain yields, particularly the susceptible Frame/Yarralinka variety, as compared to the moderately resistant Pugsley. The difference in susceptibility of the two varieties led to the large mean grain yield difference identified between the varieties.

Angas Valley-North 2005

At Angas Valley – North, soil pH and EC values greater than the critical level for toxicity did not significantly affect grain yield in the bread wheat varieties Frame/Yarralinka or Pugsley. In comparison to the Angas Valley – South site, the Angas Valley – Nth site had shallower topsoil, less carbonates, higher clay content, and an impermeable sodic layer at a depth of 20 to 30cm. The impermeable, extremely alkaline subsoil combined with a lack of soil moisture in 2005, prevented any observable root growth past 30cm. Factors affecting grain yield would therefore be expected to be associated with the topsoil, but no significant correlations were identified for topsoil pH or EC and grain yield. The lack of correlation

may have possibly been due to the wide range of values obtained for pH and EC. The sandy topsoil was highly variable in depth from a history of erosion and where the topsoil was less than 10cm deep, red-loam subsoil was present in the sample, which dramatically increased the pH and EC, but may not have been representative of the plot as a whole. Furthermore, as with the Angas Valley – South site, the bread wheats were infected with stripe rust.

Roseworthy – West 2005

At Roseworthy – West, increasing subsoil EC in the moderately to highly toxic range was found to significantly decrease grain yield in both Frame/Yarralinka ($P<0.05$) and Pugsley ($P<0.1$). The greater response of Frame/Yarralinka to subsoil EC may be associated with a lower tolerance to salinity than Pugsley. No significant response to increasing subsoil pH was identified even though subsoil pH was above the critical level. At the Roseworthy – West site, the highly toxic subsoil EC concentration appears to have dominated any possible pH affect.

Roseworthy – East 2005

At Roseworthy – East increasing topsoil pH in the slightly acidic to moderately alkaline range and increasing topsoil EC across a non-toxic range were found to be associated with a significant increase in grain yield in both Frame/Yarralinka and Pugsley. A strong positive correlation exists between topsoil pH and EC, but since neither were considered to be in a range toxic to plant growth, other factors correlated with pH and EC were considered to be responsible for the observed positive correlations with grain yield.

A distinct line existed at about the middle of the experimental site (Appendix 3) with the topsoil on one half measuring pH 6 and the other about pH 8. Topsoil EC has a similar, although less distinct trend, with EC increasing by about 50 to 100µS/m from the low pH to high pH side. The difference may be related to a change in soil type and plant available moisture, erosional activity, or a difference in management history (i.e fertiliser use, cultivation, organic cover, rotation), since the site was on land operated by The University of Adelaide and previous research plots may have dissected the current trial plot. The difference between the topsoil pH 6 and 8 sides of the experiment appears to have had a

greater effect than subsoil pH or EC, even though these were above the critical levels for toxicity.

Wanderah 2004

At Wanderah 2004, neither soil pH or EC significantly affected grain yield, and while soil pH did not reach values >pH 8.5 (considered to be the critical concentration), subsoil EC reached highly toxic levels. In 2004 the Wanderah site received below average rainfall, and with minimal stored subsoil moisture from the previous year, root growth would have been expected to remain relatively shallow. The lack of root growth observed in the subsoil may have prevented high EC from influencing grain yield. High sodicity, surface sealing, soil compaction, boron or poor weed competition, observed at the site would have contributed to the variation in grain yield.

Roseworthy 2004

At Roseworthy 2004 the site was situated on a relatively fertile, friable, red-brown, loam over clay. The topsoil pH and EC values did not reach their critical levels, along with subsoil EC, and therefore no significant correlations were identified between topsoil pH and EC or subsoil EC and grain yield. Subsoil pH did reach values >pH 8.5, but the below average seasonal rainfall for the site and low plant available soil moisture were more likely to have dominated grain yield response.

Buckleboo 2004

At Buckleboo 2004 increasing moderately alkaline topsoil pH and increasing subsoil EC in the moderately to highly toxic range were found to significantly decrease grain yield for the bread wheat variety RAC875. Buckleboo was severely water stressed in 2004 and little subsoil root growth was observed. The significance of the topsoil pH is reflected in the multiple linear regression analysis, which identified pH as accounting for 73.1% of the variation in grain yield. The greater yield response in the RAC875 variety, compared to Cascades, reflects RAC875's lower tolerance to bicarbonate.

In 2004 below average grain yields were recorded for all three sites. At Roseworthy and Wanderah the variety RAC875 significantly out-yielded Cascades, although at the severely water stressed site of Buckleboo, both had equally poor yields. The bread wheat variety Cascades was developed in Western Australia (released 1994) for medium to high rainfall areas, has low Zn efficiency, is a low sodium accumulator and has moderate bicarbonate tolerance. RAC875 was developed is South Australia (1990s) as a high yielding, drought tolerant line suited to low to medium rainfall areas, although was not released due to a bread making quality defect (high LMA). Under low rainfall conditions in 2004, RAC875 had higher yield than Cascades, despite Cascades greater bicarbonate tolerance.

In 2005 below average grain yields were again recorded, although yields were generally better than the yields recorded in the severe drought conditions of 2004. At Wanderah, Pugsley significantly out-yielded Frame/Yarralinka. Pugsley was developed in South Australia (released 2003) as a high yielding bread wheat variety suited for medium rainfall areas, moderately resistant to stripe rust (VPM gene), moderately tolerant to boron, and is a high sodium excluder, and has low bicarbonate tolerance. Frame/Yarralinka is a fixed research line, selected for high bicarbonate tolerance, but also has moderate boron tolerance and is moderately susceptible to stripe rust. A major stripe rust epidemic in 2005 across South Australia and the differences in susceptibility between Pugsley and Frame/Yarralinka, generally led to the observed superiority of Puglsey over Frame/Yarralinka at Wanderah and Angas Valley, regardless of soil pH or EC. At Angas Valley – South the severity of stripe rust on Frame/Yarralinka may have obscured any influence of subsoil pH on grain yield, in contrast to that found for Pugsley.

The lower difference in grain yeild between Pugsley and Frame/Yarralinka recorded at the Roseworthy sites was attributed to the application of the fungicide Tilt®(Propiconazole). At the Roseworthy – East site variety Pugsley only marginally out-yielded the research line Frame/Yarralinka, but at the Roseworthy – West site the grain yield of Frame/Yarralinka was significantly higher yielding than Pugsley.

The combined yield of the varieties was significantly less at the west site than the east site, and since the yield of the west site was associated with high subsoil EC, differences in Na exclusion and osmotic stress may be responsible for the yield differences. The higher sodium exclusion of Pugsley as compared to Frame/Yarralinka (based on medium Na

exclusion of Frame) may have resulted in an increase in osmotic stress in the rhizosphere, and consequently, under limited moisture, water stress occurred sooner or more frequently in Pugsley (similar to the Na149 effect in durum, section 2.5.1). Alternatively, Pugsley may have developed greater early season biomass than Frame/Yarralinka, which caused the earlier exhaustion of available soil moisture, particularly likely on the Roseworthy – West site that had a higher soil clay content (lower available soil moisture).

General comments on the response of bread and durum wheats to high soil pH.

At the 2005 sites, Wanderah, Angas Valley, and Roseworthy, the durum experiments were sown adjacent the bread wheat experiments, and at all three sites the bread wheats significantly out-yielded the durum wheats by 20-90 percent (Table 4.02, 4.07 and 4.11). The lack of adaptation in durum wheats to lower rainfall areas of South Australia is a major obstacle for the expansion and reliability of durum production. Generally, durum wheats have lower tolerance to B, NaCl and HCO_3^-, which occur frequently across most of South Australia and often occur together on the same soil types. The inability to separate a distinct high pH effect on grain yield was not surprising, since all sites suffered from low plant available moisture, and a range of abiotic stresses.

Similarly in bread wheat, below average rainfall in 2004 and 2005, severely limited plant available moisture, and combined with multiple abiotic stresses, a distinct pH effect was difficult to identify, except at Angas Valley – South with Pugsley. The identification of a pH response in the bread wheats but not for the durum wheats may be due to durums lower tolerance to bicarbonate, compared to the bread wheat varieties. The site pH at Angas Valley was extremely high, ranging from 9.8 to 10.3, which would have severely reduced root growth and limited the identification of genetic variation.

Overall, measurements of soil pH and EC for most sites failed to account for a significant proportion of the variation in grain yield. The lack of soil moisture and osmotic stress appeared to be a major yield limiting factors at most of the sites in 2004 and 2005, with many of the sites having a no subsoil moisture throughout the growing season. Often soil moisture remained in the top 30cm of the profile, which is evident in the dominance of topsoil factors for sites, such as Wanderah 2005, Angas Valley 2005, Buckleboo 2004, and Roseworthy – East 2005. Large spatial variation in soil type across sites (i.e. calcareous to

Blanchtown clay), and associated differences in factors, such as trace element availability (Zn), N and P fixation, organic matter content, boron toxicity, compaction, surface sealing, and weed growth, may have also affected the yield responses. Biotic factors were also highly prevalent, with Crown Rot (*Fusarium pseudograminearum*) observed in the durum wheats at all the experimental sites, particularly Wanderah, and stripe rust (*Puccinia striiformis*) severely infecting the bread wheats in 2005, particularly Frame/Yarralinka at Angas Valley. To gain a better understanding of the effects of high pH soils on durum and bread wheat growth in the field, further testing is required to unravel the bicarbonate component from soils were many abiotic and biotic stresses interact.

4.3 pH effect on plant nutrient acquisition of tolerant and intolerant durum lines.

4.3.1 Introduction

Plant nutrient availability depends largely on soil pH. The solubility of elements, such as, aluminium, iron and manganese increases with increasing acidity and can reach toxic concentrations. Other nutrients in acid soils, such as calcium, potassium, magnesium, and sodium, are readily leached from a weakened cation exchange complex, leading to deficiency. In alkaline soils the solubility of a number of nutrients declines, leading to deficiencies of plant available phosphorus, iron, calcium, magnesium, zinc, manganese and copper (Lindsay 1972, Marschner 1988, Troeh and Thompson 1993, Miller and Gardner 1998, Kabata-Pendias 2001).

Phosphorus at a pH>7.2 forms low-solubility calcium phosphates in calcareous soils (pH 7.5-8.5), leading to reduced levels of plant available P, however, in alkaline-sodic soils (pH >9) P availability increases due to the formation of more soluble sodium phosphates (Troeh and Thompson 1993). Iron solubility decreases about a thousand-fold for each unit rise in pH, although deficiency is commonly observed in calcareous soils due to high concentrations of HCO_3^- and the formation of relatively insoluble iron salts (Miller and Gardner 1998). Zn solubility decreases about a hundred-fold for each unit rise in pH, and similar to Fe, deficiency is common in soils with a high $CaCO_3$ concentration and

increased HCO_3^- activity (Lindsay 1972). The solubility of Mn decreases with increasing pH and deficiency in alkaline soils is common in the presence of high concentrations of

free carbonates, with Mn being adsorbed on $CaCO_3$, oxidised at MnO_2 surfaces or coprecipitated with Ca^{2+} (Marschner 1988). The concentration of Cu^{2+} in the soil is controlled by sorption/desorption reactions and depends on the surface charge of minerals, which is strongly controlled by pH (Kabata-Pendias 2001). At pH 7.5 to 8.0 the solubility of Cu^{2+} reaches a minimum, then increases above pH 8 as carbonate and anionic hydroxy complexes form (McBride 1981).

Soil pH may also indirectly affect the plant availability of other macronutrients and trace elements. In calcareous soils, soil carbonates can suppress the availability of phosphorus, zinc, manganese, copper and iron, and saturation of the cation exchange complex by Ca^{2+} can reduce the availability of Mg^{2+} and K^{+}. In sodic-alkaline soils, saturation of the cation exchange complex with Na^{+} has an even greater effect, displacing Ca^{2+} and Mg^{2+}, inducing deficiency (Naidu and Rengasamy 1993, Price 2006).

Graminaceous species (Strategy II), such as wheat, are able to tolerate high soil pH conditions due to adaptive mechanisms to acquire Zn and Fe from the soil when these are in limited amounts (Marschner 1995). Non-graminaceous species (Strategy I) generally prefer neutral to slightly acid conditions, with increasing concentrations of HCO_3^{-} in alkaline soils inducing Fe (lime-induced chlorosis) and Zn deficiency, through reduced adsorption, translocation or ion activity (Romheld 2000, Zuo *et. al.* 2007). The influence of high HCO_3^{-} on Strategy II plants as been associated with a decrease in root elongation for uptake of Fe or Zn (Alhendawi *et. al.* 1997). Variation for HCO_3^{-} tolerance and Zn- and Fe-efficiency has been identified in wheat (Cakmak *et.al*. 1996, Lui and Rathjen 1998, Genc *et. al.* 2006), with durum wheats showing a much higher susceptibility to Fe and Zn deficiency in the field than bread wheats, suggesting that durum wheats may be more responsive to the detrimental affects of high levels of HCO_3^{-}.

The following experiments aim to identify the effect of increasing soil pH on the ability of durum wheats to acquire adequate nutrition, and to determine if HCO_3^{-} tolerant durum plants have greater nutrient uptake for elements such as Zn and Fe, than HCO_3^{-} intolerant plants.

4.3.2 Materials and Methods

Five bicarbonate tolerant and intolerant durum landrace lines were used for analysis (Table 4.17). The landrace lines were selected from the hydroponic screening of a durum landrace collection, later described in section 5.4. Seed was sourced from field trials conducted in 2003.

The ten durum wheat lines were sown randomly as a single replication within a larger durum landrace collection, and the check plot Tamaroi, for a total experimental size of 300 plots (20 columns x 15 bays). The experiment was sown at eight locations, Buckleboo, Jamestown, Wanderah, Redhill, Two Wells, Angas Valley, Claypans and Coonalpyn in 2004 (Section 3.1.5).

The field trials were sown and managed as described in section 3.1. Plots were harvested at maturity in December 2004 and grain weight (g/plot) measured for each individual plot for all experiments.

Table 4.17: Five bicarbonate tolerant and five bicarbonate intolerant durum varieties, country of origin and their root length (mm) in bicarbonate treatment.

Genotype	Country of Origin	Bicarbonate Response
Tolerant		
AUS9893	Mexico	171.25
AUS7890	Iran	122.57
AUS20174	Iraq	97.15
AUS836	India	120.88
AUS7886	Iran	120.21
Intolerant		
AUS2840	India	56.15
AUS20177	Iraq	72.19
AUS8913	India	49.00
AUS A19 223	Unknown	56.00
req/11389	Unknown	60.25
Krichauff (Bread wheat)	Australia	140.22
Tamaroi (Durum wheat)	Australia	70.69

Soil samples were extracted from four of the experimental sites in September 2004, Angas Valley, Coonalpyn, Redhill and Two Wells, using the hydraulic soil coring rig (Section 3.1.4). Soil cores were taken from plots for each of the 10 lines selected for their bicarbonate response, and from an extra 20 random plots across the site for each of the four sites. Soil samples were collected from a depth of 0-10cm and 40-50cm, giving a total of 60 soil samples per site. At the remaining four sites, Jamestown, Wanderah, Claypans and Buckleboo, soil samples were taken in September 2004 from 10 random plots with a hand auger at the depths 0-10cm and 40-50cm, giving a total of 20 soil samples per site. Soil pH and EC measurements were conducted as in section 3.1.4.

Tissue samples were collected from each of the 10 bicarbonate selected lines at each of the eight sites and sent for ICP analysis (Section 3.1.3). Tissue samples were also collected from a further 20 random plots (also soil sampled) at Angas Valley, Coonalpyn, Redhill and Two Wells, to increase the sample size over the trial area.

Correlations were calculated for the concentrations of the nutrients Fe, Mn, B, Cu, Zn, Ca, Mg, Na, K and P (mg/kg YEB) and soil pH (0-10cm and 40-50cm), for the field sites combined and for the individual field sites (Section 3.4). Analysis of variance was performed to determine the association of intolerance and tolerance to bicarbonate on the nutrient concentration of the durum lines with the combined site data and for each of the individual sites.

4.3.3 Results

At Redhill, the experimental plots were not harvested due to Rhizoctonia (*Rhizoctonia solani*) and severe water stress, leading to low grain yields. At all other sites in 2004, Angas Valley, Coonalpyn, Two Wells, Buckleboo, Jamestown, Claypans and Wanderah, the grain yield was harvested, but was well below average due to drought conditions (Table 4.18).

At Angas Valley, the experimental site averaged yield 84g grain/plot, well below the long-term district average, due to severe water stress. A significantly different topsoil and subsoil pH was identified between the intolerant and tolerant plots, with intolerant plots averaging a topsoil pH of 0.46 units and subsoil pH of 0.29 units higher than the average

Table 4.18: The mean (M), standard deviation (SD) and range of soil pH (pH>8.5) and $EC_{1:5}$µS/m (EC>400) values at the depths 0-10cm and 40-50cm, and grain yield (g/plot) for the selected bicarbonate tolerant and intolerant durum landrace lines, at the eight sites Angas Valley, Coonalpyn, Redhill, Two Wells, Buckleboo, Jamestown, Claypans and Wanderah.

		Angas Valley		Coonalpyn		Redhill		Two Wells	
	Depth (cm)	M±SD	Range	M±SD	Range	M±SD	Range	M±SD	Range
pH	0-10	6.89±0.44	6.3-8.3	8.32±0.09	8.1-8.5	6.60±0.26	6.2-7.2	6.79±0.32	6.3-7.8
	40-50	9.33±0.16	8.9-9.6	8.96±0.21	8.7-9.6	8.87±0.12	8.7-9.2	9.08±0.37	7.9-9.4
$EC_{1:5}$ µS/m	0-10	62.1±28.6	37-173	136.4±13.2	116-165	143.3±32.5	98-228	112.2±27.6	69-200
	40-50	933.7±153.4	460-1150	160.4±37.6	101-306	1854.7±292.6	970-2410	944.7±308.0	270-1440
Grain Yield	Tolerant	58.0±18.6	37-77	455.2±69.9	383-554	-	-	188.6±51.6	134-270
g/plot	Intolerant	52.0±39.7	6-93	286.6±158.8	118-464	-	-	254.4±113.8	153-429
	Site	84.0±40.8	-	463.0±122.8	-	-	-	306.0±195.9	-

		Buckleboo		Claypans		Jamestown		Wanderah	
	Depth (cm)	M±SD	Range	M±SD	Range	M±SD	Range	M±SD	Range
pH	0-10	8.69±0.10	8.6-8.9	8.69±0.06	8.6-8.8	6.92±0.34	6.6-7.5	7.21±0.18	6.9-7.5
	40-50	8.82±0.19	8.5-9.2	8.78±0.10	8.7-8.9	7.48±0.20	7.1-7.7	8.48±0.31	8.2-9.0
$EC_{1:5}$ µS/m	0-10	178.6±72.4	135-360	145.9±35.3	119-223	70.0±19.4	53-117	134.4±166.5	34-383
	40-50	942.7±428.8	297-1690	207.6±74.6	125-359	112.3±31.6	81-187	797.1±508.4	206-1610
Grain Yield	Tolerant	-	-	-	-	-	-	-	-
g/plot	Intolerant	-	-	-	-	-	-	-	-
	Site	51.0±27.6	-	63.0±34.9	-	332.0±127.8	-	90.0±56.6	-

subsoil pH of the tolerant plots (Table 4.18). No significant difference was found between topsoil or subsoil EC measurements taken from plots growing the intolerant bicarbonate lines compared to the plots of intolerant bicarbonate lines (Table 4.19).

The sites Coonalpyn and Two Wells were less drought affected, with average yields for the experimental sites at 463g/plot and 306g/plot, respectively. At Coonalpyn, Redhill and Two Wells no significant difference was found between the topsoil pH and EC, and subsoil pH and EC values of plots growing tolerant bicarbonate lines compared to plots of bicarbonate intolerant lines (Table 4.19). However, at Coonalpyn, the mean grain yield of bicarbonate tolerant lines was significantly greater than the bicarbonate intolerant lines with a difference of 168g/plot.

At Buckleboo, Jamestown, Claypans and Wanderah, soil and tissue samples were taken from random plots and not the ten selected bicarbonate plots. Below average rainfall resulted in the Buckleboo site having an average experimental site grain yield of 51g/plot, Claypans 63g/plot, Jamestown 332g/plot and Wanderah 90g/plot (Table 4.18).

Table 4.19: Difference between selected bicarbonate tolerant and intolerant lines for soil pH and EC at the depths, 0-10cm and 40-50cm, and mean grain yield (g/plot), for the sites, Angas Valley, Coonalpyn, Redhill and Two Wells.

			Experimental Site			
	Depth (cm)	Bicarbonate	Angas Valley	Coonalpyn	Redhill	Two Wells
pH	0-10	Tolerant	**6.65**	8.32	6.60	7.07
		Intolerant	**7.12**	8.34	6.81	6.80
	40-50	Tolerant	**9.08**	8.88	8.83	9.14
		Intolerant	**9.37**	8.86	8.88	9.02
$EC_{1:5}$ µS/m	0-10	Tolerant	52.0	136.0	149.4	136.8
		Intolerant	79.0	134.6	132.4	114.6
	40-50	Tolerant	948.0	191.0	1954.0	1174.0
		Intolerant	912.0	165.0	1825.0	1054.0
Grain yield	g/plot	Tolerant	58	**455**	0*	189
		Intolerant	52	**287**	0*	254

l.s.d(0.05) for pH(0-10)=0.47, pH(40-50)=0.21, EC(0-10)=43.8, EC(40-50)=303.5, and yield (g/plot)=99.4.

* Plots not harvested.

Analysis (ICP) of tissue samples taken from each of the ten bicarbonate tolerant and intolerant lines, and the 20 random plots from the eight sites were correlated with soil pH of the plots (Table 4.20).

Table 4.20: Correlation (r) between tissue element concentrations (mg/kg) sampled at eight sites and soil pH at the depths, 0-10cm and 40-50cm, for the selected bicarbonate lines (10 samples) and for the total lines tissue sampled (30 samples), which also includes the bicarbonate lines.

	Bicarbonate lines		Total lines	
	pH 0-10cm	pH 40-50cm	pH 0-10cm	pH 40-50cm
Fe	0.433**	0.162^{ns}	0.191**	-0.433***
Mn	-0.639***	-0.060^{ns}	-0.499***	-0.357***
B	-0.234^{ns}	0.333*	-0.297***	0.316***
Cu	-0.224***	0.310*	-0.144^{ns}	-0.087^{ns}
Zn	-0.444**	-0.126^{ns}	-0.082^{ns}	-0.480***
Ca	0.680***	-0.149^{ns}	0.348***	-0.335***
Mg	-0.272^{ns}	0.332*	-0.298***	0.207**
Na	-0.500***	0.234^{ns}	-0.192**	0.179*
K	0.727***	-0.146^{ns}	0.601***	-0.229**
P	-0.364***	-0.147^{ns}	-0.505***	0.136^{ns}

ns not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

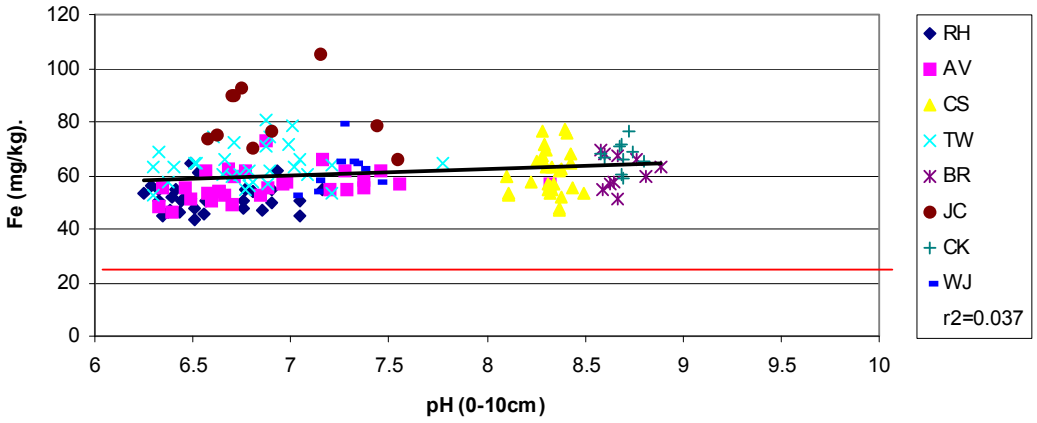
Iron (Fe)

The tissue Fe concentration (mg/kg) of the combined samples for the eight sites significantly increased with increasing topsoil pH in the mildly acidic to moderately alkaline range, but significantly decreased with subsoil pH in the neutral to strongly alkaline range (Figure 4.14). Correlations (r) between the tissue Fe concentration and pH values of the eight individual sites were not found to be significant for either topsoil pH or subsoil pH.

There was no significant (P<0.05) difference between the mean Fe tissue concentration of the intolerant and tolerant bicarbonate lines (Table 4.21). Individual sites where found to significantly differ in the mean Fe tissue concentration, but not between intolerant and tolerant bicarbonate lines at each site (Table 4.21). None of the eight sites had plots with

tissue concentrations of Fe below the deficiency level (25mg/kg), even at the highly calcareous sites of Claypans, Coonalpyn and Angas Valley (Figure 4.14).

(a) pH (0-10cm)



(b) pH (40-50cm)

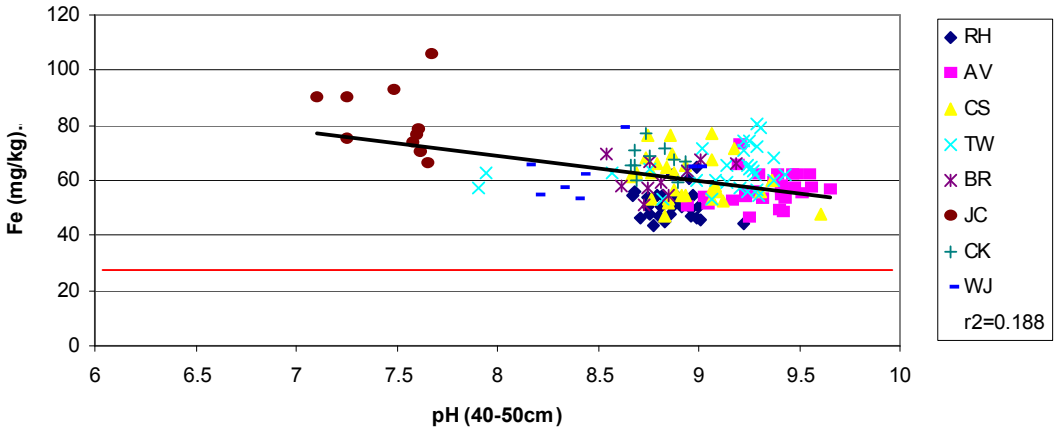


Figure 4.14: Tissue Fe concentration (mg/kg) for pH at (a) 0-10cm and (b) 40-50cm for the sites Redhill (RH), Angas Valley (AV), Coonalpyn (CS), Two Wells (TW), Buckleboo (BR), Jamestown (JC), Claypans (CK) and Wanderah (WJ). Critical deficiency level = 25mg/kg (Jones *et.al.* 1991)

Manganese (Mn)

The Mn concentration (mg/kg) for all samples significantly decreased with increasing topsoil and subsoil alkaline pH (Figure 4.15). Significant correlations for individual sites were only found for topsoil pH at Jamestown (pH 6.6 – 7.5) and subsoil pH at Coonalpyn (pH 8.7 to 9.1).

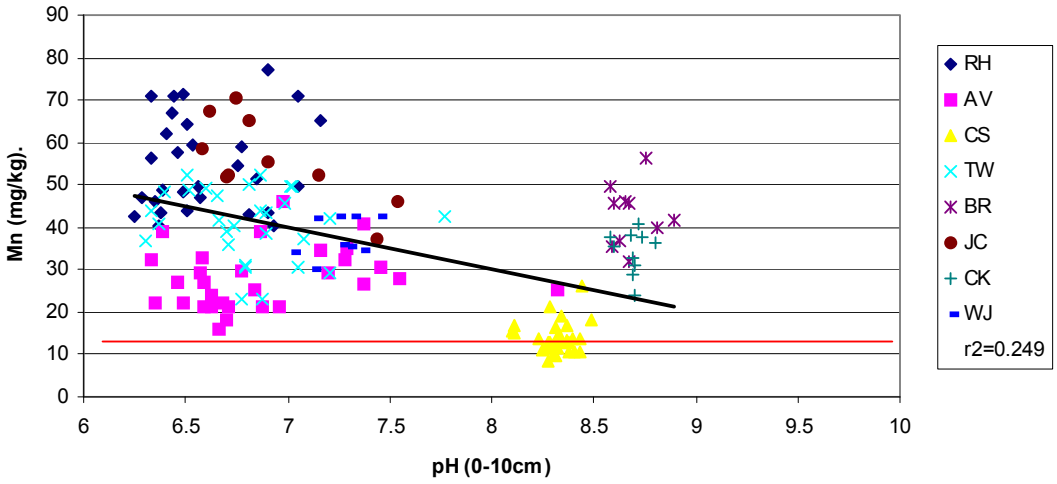
Table 4.21: Tissue nutrient concentration (mg/kg) of intolerant and tolerant bicarbonate lines for the sites Angas Valley, Coonalpyn, Redhill, and Two Wells.

Concentration		Experimental Site				
mg/kg	Bicarbonate	Angas Valley	Coonalpyn	Redhill	Two Wells	Mean
Fe	Tolerant	52.6	67.6	55.0	68.2	60.9
	Intolerant	59.2	65.0	51.4	64.0	59.9
Mn	Tolerant	**20.4**	11.4	53.6	45.4	32.7
	Intolerant	**30.2**	13.8	53.8	39.6	34.4
B	Tolerant	10.9	3.0	8.6	59.8	20.6
	Intolerant	12.8	3.3	11.0	66.2	23.3
Cu	Tolerant	3.98	2.80	4.54	**7.22**	4.63
	Intolerant	4.28	2.50	4.68	**6.12**	4.40
Zn	Tolerant	19.40	16.00	21.20	23.20	**19.95**
	Intolerant	15.32	13.80	17.74	19.40	**16.57**
Ca	Tolerant	**1364**	3060	1284	1666	1844
	Intolerant	**2106**	2860	1622	2046	2158
Mg	Tolerant	**962**	794	934	1144	958
	Intolerant	**1246**	966	958	1078	1062
Na	Tolerant	1660	818	1049	2055	1396
	Intolerant	1702	660	1674	2446	1620
K	Tolerant	22500	33400	**24000**	**27400**	**26825**
	Intolerant	20960	30200	**19600**	**22420**	**23295**
P	Tolerant	2562	2880	3026	3400	2967
	Intolerant	2820	2440	3560	4040	3215

l.s.d(0.05) for Fe=8.41, Mn=9.62, B=24.40, Cu=1.06, Zn=4.15, Ca=721.4, Mg=242.4, Na=966.2, K=3441.1, P=681.9.

There was no significant ($P<0.05$) difference between the mean Mn concentration of the tissue of intolerant and tolerant bicarbonate lines (Table 4.21), although individual sites were found to significantly differ in the mean Mn tissue concentration. Intolerant bicarbonate lines at AV, CS, and RH had a higher tissue Mn concentration, although only the difference at Angas Valley was found to be statistically significantly (Table 4.21). Coonalpyn was the only site that recorded tissue concentrations of Mn below the deficiency level, although concentrations of tissue Mn below 24mg/kg at Angas Valley would be considered marginal (Weir and Cresswell 1994).

(a) pH (0-10cm)



(b) pH (40-50cm)

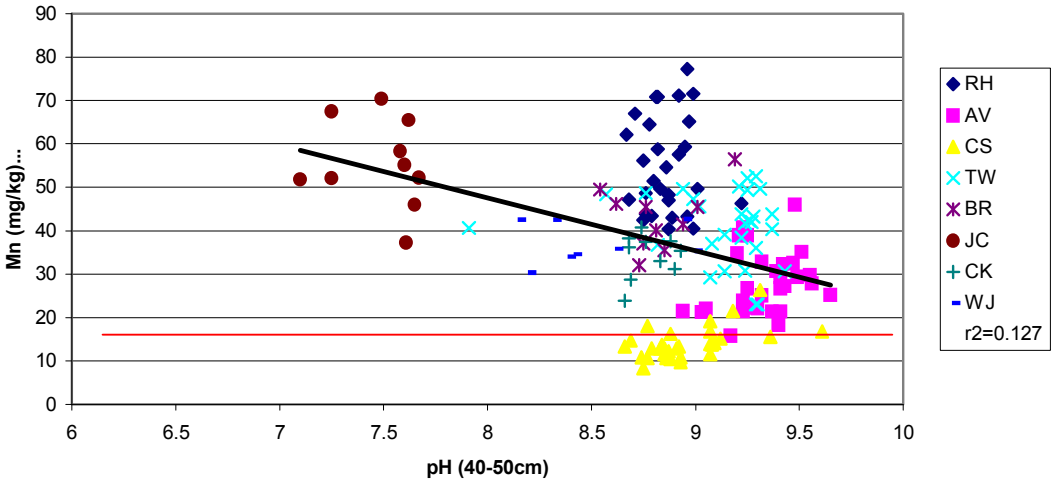


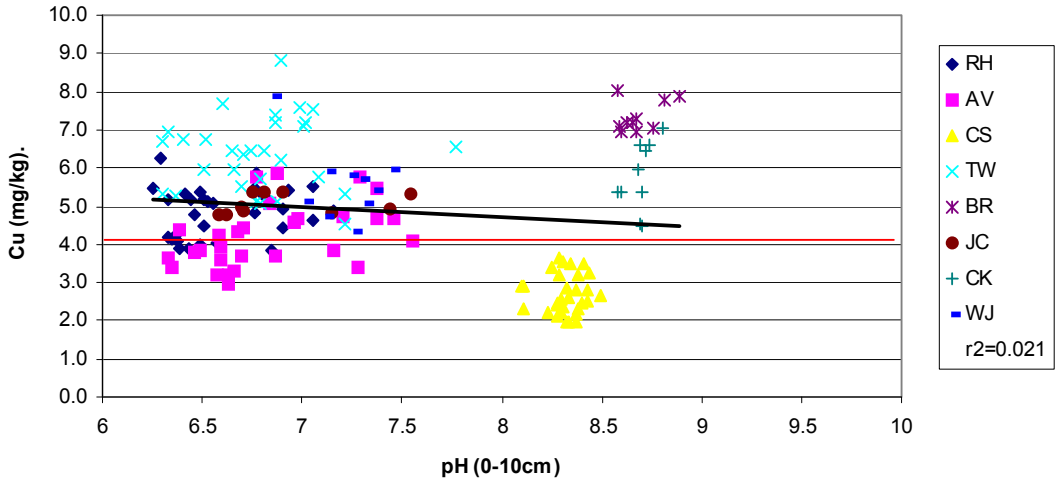
Figure 4.15: Tissue Mn concentration (mg/kg) for pH at (a) 0-10cm and (b) 40-50cm for the sites Redhill (RH), Angas Valley (AV), Coonalpyn (CS), Two Wells (TW), Buckleboo (BR), Jamestown (JC), Claypans (CK) and Wanderah (WJ). Critical deficiency level = 12mg/kg (Weir and Cresswell 1994)

Copper (Cu)

The tissue Cu concentration (mg/kg) of the combined sites did not significantly ($P<0.05$) differ with increasing topsoil or subsoil pH (Figure 4.16), although at Angas Valley the Cu concentration in tissues was found to increase with increasing topsoil pH from 6.3 to 7.5. Furthermore, correlations between the Cu tissue concentration and soil pH of only the plots

with the selected bicarbonate lines found that the Cu concentration significantly decreased with increasing topsoil pH and increased with increasing subsoil pH (Table 4.20).

(a) pH (0-10cm)



(b) pH (40-50cm)

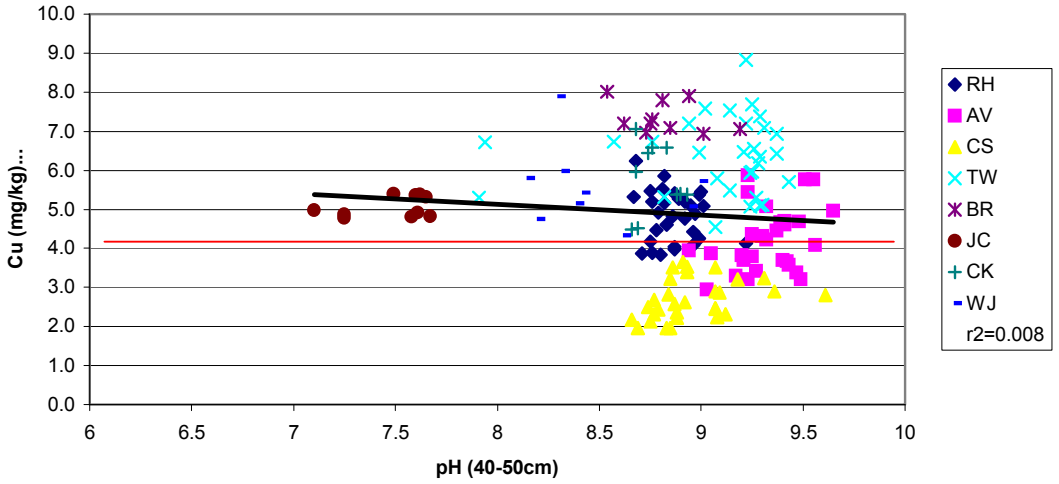


Figure 4.16: Tissue Cu concentration (mg/kg) for pH at (a) 0-10cm and (b) 40-50cm for the sites Redhill (RH), Angas Valley (AV), Coonalpyn (CS), Two Wells (TW), Buckleboo (BR), Jamestown (JC), Claypans (CK) and Wanderah (WJ). Marginal deficiency level = 4mg/kg (Weir and Cresswell 1994).

No significant difference ($P<0.05$) was found between the mean Cu tissue concentration of intolerant and tolerant bicarbonate lines, although individual sites were found to significantly differ (Table 4.21). Only intolerant bicarbonate lines at Two Wells were found to have significantly less tissue Cu than tolerant lines (Table 4.20). All tissue

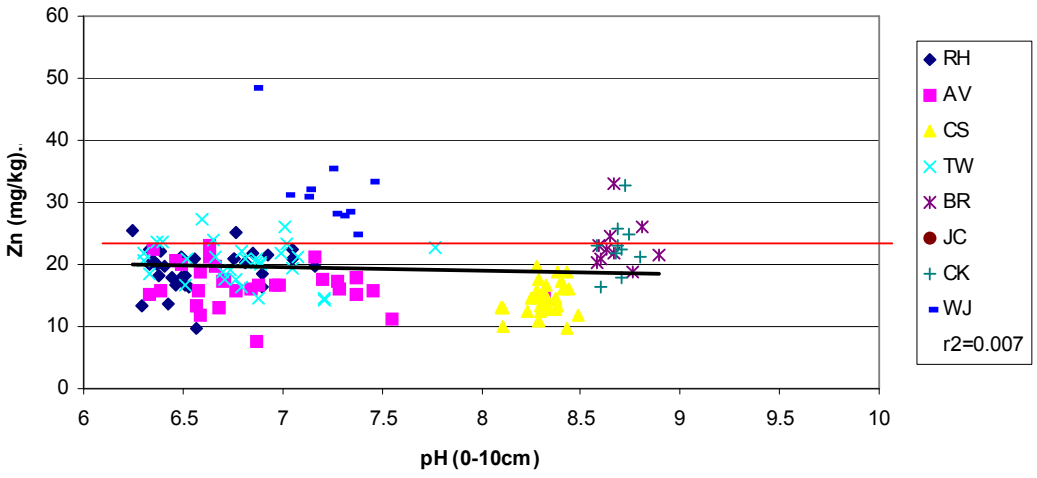
concentrations of Cu at Coonalpyn were below or within the marginal range. At Angas Valley, almost half of tissue samples analysed were in the marginal range.

Zinc (Zn)

Tissue Zn concentration (mg/kg) was unaffected (P>0.05) by topsoil pH, but significantly decreased (P<0.05) with increasing subsoil pH (Figure 4.17). For individual sites, Wanderah had tissue Zn concentrations that decreased with increasing topsoil pH, and Redhill had tissue Zn concentrations that decreased with increasing subsoil pH. Contrary to the combined tissue samples (bicarbonate and random), when the selected bicarbonate line tissue samples alone were analysed, an increase in topsoil pH was correlated with a decrease in the tissue Zn concentration (Table 4.20).

Tolerant bicarbonate lines were found to have a significantly higher (P<0.05) mean Zn concentration than intolerant lines (Table 4.21). The bicarbonate intolerant lines had lower Zn tissue concentrations than the tolerant lines at all sites, although the difference was not statistically significant on an individual basis (Table 4.21). All sites, except Wanderah, recorded tissue concentrations of Zn below the marginal deficiency level, with Angas Valley and Coonalpyn classified as extremely low (<16mg/kg Zn).

(a) pH (0-10cm)



(b) pH (40-50cm)

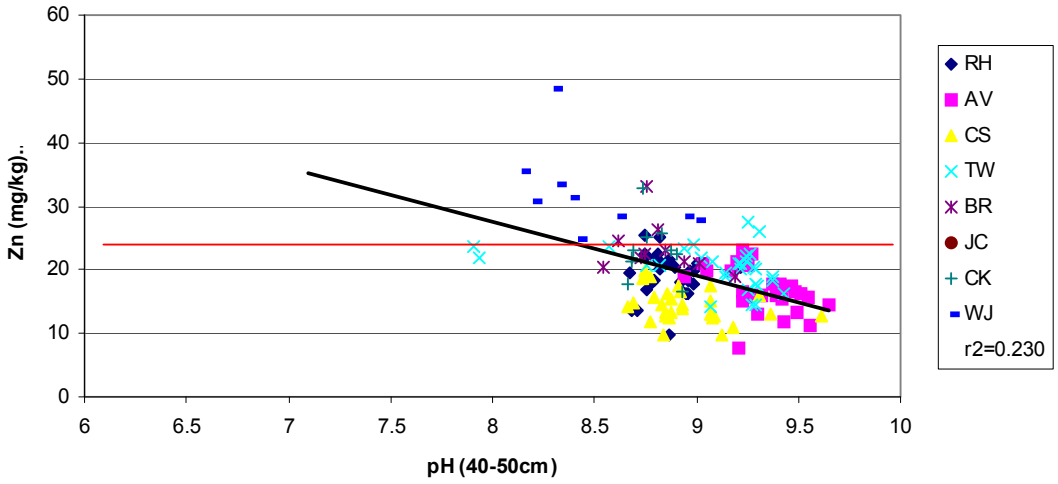
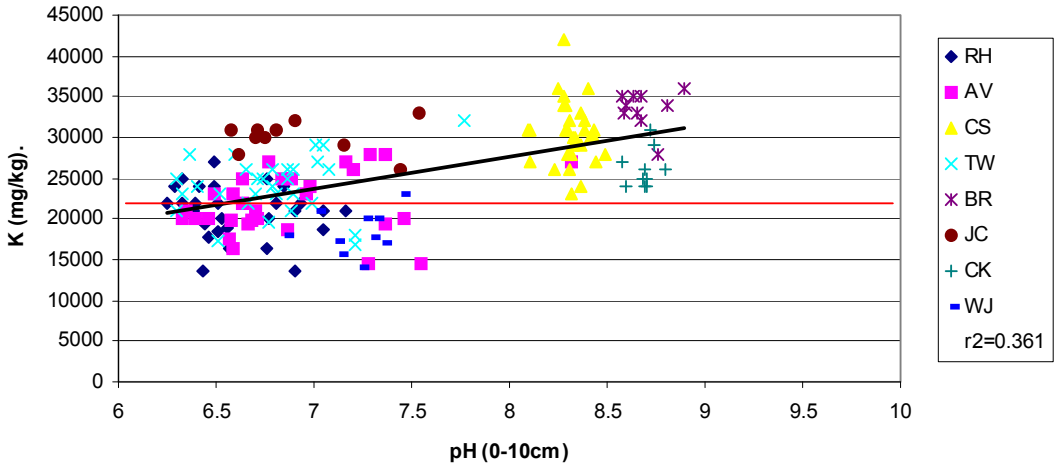


Figure 4.17: Tissue Zn concentration (mg/kg) for pH at (a) 0-10cm and (b) 40-50cm for the sites Redhill (RH), Angas Valley (AV), Coonalpyn (CS), Two Wells (TW), Buckleboo (BR), Jamestown (JC), Claypans (CK) and Wanderah (WJ). Marginal deficiency level = 24mg/kg (Wilhelm *et. al.* 1993).

Potassium (K)

Tissue K concentration (mg/kg) across the eight sites significantly increased with increasing topsoil pH, but significantly decrease with increasing subsoil pH (Figure 4.18).

(a) pH (0-10cm)



(b) pH (40-50cm)

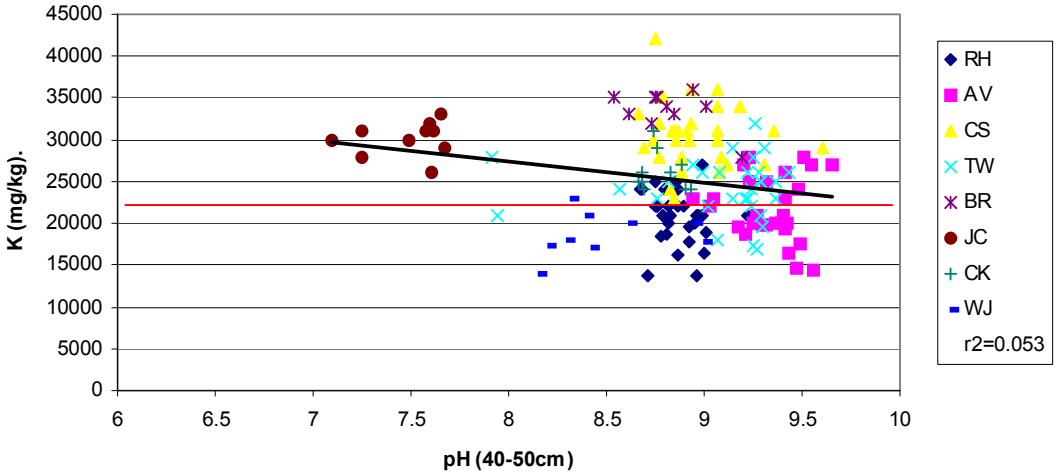


Figure 4.18: Tissue K concentration (mg/kg) for pH at (a) 0-10cm and (b) 40-50cm for the sites Redhill (RH), Angas Valley (AV), Coonalpyn (CS), Two Wells (TW), Buckleboo (BR), Jamestown (JC), Claypans (CK) and Wanderah (WJ). Marginal deficiency level = 23000mg/kg (Weir and Cresswell 1994).

Similar to Zn, bicarbonate tolerant lines had a significantly higher ($P<0.05$) mean K tissue concentration than intolerant lines (Table 4.21). Tolerant lines tended to have a higher K tissue concentration than the intolerant lines at all sites, although the difference was found to be statistically significant for the only the Redhill and Two Wells sites (Table 4.21). Most of the tissue samples from Redhill and Angas Valley recorded values within the marginal range, with Two Wells also recording some tissue K concentrations under 23000mg/kg.

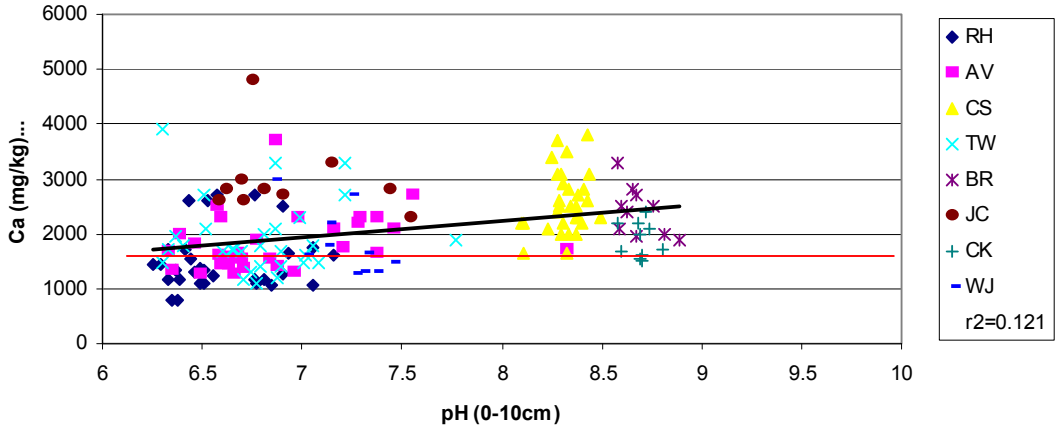
Calcium (Ca)

Similar to K, the Ca tissue concentration (mg/kg) generally increased with increasing topsoil pH, but significantly decreased with increasing subsoil pH (Figure 4.19). At the Wanderah site, however, the Ca concentration decreased with increasing topsoil pH from 6.9 to 7.4.

Intolerant bicarbonate lines were generally found to have a higher mean Ca tissue concentration than tolerant lines ($P<0.1$) (Table 4.21). Between intolerant and tolerant bicarbonate lines at each site, the intolerant lines were found to have a higher Ca tissue concentration than the tolerant lines at sites, AV, RH and TW, although the difference was found to be statistically significant for only the Angas Valley site (Table 4.21). Most of the

tissue samples from Redhill, Angas Valley and Two Wells recorded values below the marginal range, with Coonalpyn, Wanderah and Claypans also recording some tissue Ca concentrations under 1800mg/kg.

(a) pH (0-10cm)



(b) pH (40-50cm)

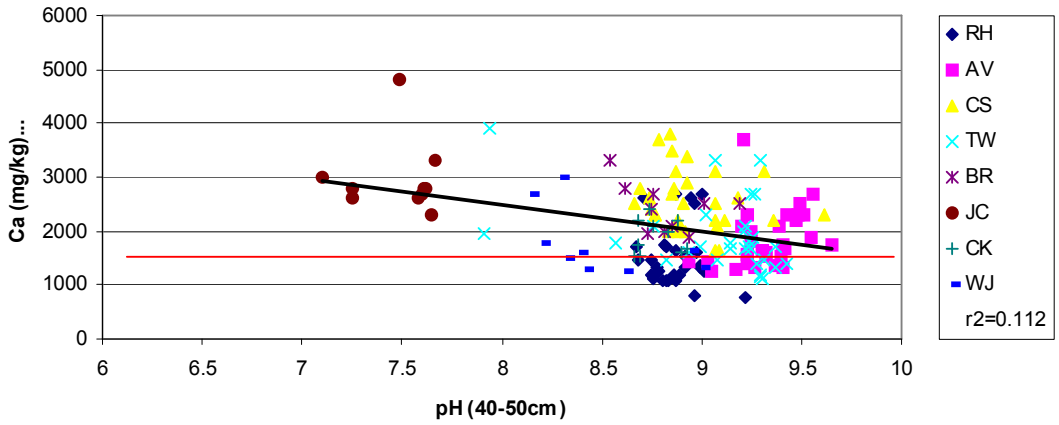
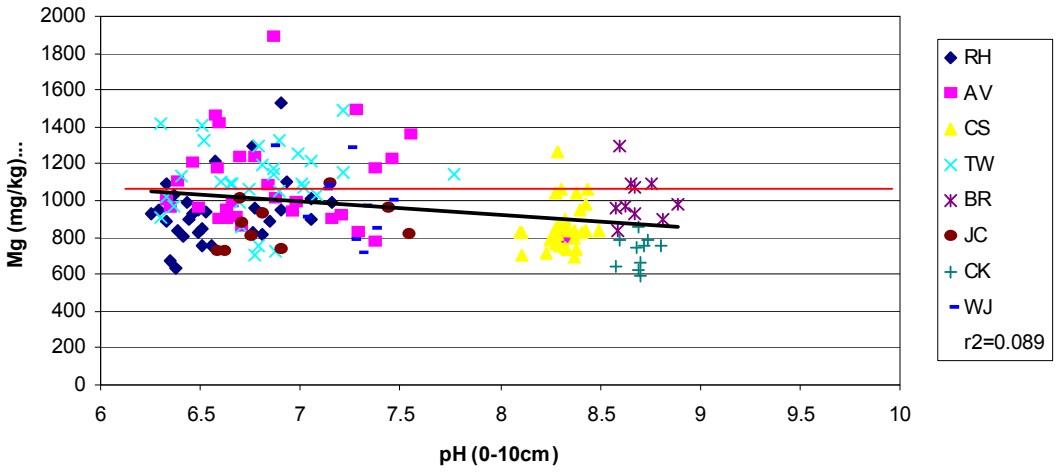


Figure 4.19: Tissue Ca concentration (mg/kg) for pH at (a) 0-10cm and (b) 40-50cm for the sites Redhill (RH), Angas Valley (AV), Coonalpyn (CS), Two Wells (TW), Buckleboo (BR), Jamestown (JC), Claypans (CK) and Wanderah (WJ). Marginal deficiency level = 1800mg/kg (Weir and Cresswell 1994).

Magnesium (Mg)

Tissue Mg concentration (mg/kg) decreased with increasing topsoil pH, however, contrary to most of the other nutrients, the tissue concentration of Mg was found to significantly increase with increasing subsoil pH (Figure 4.20). The exception was Wanderah, where the Mg concentration in tissues decreased with increasing subsoil pH from 8.1 to 9.0.

(a) pH (0-10cm)



(b) pH (40-50cm)

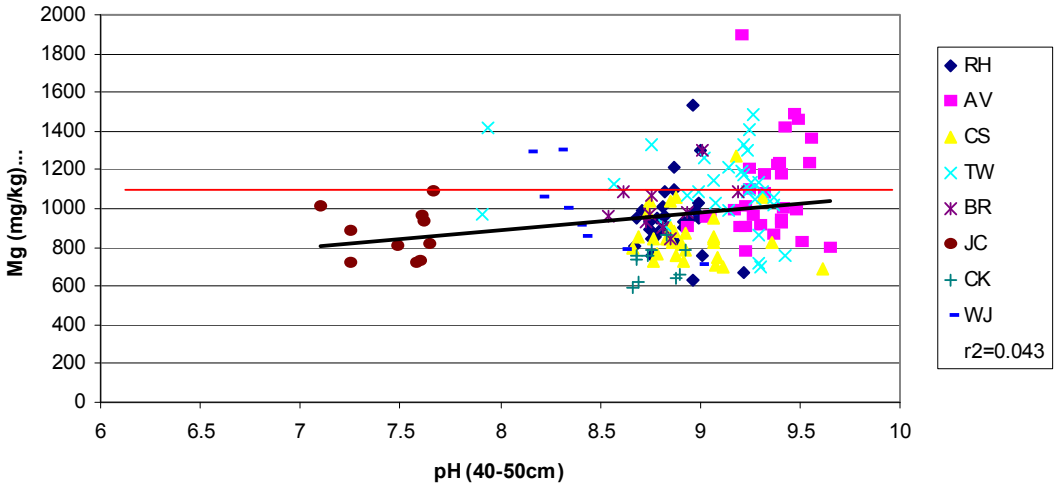


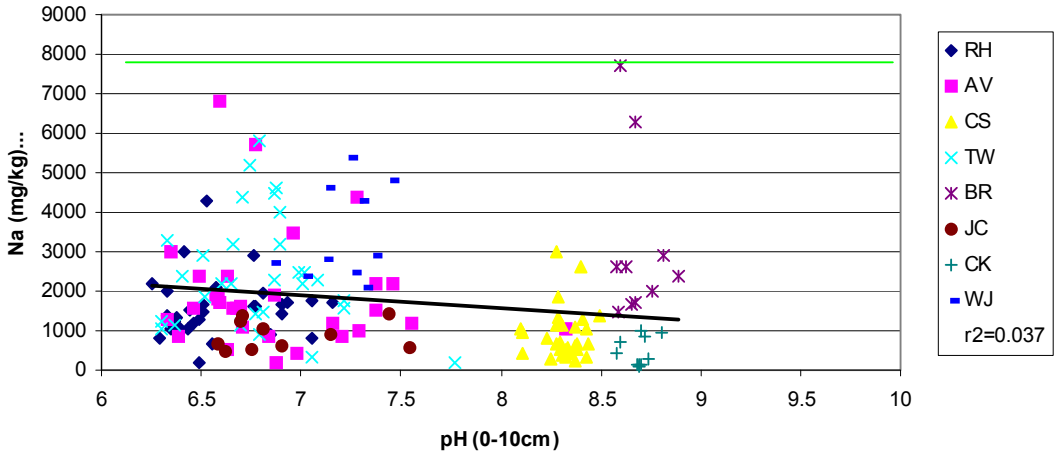
Figure 4.20: Tissue Mg concentration (mg/kg) for pH at (a) 0-10cm and (b) 40-50cm for the sites Redhill (RH), Angas Valley (AV), Coonalpyn (CS), Two Wells (TW), Buckleboo (BR), Jamestown (JC), Claypans (CK) and Wanderah (WJ). Critical deficiency level = 1100mg/kg (Weir and Cresswell 1994).

Bicarbonate tolerant lines recorded a lower mean Mg tissue concentration that intolerant lines (Table 4.21). For individual sites, tolerant lines had a lower Mg tissue concentration than the intolerant lines at sites, AV, CS and RH, although the difference was found to be statistically significant for only the Angas Valley site (Table 4.21). Most of the tissue samples from Redhill, Angas Valley, Two Wells and Coonalpyn recorded values near or below the critical level, or within the marginal range of 1100 to 1200mg/kg.

Sodium (Na)

The tissue Na concentration (mg/kg) of the eight sites combined significantly decreased with increasing topsoil pH, but significantly increased with increasing subsoil pH (Figure 4.21). At the Two Wells site increasing subsoil pH from 8.6 to 9.4 was associated with a decrease in the tissue Na concentration.

(a) pH (0-10cm)



(b) pH (40-50cm)

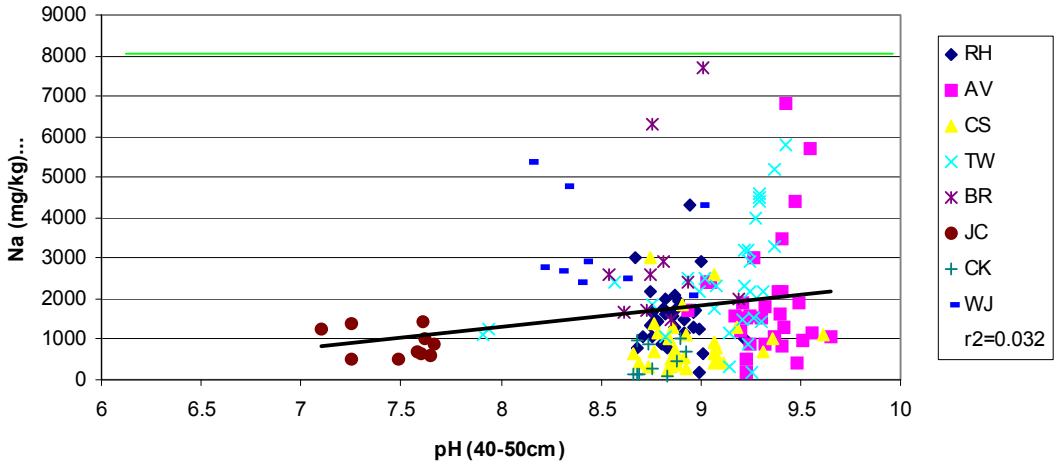


Figure 4.21: Tissue Na concentration (mg/kg) for pH at (a) 0-10cm and (b) 40-50cm for the sites Redhill (RH), Angas Valley (AV), Coonalpyn (CS), Two Wells (TW), Buckleboo (BR), Jamestown (JC), Claypans (CK) and Wanderah (WJ). Critical toxicity level = 8000mg/kg (Weir and Cresswell 1994).

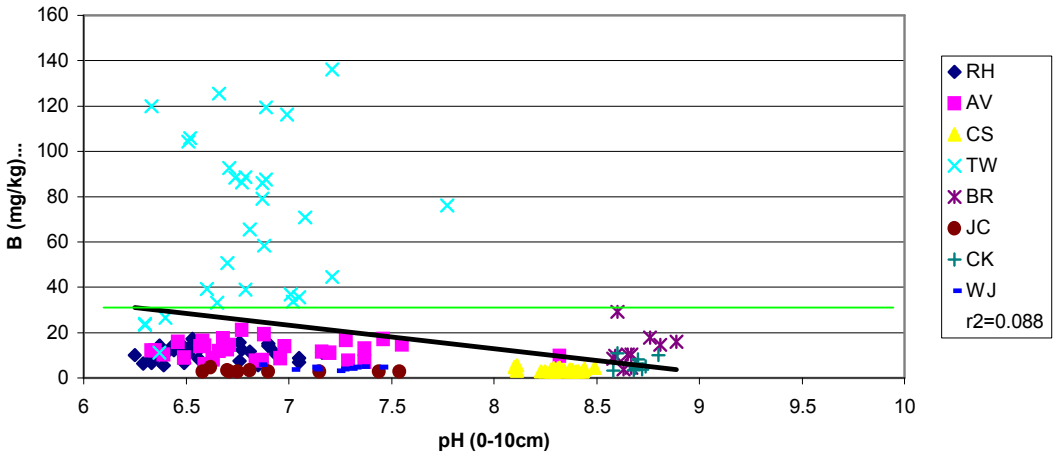
The mean Na concentration of the tissue in the bicarbonate intolerant lines was higher than the tolerant lines, but again, not at a statistically significant level (Table 4.21). At AV, TW

and RH, intolerant lines were found to have a higher Na tissue concentration than the tolerant lines, although the difference was not found to be statistically significant for any site. No sites had samples with tissue Na concentrations greater than 8000mg/kg, considered toxic.

Boron (B)

Similar to Na, B concentration (mg/kg) significantly decreased with increasing topsoil pH, but significantly increased with increasing subsoil pH (Figure 4.22).

(a) pH (0-10cm)



(b) pH (40-50cm)

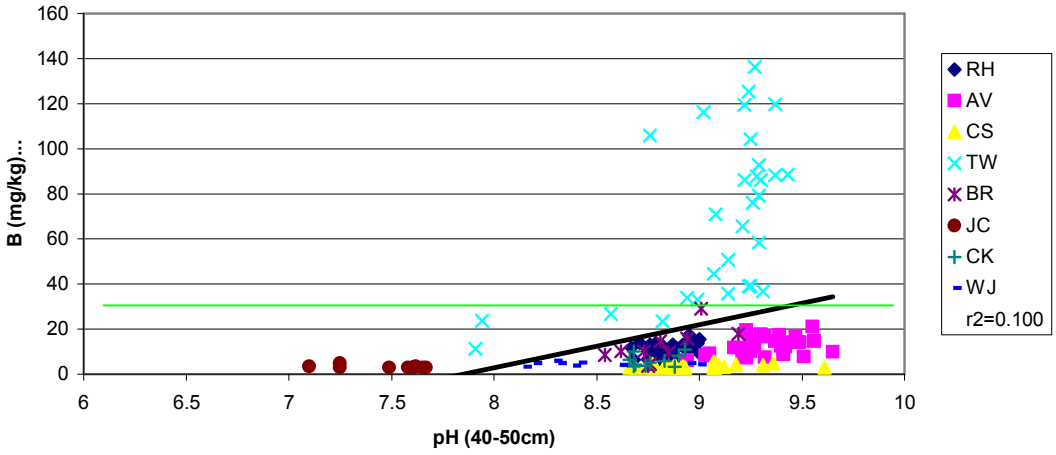


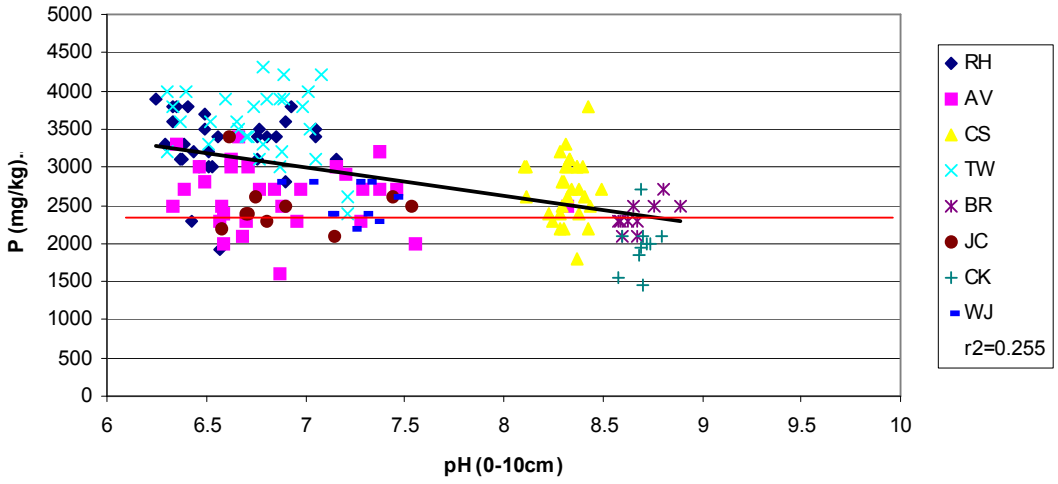
Figure 4.22: Tissue B concentration (mg/kg) for pH at (a) 0-10cm and (b) 40-50cm for the sites Redhill (RH), Angas Valley (AV), Coonalpyn (CS), Two Wells (TW), Buckleboo (BR), Jamestown (JC), Claypans (CK) and Wanderah (WJ). Critical toxicity level = 30-100mg/kg

Intolerant bicarbonate lines generally had a higher mean B tissue concentration than tolerant bicarbonate lines (P<0.2) (Table 4.21). The bicarbonate intolerant lines were found to have a higher B tissue concentration than the tolerant lines at each of the four sites, but again, the difference was not statistically significant (P>0.05) (Table 4.21).

Phosphorus (P)

Tissue P concentration (mg/kg) significantly (P<0.05) decreased with increasing topsoil pH, but increased with increasing subsoil pH (P<0.2) (Figure 4.23).

(a) pH (0-10cm)



(b) pH (40-50cm)

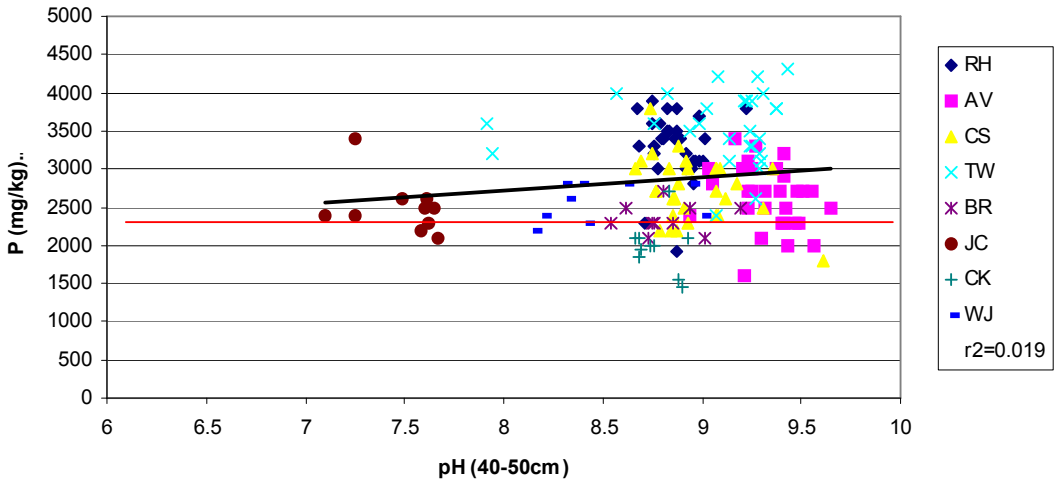


Figure 4.23: Tissue P concentration (mg/kg) for pH at (a) 0-10cm and (b) 40-50cm for the sites Redhill (RH), Angas Valley (AV), Coonalpyn (CS), Two Wells (TW), Buckleboo (BR), Jamestown (JC), Claypans (CK) and Wanderah (WJ).Critical deficiency = 2400mg/kg (Weir and Cresser 1994).

Tolerant bicarbonate lines had a higher mean P tissue concentration than intolerant lines, but not at a statistically significant level (Table 4.21). On an individual site basis the intolerant lines generally had a lower P tissue concentration than the tolerant lines at the sites Angas Valley, Redhill and Two Wells ($P<0.2$) (Table 4.21). The sites Angas Valley and Coonalpyn recorded tissue P concentration below the critical deficiency level of 2400mg/kg, although most of the tissue samples for Angas Valley and Coonalpyn sites were within the marginal P concentration range of 2400 to 2900mg/kg.

4.3.4 Discussion

Grain yields in 2004 were well below the long-term district average with the Redhill site not harvested, and the sites Angas Valley, Buckleboo and Claypans yielding less than 63g/plot (0.2t/ha) for the landrace field trials. The international durum landraces used in these experiments were particularly affected by poor rainfall, and are poorly adapted to South Australian soil and climatic conditions, resulting in yields up to 5 times lower than the bread wheats, and 3 times lower than the commercial durum lines in adjacent experiments. The reduced shoot and root growth observed, combined with many abiotic and biotic stresses, were expected to have a major affect on the acquisition, translocation and utilisation of nutrients through the 2004 season.

The intolerant and tolerant bicarbonate lines were selected on the basis of relative root length in HCO_3^-/CO_3^{2-} solution at pH 9.0 to 9.2, with nutrients Ca, Zn and B. However, despite the intolerant lines recording 50% less root length than tolerant lines in hydroponics, no significant differences were found between the grain yield of intolerant and tolerant lines at any field site. The lack of difference between bicarbonate tolerant and intolerant lines was not unexpected, since the generally poor adaptation of the durum landraces would allow factors, such as crown rot, heat stress and lodging, all affecting final grain yield, including bicarbonate tolerance. However, these factors were not tested.

Soil measurements at the eight field sites found no significant difference between pH or EC for the plots growing intolerant or tolerant lines for any of the field sites, at either depth, expect pH (40-50cm) at Angas Valley. At Angas Valley the plots in which the intolerant lines were grown averaged a pH of 0.3 units higher than those plots for the

tolerant lines, which would affect the comparison between nutrient uptake of tolerant and intolerant lines.

Nutrient trends with topsoil pH for the combined site data

The eight field sites could be separated into two distinct ranges for topsoil pH, pH 6.3 to 7.5, including RH, AV, TW, JC, and WJ, which had a loam to clay-loam topsoil, and pH 8.2 to 8.8, including CS, BR and CK, which had lighter, calcareous, sandy to sandy loam topsoil. When the tissue data from the thirty plots per site (20 random + 10 bicarbonate, RH, AV, CS, TW) or 10 plots per site (10 random, BR, JC, CK, WJ) were plotted against pH 0-10cm across all sites (total 160 samples), sites with high pH, calcareous, and sandier topsoil had a lower tissue nutrient concentrations. The lower tissue concentration reflected reduced solubility for P, Fe, Zn, Mn, and Cu at higher soil pH and suppression by carbonates, reduced Mg and K availability from Ca saturation, and leaching of nutrients from the coarse, sandy soil particles. The nutrients Mn, Cu, Zn, Mg, P, and B did follow a negative trend, although most of the correlations were strongly influence by the Coonalypn site with Molineaux to Woorinen soil type and low fertility.

The nutrients Fe and K had a significant positive trend, however, where an increase in the tissue nutrient concentration corresponded with an increase in topsoil pH from 6.3 to 8.9. Topsoil Fe varied little between sites, and subsequent removal of the RH data, indicated no significant difference in Fe tissue concentration across the sites. The low concentration Fe at the RH site may have been due to low organic matter, since the site has high sodicity and salinity, and Fe solubility is strongly related to the formation of chelated Fe derived from organic matter (Marschner 1997). The increase in K tissue concentration with increasing topsoil pH is somewhat unexpected. In the calcareous topsoils of CS, CK, and BR, K would be expected to have reduced availability due to Ca^{2+} saturation, resulting in a lower tissue concentration, and RH, AV, TW, WJ and JC would be expected to have higher soil K concentrations due to a greater clay content in the surface soil. The higher K tissue concentration for CS, CK and BR, as compared to the sites RH, AV, TW, and WJ, may have been related to higher salinity at the sites RH, AV, TW and WJ, compared to CS, CK, BR and JC, with high Na^{+} reducing the overall uptake of K (Section 2.5.1). Alternatively, the low tissue K at CK and BR may have been due to the inability of plant roots to access K, since K is relatively immobile in the soil and moves primarily by

diffusion (Marschner 1997), and CK and BR had the lowest rainfall and grain yield. CS, being a siliceous sand A horizon, is inherently low in K along with several other nutrients (Section 2.2.3). The increase in Ca tissue concentration with increasing pH was not unexpected, due to the higher $CaCO_3$ concentration driving the soil pH to values around pH 8.4 in the topsoil of sites CS, CK and BR.

Nutrient trends with subsoil pH for the combined site data.

Soil samples taken from the subsoil (40-50cm) of the eight sites found that the subsoil pH of the sites was mildly to strongly alkaline, between pH 8.1 and 9.6, except for JC, which had a subsoil pH between 7.1 and 7.7. The high subsoil pH values for the sites reflected the presence of calcium (pH 7.5 –8.5) and sodium (pH >9) carbonates (Section 2.5.4). The trends in the tissue nutrient concentrations, as with the topsoil, would be expected to be negative as pH increased from 7.1 to 9.6 for Fe, Mn, Zn, K, P and Mg. The results obtained for the tissue nutrient concentrations indicated a negative trend with increasing subsoil pH for Fe, Mn, Zn, K, and Ca, but a significant positive trend for Mg, and no trend for P. The lower subsoil pH of the JC site, however, strongly influenced the significance of the correlations. JC had high Fe, Mn, K and Ca, equal Cu and P, and lower Mg compared to the other sites. Removal of the JC data resulted in Fe, Ca and P correlations with pH being non-significant, and lowered the significant trend for Mn, K and Mg.

The bulk of available Cu forms in the soil solution are soluble organic complexes, with both pH and organic matter controlling availability. The absence of a negative trend between soil pH and tissue Cu concentration may reflect differences in the amount of soil organic matter between the sites, soil types, or the plant requirement for only trace amounts, which were adequate in most of the sites with high subsoil pH. The absence of a trend in the P data for the subsoil was likely due to high available P in the topsoil resulting from fertilisation.

The negative trend for Ca, particularly with the inclusion of the low pH JC data, was likely associated with increasing concentrations of $NaHCO_3$ and Na_2CO_3 at sites with pH > 8.3, which compete with Ca^{2+} and reduce plant Ca availability.

A significant positive trend for shoot tissue Mg concentration occurred over the pH range 7.1 to 9.6, and to a lesser extent from pH 8.1 to 9.6. Saturation of adsorption sites with Ca^{2+} or Na^{+} displaces Mg^{2+} reducing plant availability, leading to the expectation that a higher pH from $CaCO_3$ and Na_2CO_3 would reduce the tissue Mg concentration and produce a negative trend. However, Mg^{2+} also interacts strongly with other cations, such as, Mn^{2+}, K^{+} and NH^{4+}, which can reduce Mg^{2+} availability. The low Mg concentrations at sites, such as JC, may indicate a cation imbalance, resulting from heavy fertilisation with materials lacking Mg^{2+}, such as ammonium, or soil being high in available K.

Tissue B and Na concentrations positively correlated with increasing subsoil pH. Increasing pH is directly correlated with increasing Na and at pH >9 the carbonates Na_2CO_3 and $NaHCO_3$ dominate soil solution chemistry. The increasing B concentration is not directly related to pH, but from the accumulation of B at the surface of the B horizon from reduced leaching through the highly sodic subsoil.

Nutrient trends with soil pH for individual sites

The correlations for tissue nutrient concentrations for individual sites failed to identify significant trends for most nutrients at the majority of sites. The inability to detect significant correlations for individual sites is likely due the high variability of soil data and nutrient concentration, and low number of sample plots (30 for RH, AV, CS, TW and 10 for BR, JC, CK, WJ). Trends detected generally followed a similar pattern to the results of the combined sites, except for the sites CS, AV, and WJ.

Contrary to the combined site results, there was a positive trend for Mn to increase with increasing pH at the depth of 40-50cm over the pH range 8.7 and 9.6 for CS. CS was the only site deficient in Mn, so would have been sensitive to changes in plant Mn availability. At a pH >8.3 increasing concentrations of soil Na_2CO_3, increase Mn solubility, compared to high $CaCO_3$.

AV had a positive trend for Cu and pH at the depth of 0-10cm over the pH range 6.3 to 7.5. The topsoil Cu concentration was generally found to be site specific rather than associated with soil pH. The positive trend at AV may be due to variable soil type or organic matter

concentration across the experimental site, since Cu solubility was not expected to change as much over the narrow pH range.

WJ had a negative trend for Ca and pH at the depth of 0-10cm over the pH range 6.9 to 7.4 and also a negative trend for Mg and pH at the depth of 40-50cm over the pH range 8.1 to 9.0. The WJ site had a large difference in soil type across the site, from a calcareous sandy rise to a heavy, magnesia prone, clay flat, which was reflected the changes in concentration of Ca^{2+} and Mg^{2+}.

Nutrient trends with soil pH for the selected bicarbonate lines

Trends in tissue nutrient concentration using only the bicarbonate lines (10 lines, RH, AV, CS, TW) when plotted against pH 0-10cm for all sites combined (total 40 samples), were similar to those for total lines for combined sites, although B and Mg were not statistically significant. At the depth 40-50cm, however, some variations were observed between increasing pH and tissue nutrient concentration. The trends for Mg, B and P remained the same, but Fe, Mn, Zn, K, Ca, and Na became non-significant, and Cu changed from a negative trend to a positive trend. The non-significance for many of the nutrients occurred in the narrower pH range (pH 7.1 – 9.6) for the total sites and samples, to pH 8.6 - 9.6, for the four sites with bicarbonate lines. The latter pH range would have had a marked increase in Na_2CO_3 and $NaHCO_3$ concentrations, resulting in a concurrent increase in the solubility of some ions, such as, Fe, Mn, and Cu compared to high $CaCO_3$ soils (pH 8.0 – 8.4).

Bicarbonate tolerance and tissue nutrient concentrations

Zinc (Zn)

Tissue Zn concentrations were below the marginal level of 24mg/kg (Reuter and Robinson 1997) at the sites AV, BR, CK, CS, JC, RH and TW. At the sites AV, CS, RH and TW, intolerant bicarbonate lines had less Zn than tolerant lines, but not at a statistically significant level, except when all sites were combined. The reduced tissue Zn concentration in intolerant lines, the general decrease with increasing alkaline pH at most of the sites, and deficient levels of Zn, suggests that an increase in the ability of tolerant lines to acquire Zn may be an adaptive response for improved growth on high pH soils

(Cakmak *et. al.* 1996, Genc *et. al.* 2006). Greater root damage from HCO_3^- toxicity in intolerant lines may reduce Zn uptake (Forno *et. al.* 1975, Marschner 1995). Similarly, the reduced root length in intolerant lines resulting from HCO_3^- toxicity may reduce the root surface area for Zn absorption (Cakmak *et. al.* 1996).

Iron (Fe)

Iron deficiency is occasionally observed in durum wheats on highly calcareous soils or siliceous sands, particularly during periods of rapid tissue growth. However, RH, AV, CS and TW, all had tissue Fe concentrations in the adequate range (25-100mg/kg, Reuter and Robinson 1997), even at the highly calcareous and low fertility site of CS. Graminaceous species (Strategy II), such as wheat, are able to acquire iron via phytosiderphore release. The phytosiderphore release results in there being little soil correlation between HCO_3^- concentration and plant iron deficiency (Cramer 2002), due to decreased root elongation, which reduces the capacity for uptake of Fe (Alhendawi *et. al.* 1997). No significant difference in Fe concentration between the intolerant and tolerant bicarbonate lines was identified for any site. The inability to detect a Fe response between the intolerant and tolerant bicarbonate lines may have resulted from the adequate levels of plant available Fe at the experimental sites, a low requirement for Fe from severely reduced (droughted) growth, or the durum wheats having adequate mechanisms for the acquisition of Fe under low Fe soil concentrations, which compensated for reduced root growth resulting from HCO_3^- toxicity.

Manganese (Mn)

Manganese deficiency below the critical level of 12mg/kg was found at only the CS site, although Mn at AV was in the marginal range. The Molineaux type sands at CS, and to a lesser extent AV, are commonly low in trace elements due to the relatively recent (Quarternary) reworking of the dune systems. At the sites, CS and AV, intolerant bicarbonate lines had more Mn than tolerant lines, but statistically significant for only the AV site. The significant decrease in Mn tissue concentration with increasing pH for both topsoil and subsoil pH would suggest that improved acquisition of Mn would improve plant tolerance to high pH soils by reducing the incidence of Mn deficiency. However, for the durum landraces tested, increased uptake of Mn was associated with intolerance to

HCO_3^- and/or high pH. The increase in Mn tissue concentration for intolerant lines for AV may have been associated with the maintenance of the cationic balance. Alternatively, AV may be an atypical result associated with this particular soil type or organic matter content, since at TW tolerant lines had a greater concentration of Mn than intolerant lines, although the results were only significant at $P<0.2$.

Copper (Cu)

Copper deficiency is rarely observed in cereal crops in the field and only on soils either inherently low in total copper or on soils high in organic matter. Cu deficiency has been recorded in the Coonalpyn area, although areas recognised as deficient have often had Cu applied to the soil, which produces a long lasting residual benefit in remedying Cu deficiency. Similar to Mn, tissue concentrations of Cu at CS were in the marginal range of 2-4mg/kg, with AV also recording tissue Cu concentrations in the low range. No significant difference was identified between intolerant and tolerant bicarbonate lines at these low Cu sites, however, at the highest tissue Cu site (TW), intolerant lines had significantly less Cu that the tolerant lines. Tolerant bicarbonate lines may have a greater capacity to absorb Cu compared to intolerant lines, similar to Zn, due to greater root growth. The inability to identify a difference between intolerant and tolerant bicarbonate lines at the other sites may be associated with a failure to discriminate a difference in Cu at marginal levels, since RH, AV and CS, had an average Cu concentration of 4.61, 4.13 and 2.65mg/kg, respectively. As previously described, organic matter content also may have had a significant role in Cu availability at the experimental sites.

Phosphorus (P)

A significant decrease in P with increasing topsoil pH between 6.3 and 8.4 was likely associated with an increase in the soil $CaCO_3$ concentration, which has a high affinity for forming insoluble P carbonate. At the highly calcareous sites CS and AV, tissue P concentrations were around the critical level of 2400mg/kg (Reuter and Robinson 1997). At the sites AV, RH and TW, intolerant bicarbonate lines has less P than tolerant lines ($P<0.1$). Reductions in root growth resulting from HCO_3^- toxicity would have been expected to reduce tissue P concentration in intolerant lines, since P is relatively immobile

in the soil and with a lack of soil moisture throughout the 2004 season, a reduction in root growth would have decreased P uptake (Rengel and Marschner 2005).

Potassium (K)

The concentration of tissue K was found to be within the marginal range of 15000 to 23000mg/kg (Reuter and Robinson 1997) for the sites AV and RH, and to a lesser extent TW. At all sites, intolerant bicarbonate lines had less K than tolerant lines, although only at RH and TW were these statistically significant. The results suggest that bicarbonate tolerant lines are better able to absorb K under high pH conditions compared to intolerant types. However, tissue K concentration is often associated with the level of soil salinity. The sites TW, RH and AV all had EC values greater than the critical toxicity level in the subsoil, suggesting a Na response may be occurring, particularly since intolerant bicarbonate lines also had a slightly higher tissue Na concentration. The higher concentration of K in tolerant lines may be a regulatory response to overcome high HCO_3^- or for maintaining cationic balance (Section 2.4.3).

Calcium (Ca)

The Ca concentration in tissues increased over the topsoil pH range 6.3 to 8.4, with increasing $CaCO_3$ concentration, and decreased over the subsoil pH range 8.6 to 9.6, with increasing Na_2CO_3 concentration. Despite the highly calcareous nature of the sites, RH, AV, and TW were below the marginal concentration for Ca deficiency (1800mg/kg, Reuter and Robinson 1997). At these sites, pH >8.6 and $EC_{1:5}$>400µS/m would suggest that Na^+ saturation was displacing Ca^{2+} and inducing deficiency. However, as with K, the EC did not vary significantly between plots in which intolerant and tolerant lines were grown at each site and does not explain the differences found in Ca uptake. At the sites AV, RH and TW, intolerant bicarbonate lines had greater Ca concentrations than tolerant lines, although only AV had a difference were $P<0.05$. A reduction in the amount of Ca taken up by roots may indicate an adaptive response in the tolerant lines, where reduced Ca^{2+} uptake under high HCO_3^- concentration in calcareous soils allows increased uptake of other essential trace elements. Alternatively, damage of roots by HCO_3^- toxicity in intolerant lines may reduce the plants capacity to control ion transport allowing high amounts of Ca to enter the roots.

Magnesium (Mg)

Tissue Mg concentrations were found below the critical level of 1100mg/kg (Reuter and Robinson 1997) were measured at all four sites. Magnesium interacts strongly with other cations, with high soil Ca^{2+}, Na^{+}, K^{+} or NH_4^{+} capable of inducing deficiency. All sites had subsoil pH values >8.6 (high Na_2CO_3), which would have greatly reduced the plant available Mg (Bohn *et. al.* 2001). At the sites AV, CS and RH, intolerant bicarbonate lines had less Mg than tolerant lines, although only AV had $P<0.05$. A decrease in the amount of Mg of tolerant lines, similar to Ca, may be associated with ionic imbalance and reduced uptake at the root surface.

Sodium (Na)

The tissue concentration of Na is generally strongly associated with the total uptake of cations and anions into plant shoots. At the sites AV, RH and TW, measurements of soil $EC_{1:5}$ were well above the critical toxicity concentration of 400µS/m, averaging >900µS/m. However, no sites recorded tissue Na concentrations above the critical toxicity level of 8000mg/kg (Reuter and Robinson 1997). TW recorded the highest mean Na tissue concentration of 2250mg/kg. Low rainfall in 2004 for all sites may have restricted root growth to the topsoil layers and minimised NaCl movement from subsoil layers (Rengasamy 2002). At the sites AV, RH and TW, bicarbonate intolerant lines had a higher Na tissue concentration than tolerant lines, but only at $P<0.2$. Root damage by HCO_3^{-} toxicity may reduce the plants capacity to restrict or discriminate the uptake of Na^{+}, or reduce the removal of Na^{+} from the roots. The higher Na^{+} tissue concentration in intolerant lines may have had a minor role in reducing the K^{+} concentration in intolerant lines, although Ca^{2+} and Mg^{2+} also had higher tissue concentrations in intolerant lines.

Boron (B)

High B may have also had a role in damaging or restricting plant root growth. The tissue concentration of B was found to significantly increase with increasing subsoil pH. Two Wells was the only site to record tissue B concentration above the critical toxicity level of 30mg/kg, although Angas Valley and Redhill recorded B concentrations in the high range of 11 to 20mg/kg. The critical concentration for B toxicity is approximately 30-100mg/kg,

however, leaching of B from foliage by rain may invalidate field derived tissue samples for diagnosing B (Reuter and Robinson 1997). At all sites, intolerant bicarbonate lines had a higher tissue B concentration than tolerant lines, but only at $P<0.2$. The high B tissue concentrations suggest that the durum landrace lines have minimal tolerance to high soil B and damage by HCO_3^- toxicity may further expose roots to B damage, or vice versa.

General Summary

Overall, the results suggests that the durum landrace lines selected for tolerance to bicarbonate solution absorb less Ca^{2+}, Mg^{2+}, Na^+, and possibly Mn^{2+}, and more Zn^{2+}, K^+, and possibly Fe^{3+} and Cu^{2+}, in the field than those lines selected with lower tolerance to bicarbonate solution. However, the lack of consistent statistically significant differences between intolerant and tolerant lines and between sites prevents any firm conclusions from being draw, although enough evidence exists to warrant further investigation. The low soil moisture throughout the 2004 growing season and very low yields for the durum landraces probability influenced the level of significance in the results. Soil type, nutrition, organic content, salinity and pH of the topsoil would have been particularly important throughout the 2004 growing season, with moisture mainly observed only in the top 30cm of the profile.

4.4 Relationship between HCO_3^-, CO_3^{2-}, EC and alkaline pH in the field.

4.4.1 Introduction

The measurement of soil pH at field sites has been used extensively as an indicator of the HCO_3^- and CO_3^{2-} concentration of the soil solution. The decision to use soil pH instead of actual HCO_3^- and CO_3^{2-} values was due to the large number of soil samples collected from the various experiments and the ease of measuring pH as a 1:5 soil water extract compared to the more time intensive measurement of soil carbonates. In most soil solutions, the pH of alkaline soils is directly related to the concentration of HCO_3^- and CO_3^{2-} (Orlov 1992).

Alkaline soils occur in areas where rainfall is insufficient to leach base forming cations, such as, Ca^{2+}, Mg^{2+}, K^+ and Na^+, from the soil profile (Bohn *et. al.* 2001). The accumulation of cations on the exchange sites of the soil particles allows more OH^- ions

into solution. Soils dominated by $CaCO_3$ have a pH_w of no greater than 8.3 and a concentration of HCO_3^- commonly ranging from 1-4mM (Mengel *et. al.* 1984, McCray and Motacha 1992, Bohn *et. al.* 2001). The presence of Na^+ on the exchange sites in soils inevitably leads to an increase in pH due to the higher solubility of Na_2CO_3 and $NaHCO_3$, compared to $CaCO_3$ (Orlov 1992). In sodic soils the concentration of HCO_3^- can rise to 10mM and higher, although high HCO_3^- may also increase with high organic matter, waterlogged soils, or in the rhizosphere (Mengel *et. al.* 1984, McCray and Motacha 1992). The concentration of CO_3^{2-} also rises with pH and becomes the dominant carbonate species in solution when pH is higher than 9.5 (Bohn *et. al.* 2001). The interaction and equilibrium between the H_2O - CO_2, Ca^{2+} - CO_3^{2-}, and Na^+ - CO_3^{2-} systems, act to control the concentrations of CO_3^{2-}, HCO_3^-, and OH^- in the alkaline solution, such that,

$$pH = \log [CO_3^{2-} + HCO_3^-] + 7.87 - \log Pco_2 - 0.5I^{0.5}$$ (Chorom and Rengasamy 1997)

The predominance of carbonates in the anion composition of soils and their interaction with the cations Ca^{2+}, Mg^{2+} and Na^+ can be used to explain the alkalinity of most soil solutions (Orlov 1992).

The aim of the following experiment was to determine if soil pH measurements were a suitable indicator for predicting the concentration of both HCO_3^- and CO_3^{2-}. In South Australia's calcareous and sodic soils, increasing alkaline soil pH is expected to coincide with an increase in both HCO_3^- and CO_3^{2-}.

4.4.2 Materials and Methods

Soil samples used for the measurement of soil bicarbonate and carbonate were randomly selected from the samples taken in Experiment 2 (RAC875/Cascades), Section 4.2.2. These soil samples were extracted using the hydraulic soil coring rig at a depth of 0-10cm and 40-50cm. The samples were collected in September 2004 at the sites Buckleboo (BR), Wanderah (WJ) and Roseworthy (RAC) (Section 3.1.5). From the 113 plots sampled in the RAC875/Cascades experiment, ten were randomly selected across each site for carbonate testing. Soil samples were prepared and measured for pH and EC as described in Section 3.1.4.

The bicarbonate and carbonate concentrations were measured by P. Rengasamy, University of Adelaide. A 20g soil sample was mixed with 100mL of RO H_2O and shaken for 1hr, end over end. The soil solution was filtered through a 0.45 micron membrane filter and the filtrate collected. A 20mL aliquot was measured into a conical flask, methyl orange added, and titrated against 0.1M HCl. The result provided the concentration of soluble HCO_3^- in the soil solution. Another 20mL aliquot was measured into a conical flask, phenolphthalein added, and again titrated against 0.1M HCl, which provided the concentration of soluble HCO_3^- and CO_3^{2-} combined. The concentration of CO_3^{2-} was therefore calculated by subtracting the results of the methyl orange test from the phenolphthalein test (Rayment and Higginson 1992).

The data was graphed and correlations (r) identified. The concentration (mg/kg) of carbonate and bicarbonate was plotted against pH for both the topsoil (0-10cm) and subsoil (40-50cm) for each of the three sites and for the sites combined. The soil $EC_{1:5}$(µS/m) was also plotted against pH for both the topsoil and subsoil for each of the three sites.

4.4.3 Results

A significant positive correlation was identified between the concentration (mg/kg) of CO_3^{2-} and HCO_3^- and pH for the topsoil (0-10cm) for the three sites combined (Figure 4.24). The CO_3^{2-} concentration was zero over the pH range 6.6 to 8.55 before a sharp increase to 0.37mg/kg over the pH range 8.55 to 8.9. The HCO_3^- concentration had a significant increase over the total pH range measured, from pH 6.6 to 8.9, although a high degree of variation existed. The concentration of HCO_3^- increased on average from 1.19 to 1.60mg/kg over 2.4 pH units, but varied from 0.60 to 2.15mg/kg with a correlation (r) of 0.368 and significance at the 0.05% level.

The correlation for the concentration of HCO_3^-, CO_3^{2-}, and pH at individual sites identified few significant trends. The topsoil pH at RAC ranged from 6.6 to 7.6 and had a zero CO_3^{2-} concentration and a non-significant increase in HCO_3^-. The topsoil pH at WJ ranged from 7.0 to 8.0 and also had zero CO_3^{2-}, but a significant increase in HCO_3^-. The topsoil pH at BR was much higher, ranging from 8.4 to 8.9 and had a significant increase in CO_3^{2-}, but over the narrow pH range had a significant decrease in HCO_3^-.

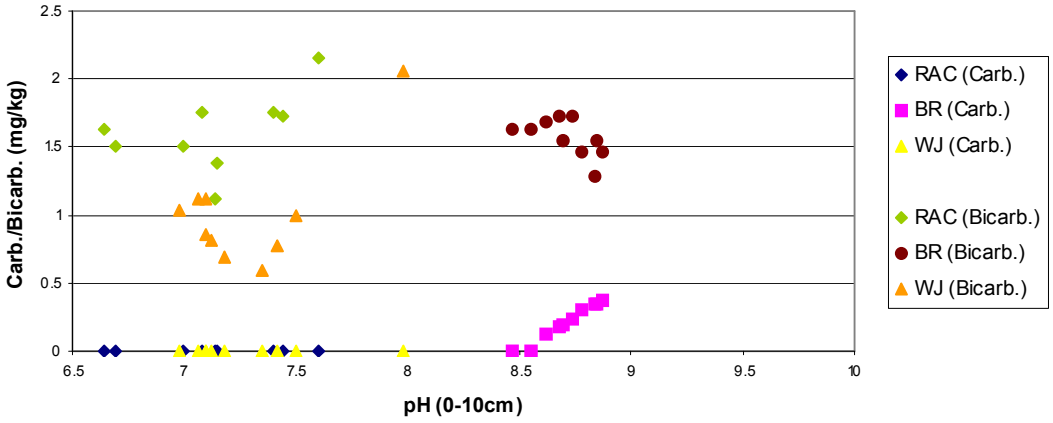


Figure 4.24: Concentration (mg/kg) of carbonate and bicarbonate for topsoil pH(0-10cm) at Roseworthy (RAC), Buckleboo (BR) and Wanderah (WJ).

A significant positive correlation was also identified when the concentration (mg/kg) of CO_3^{2-} and HCO_3^- was plotted against pH for the subsoil (40-50cm) for the three sites combined (Figure 4.25). Similarly, the CO_3^{2-} concentration was zero over the pH range 8.3 to 8.55 before a sharp increase to 1.10mg/kg over the pH range 8.55 to 9.6. The HCO_3^- concentration had a significant increase over the total pH range measured, from pH 8.3 to 9.6, although with less variation and a lower concentration than the topsoil values. The concentration of HCO_3^- increased on average from 0.60 to 1.20mg/kg over 1.3 pH units, but varied from 0.43 to 1.29mg/kg with a correlation of 0.630 and significance at the 0.001% level. The correlation for HCO_3^-, CO_3^{2-}, and pH at individual sites identified significant trends only for CO_3^{2-}. Individual sites all had a non-significant increase in HCO_3^-.

Buckleboo and Wanderah both recorded a positive correlation between $EC_{1:5}$µS/m and pH with values greater than 400µS/m ($EC_{1:5}$) (Figure 4.26). BR had a significant (0.001% level) increase in $EC_{1:5}$ over the pH range 8.5 to 9.6 for topsoil and subsoil values combined, but not when topsoil and subsoil values were correlated separately. WJ also had a significant (0.01% level) increase in $EC_{1:5}$ over the pH range 7.0 to 8.7 for topsoil and subsoil values combined, and similar to BR, not when topsoil and subsoil values were correlated separately. WJ also recorded a higher average $EC_{1:5}$ ranging from 120 to 2930µS/m over a lower pH range 7.0-8.7, compared to BR, with an $EC_{1:5}$ ranging from 150 to 1850µS/m and pH range of 8.5 to 9.6. RAC had the lowest $EC_{1:5}$ values ranging

from 70 to 310µS/m, even though pH values were recorded over a wide range from pH 6.6 to 9.2.

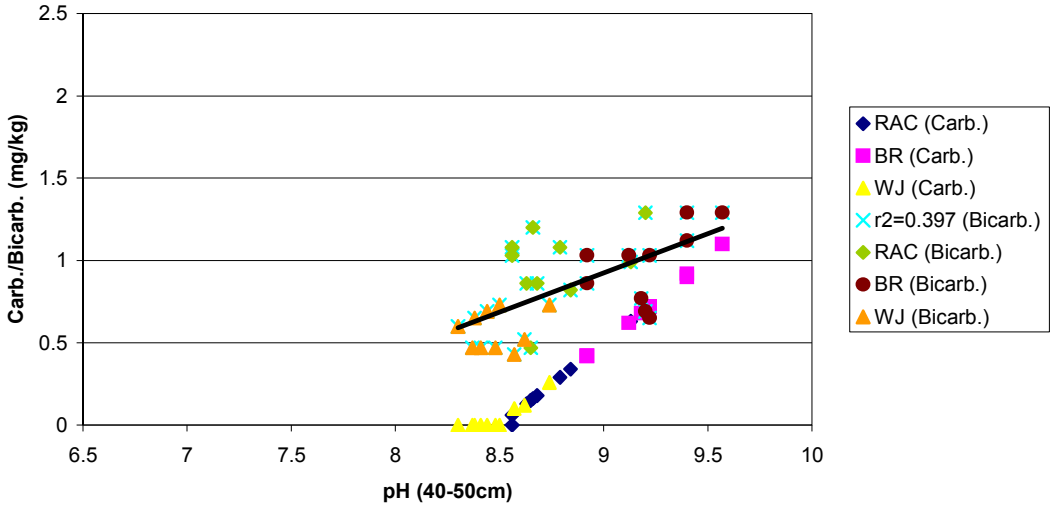


Figure 4.25: Concentration (mg/kg) of carbonate and bicarbonate for subsoil pH(40-50cm) at Roseworthy (RAC), Buckleboo (BR) and Wanderah (WJ).

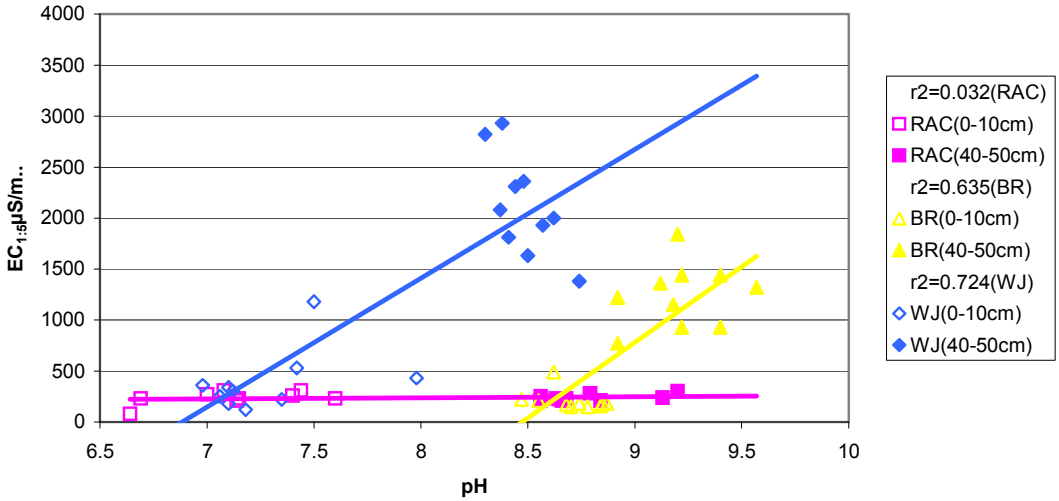


Figure 4.26: Soil $EC_{1:5}$µS/m and pH at depth 0-10cm and 40-50cm at the sites Roseworthy (RAC), Buckleboo (BR) and Wanderah (WJ).

4.4.4 Discussion

The concentration of bicarbonate (HCO_3^-) increased with increasing pH in the topsoil (pH 6.6 to 8.9) and subsoil (pH 8.3 to 9.6). No significant trends were identified for individual sites for HCO_3^- due to a low number of values, a narrow pH range, and high variation

around the trend line. The combined trends were also mostly driven by the Buckleboo data, which recorded subsoil pH values >9, and was the only site with topsoil values in the alkaline pH range.

A greater level of variation for HCO_3^- was identified in the topsoil as compared to the subsoil, along with a higher overall concentration of HCO_3^-. The difference in the concentration of HCO_3^- and variability in the concentrations may be associated with a greater amount of organic matter, greater biological activity, the application of fertilisers, and variable concentrations of cations, such as Ca^{2+} and Na^+ (Ponnamperuma 1972, Mashhady and Rowell 1978, Bohn *et. al.* 2001). The breakdown of organic matter can increase Pco_2, shifting the system equilibrium, increasing the activity of H^+, and increasing the concentration of HCO_3^-, CO_3^{2-} and OH^- (Ponnamperum 1967).

The carbonate (CO_3^{2-}) concentration was also found to increase with increasing alkaline pH in both the topsoil and subsoil, although only at pH 8.5. Carbonate becomes soluble at pH > 8.5, since the solubility product of $CaCO_3$ in equilibrium with H_2O and CO_2 gives a pH value of 8.3 (Bohn *et. al.* 2001). Soil solution with a pH value higher than 8.3 indicates the presence of Na^+, and the formation of soluble Na_2CO_3 and $NaHCO_3$ (Orlov 1992). The solubility of CO_3^{2-} is closely related to the pH of the soil solution, and the concentration increases at a more rapid rate than HCO_3^-. At a pH >9.5, CO_3^{2-} becomes the dominant carbonate species in solution (Bohn *et. al.* 2001).

A pH of 8.5 has generally been regarded as the critical level for toxicity where bicarbonate significantly reduced root length in hydroponic solutions (Lui and Rathjen 1999, Section 5.2). In soil solutions with increasing pH >8.5, the concentration of CO_3^{2-} increases rapidly, however, the solution pH at the soil-root interface can be up to 2 units different from the bulk soil, and the root apoplasts are strongly buffered in the slightly acidic range, which can influence the form of carbonate species (mostly HCO_3^-) in contact with the plant (Grignon and Sentenac 1991, Guern *et. al.* 1991). An increase in HCO_3^- and CO_3^{2-} from increasing soil pH is likely to increase the concentration of HCO_3^- at the root surface.

The relationship between carbonate concentration and pH may also be affected by other factors, such as organic matter, fertiliser and waterlogging, ion concentrations, soil type, and soil moisture. Waterlogging may occur on the top of the dense, sodic-alkaline subsoil,

although this was unlikely to be a influencing the carbonate concentration due to low rainfall in 2004. Soil salinity (EC) can have a significant influence on pH and HCO_3^-/CO_3^{2-} activity. The presence of Na^+ on the exchange sites in soils leads to an increase in pH due to the higher solubility of Na_2CO_3 and $NaHCO_3$, compared to $CaCO_3$ (Orlov 1992). The soil pH is related to the available fraction of Na^+ in soils, which is not electrically balance by SO_4, Cl, and NO_3, the 'non-alkaline' anions (Mashhady and Rowell 1978). However, practically all anions of weak acids might take part in the formation of alkalinity. Sulphide, phosphate and carbonate ions are the strongest bases in soils, although silicates and borates can also have a significant influence (Orlov 1992).

At Buckleboo the soil $EC_{1:5}$ at toxic levels increased over the pH range 8.5 to 9.6, indicating that an increasing Na^+ concentration was likely responsible for the increase in pH. Wanderah, similar to Buckleboo, had soil $EC_{1:5}$ values at toxic levels, which increased over a lower alkaline pH range (7.0 to 8.7). However, at Roseworthy an increase in soil pH was not associated with an increase in soil EC. Soil EC remained relatively constant in a non-toxic range for an increase in pH from 6.6 to 9.2. The alkaline pH at Roseworthy may be attributed to other factors, such as ionic composition involving sulphides, phosphates, carbonates, silicates or borates.

The alkalinity or pH of most soil solutions can be explained by the predominance of carbonates in the anion composition, with a direct relationship between the concentration of CO_3^{2-} and HCO_3^-, and pH. However, other bases, interactions with the cations, Ca^{2+}, Na^+ and Mg^{2+}, organic matter, and waterlogging, can alter the nature of the relationship, such as increasing the concentration of HCO_3^- at a fixed pH. The pH may be used as an estimate for assessing the concentrations HCO_3^- and CO_3^{2-}, but not as an exact measure of their concentrations in the soil solution.

Chapter 5

Screening bread and durum wheat seedlings for bicarbonate toxicity.

5.1 Introduction

In high pH soils, plant growth can be limited directly by high OH^- concentrations and indirectly by nutritional disorders and HCO_3^-/CO_3^{2-} toxicity. The direct effects of high OH^- concentrations are relatively unknown, but are thought to limit root growth. Kopittke and Menzies (2004) examining OH^- toxicity in mungbeans (*Vigna radiata* L.) measured a reduction in root length at a bulk solution pH ≥8.5. More commonly, growth inhibition at alkaline pH has been studied in relation to nutrient disorders and HCO_3^- toxicity, although often these studies fail to separate the direct effects of OH^- toxicity from the effects of HCO_3^- toxicity and nutritional disorders on plant growth in solution experiments. In high pH solutions the presence of high HCO_3^- concentrations causes equilibrium reactions to form OH^-. Furthermore, cations such as Fe^{3+}, Cu^{2+}, Mn^{2+} and Zn^{2+}, can be precipitated as carbonates and reach deficient levels, contributing to the observed reduction in root length.

Studies of HCO_3^- toxicity in solution culture experiments have been performed on a number of species, including lupin (Tang *et. al.* 1993, 1996), rice, rye, wheat (Lui and Rathjen 1998, Hajibolard *et. al.* 2003), barley, sorghum, maize (Alhendawi *et. al.* 1997) and various calcicole and calcifuge species (Lee and Woolhouse 1968). Lupins are particularly sensitive to high pH (calcareous) soils and in experiments by Tang *et. al.* (1996) HCO_3^- at 4-6mmol $KHCO_3$/L was found to reduce both root elongation and shoot growth after 28 days. Solution pH of the treatments (including basal nutrients) varied with HCO_3^- concentrations and was not adjusted, but buffered with $CaCO_3$ and replaced every 7 days. Similarly, Alhendawi *et. al.* (1997) measured a reduction in root length, fresh and dry weight, in barley, sorghum and maize, after 8 days with HCO_3^- at 5-20mM $NaHCO_3$. The treatment solution contained basal nutrients, was adjusted daily to pH 8 with NaOH, and replaced every two days. Lee and Woolhouse (1968) were also able to demonstrate a reduction in root growth after 12 days for calcicole and calcifuge species grown on filter paper with 5-20m-equiv./L $NaHCO_3$, or in solution with 1-10m-equiv./L $NaHCO_3$ and pH <8.4.

The results of Hajibolard *et. al.* (2003) suggest that for the more pH tolerant species, wheat and rye, root growth is not affected by HCO_3^- at a concentration of 10mM $NaHCO_3$ after 8 days. The treatment solution contained basal nutrients, was adjusted 2-3 times/day with KOH to pH 8, and replaced every 3 days. The inability to identify a reduction in root growth may have been due to the low pH of the nutrient solution, since Lui and Rathjen (1999) were able to identify a reduction in root length for wheat with a HCO_3^- concentration of 5mM and solution pH of 8.7. Root growth of wheat seedlings was reduced by both 5mM $NaHCO_3$ and $KHCO_3$ in the treatment solution with basal nutrients and buffered with 0.5g $CaCO_3$/L.

Alkaline soils represent some of the lowest yielding cereal cropping areas in South Australia, with soil pH values often higher than pH 8.5 and HCO_3^- concentrations exceeding 5mM, particularly in the subsoil. Improving the level of tolerance to HCO_3^- toxicity in bread and durum wheats offers the potential for increasing productivity on alkaline soils. Screening for tolerance directly in the field is difficult due to the numerous physical and chemical properties interacting to influence plant growth. The development of a simple, representative solution screen would allow targeted selection for HCO_3^- tolerance for a greater number of lines in a short period of time.

The aim of this chapter was to develop an accurate, efficient, low cost, hydroponic screening method for bread and durum wheat that could be used for the selection of bicarbonate tolerance in a commercial breeding program. The screen was initially developed to determine both the level of tolerance in current commercial varieties and whether HCO_3^- tolerance contributed to the dominance of particular varieties in the field. The second objective was to identify a greater level of tolerance to bicarbonate for durum wheat in advanced breeding material and durum landraces than currently exists in commercial varieties as a prelude to inclusion in a breeding program.

5.2 Development of a high through-put, low-cost, hydroponic screening method.

5.2.1 Introduction

The development of a simple solution screening method was initially based on the methods of Lui and Rathjen (1998), which were further developed by Cooper and Das (pers.

comm.), and modified using components of the screening methods for aluminium (Ma *et. al.* 2003) and boron (Campbell *et. al.* 1994, Chantachume *et. al.* 1995), currently employed by the Waite Durum breeding group, University of Adelaide. Lui and Rathjen (1998) selected bicarbonate tolerant wheat lines using 5mM $NaHCO_3$, 0.5g $CaCO_3$/L and a complete nutrient solution, maintained at pH 8.7. Ma *et. al.* (2003) used a HCO_3^- solution for studies of Al toxicity at high pH. Solutions contained 1mmol/L $NaHCO_3$, 80µmol/L Na_2CO_3 and 0.5g/L $CaCO_3$, with or without Al. Plant nutrients were not added to the solution. Solution pH was maintained at pH 9.2 by passing air through a 10mol/L KOH solution to remove CO_2 before entering the 22L tank, and the twice-daily addition of Na_2CO_3. Seeds were pre-germinated for 4 days before being transferred to tanks, treatment solution was added after 2 days in the 22L tanks, and root length measured after a further 10 days. Chantachume *et. al.* (1995) in boron screens identified the need for 500µM $Ca(NO_3)_2.4H_2O$, 2.5µM $ZnSO_4.7H_2O$ and 15µM H_3BO_3 as a base nutrient solution when screening bread wheat for up to 10 days in solution culture experiments.

From previous experiments using HCO_3^- and CO_3^{2-} as the treatment solution for screening wheat at the University of Adelaide, and experiments with wheat and other species by various authors, a number of questions remained unanswered. These questions included, what was the optimum pH for screening bread and durum wheat lines? How many days were needed in the treatment solution before root measurements could be taken? Were nutrients required for 10-15 days growth? Was there a difference between using $NaHCO_3$ and $KHCO_3$? Was it better to use the total root length or relative root length for assessing tolerance? What was the effect of seed source on the total root length? Was the solution test reproducible? And did the solution test represent HCO_3^- tolerance in the field? The aim of this chapter was to address these questions.

5.2.2 Materials and Methods

pH for screening

The effect of the treatment pH on root length was tested over the pH range 7.5 to 9.5 using 10 bread and durum wheat varieties. General methods are described in Section 3.3. Seven tanks were set up for the treatments with a final solution pH of 7.5, 8.0, 8.4, 8.6, 8.8, 9.0, and 9.5. The treatments contained 1mM $NaHCO_3$, 80µM Na_2CO_3, 500µM

$Ca(NO_3)_2.4H_2O$, 2.5µM $ZnSO_4.7H_2O$ and 15µM H_3BO_3, and were adjusted to the selected pH with NaOH. The ten wheat varieties included three durum wheats, Tamaroi, Yallaroi, and Daki-Cyn, and seven bread wheats, Krichauff, Molineux, Janz, Excalibur, Frame, BT-Schomburgk, and Trident. The seeds were placed onto the trays in a complete randomised block design with six replications per tank (i.e. six wells with three seeds/variety/tray). Absolute root length was measured after 10 days in the treatment solution. The average of all varietal means and the standard deviation was determined for each pH treatments, and the statistical difference between treatments calculated. The mean root length of each variety was also plotted over the range of pH values.

Number of days in solution

The effect of time in treatment on root length was tested from three to eighteen days for five wheat varieties. The wheat varieties included two durum wheats, Tamaroi and Daki-Cyn, and three bread wheat varieties, Frame, Krichauff and Excalibur. Six tanks were set up containing 1mM $NaHCO_3$, 80µM Na_2CO_3, 500µM $Ca(NO_3)_2.4H_2O$, 2.5µM $ZnSO_4.7H_2O$, 15µM H_3BO_3, adjusted with NaOH to pH 8.8 daily. Each tank represented one replication to give a total of six replications for the experiment. One tray was placed in each tank and contained sixteen groups of the five varieties. From three to eighteen days, one group of the five varieties per tray was measured for absolute root length (16 treatments). The mean root length for each variety was plotted against the number of days in the treatment solution.

Nutrient supplements

The effect of using $CaCO_3$ as a high pH solution buffer (Tang *et. al.* 1996, Lui and Rathjen 1998, Ma *et. al.* 2003) and Ca, B and Zn as a simple nutrient solution (Chantachume *et. al.* 1995, Campbell *et. al.* 1998) for bicarbonate testing was assessed. Ten wheat varieties where used, which included, three durum wheats, Tamaroi, Yallaroi, and Daki-Cyn, and seven bread wheats, Krichauff, Molineux, Janz, Excalibur, Frame, BT-Schomburgk, and Trident. The seeds were placed onto the trays in a complete randomised block design with six replications per tank (i.e. six wells with three seeds/variety/tray) over three treatments (tanks). The three treatments were 5mM $CaCO_3$, the base nutrients of 500µM $Ca(NO_3)_2.4H_2O$, 2.5µM $ZnSO_4.7H_2O$, 15µM H_3BO_3, and nil. All treatments also contained

1mM $NaHCO_3$ and 80µM Na_2CO_3, adjusted with NaOH to pH 8.8 daily. Absolute root length was measured after 10 days in the treatment solution. The mean root length of each variety and for the varieties combined was calculated for each treatment.

Bicarbonate or hydroxide

The effect of sodium carbonates versus sodium hydroxide was determined using forty wheat lines and three treatments. The wheat lines included, three bread wheat varieties, Krichauff, Cascades and RAC875, two advanced durum wheat breeding lines, R622Sh/Tm//WLYY9Tm2 and C8MMDYK/BTWLYY9//Tm, five durum wheat landrace selections, AUS7890/13, AUS7823/1, AUS9893, AUS8634/1 and AUS4897/19, and thirty double haploid lines from the RAC875/Cascades bread wheat population. The three treatments included, 1mM $NaHCO_3$ and 80µM Na_2CO_3 adjusted to pH 9.2 with Na_2CO_3, NaOH adjusted to pH 9.2 with NaOH, and a RO control (pH 8.4) that was not pH adjusted. All treatments contained the base nutrients of 500µM $Ca(NO_3)_2.4H_2O$, 2.5µM $ZnSO_4.7H_2O$, and 15µM H_3BO_3. Root length was measured after 10 days in the treatment solution. The mean root length was calculated for each variety and for the varieties combined for each of the three treatments. Relative root length (%) was also determined for each variety by dividing the root length in the HCO_3^- or NaOH treatment by the root length of the RO control treatment and multiplying by 100.

Absolute root length or relative root length

The correlation between the root length of control solutions (RO), root length in bicarbonate solutions (RL) and the calculation of relative root lengths (RRL) was determined by assessing the results of several experiments. The RO, RL and RRL values were compared for nine bread and durum wheat populations, including RAC875/Cascades, Berkut/Krichauff, Kukri/Excalibur, Worrakatta/TmWLYY9//WLYY9Tm, Frame/ Yarralinka//Pugsley, Meering/90072, Kukri/RAC875, Meering/Yitpi and Stylet/Westonia, and a collection of fifty commercial bread wheat varieties and twenty-four *Triticum dicoccoides* lines. Each population or group of varieties were tested in separate experiments. The assessments on all the populations were conducted with four replications, except RAC875/Cascades, which had six replications, and the *T. dicoccoides*, which only had two replications. The bicarbonate treatment solution contained 5mM $NaHCO_3$, 1mM

Na_2CO_3, 5mM $CaCO_3$, 2.5µM $ZnSO_4.7H_2O$, 15µM H_3BO_3, adjusted with Na_2CO_3 to pH 9.1-9.2 daily. The control treatment contained 5mM $CaCO_3$, 2.5µM $ZnSO_4.7H_2O$, 15µM H_3BO_3, and pH not adjusted (8.4). The root length of the bicarbonate treatment (RL) and control treatment (RO) were measured after 10 days. The relative root length (%) for each line in the populations was calculated by dividing RL by RO and multiplying by 100. Correlations (r) were calculated between RO, RL and RRL for each of the populations.

Seed source

The effect of seed source on root length was determined by testing the seed of nine wheat varieties collected from 13 field sites. The nine wheat varieties included, four durum wheat varieties, Tamaroi, Arrivato, Yallaroi and Gundaroi, and five bread wheat varieties, Frame, Janz, Krichauff, Excalibur and Machete. Seed was assessed from the 2003 SARDI S4 trials (National variety trials) from the field sites, Booleroo, Cummins, Kimba, Lock, Minnipa, Minarto, Paskerville, Spalding, Turretfield, Ungarra, Urania, Wokurna, and Wunkar. Seeds were selected for similar size. The bicarbonate treatment solution contained 1mM $NaHCO_3$, 80µM Na_2CO_3, 500µM $Ca(NO_3)_2.4H_2O$, 2.5µM $ZnSO_4.7H_2O$, and 15µM H_3BO_3, adjusted with NaOH to pH 8.8 daily. The experiment consisted of five replications that were measured after 10 days in treatment. The mean and standard deviation of root length was calculated for each variety and for the varieties combined for each of the thirteen field sites.

Reproducibility

The consistency of root length measurements between bicarbonate treatments with the same line, but conducted as a different experiment at a different time, using a different seed source, was assessed. The double haploid bread wheat population RAC875/Cascades with 92 lines was tested in four separate completely randomised experiments.

- Experiment 1 used seed sourced from 2003 field trials. The treatment solution contained 5mM $NaHCO_3$, 1mM Na_2CO_3, and 5mM $CaCO_3$, adjusted with Na_2CO_3 to pH 9.1-9.2 daily. The experiment had two replications (tanks) and roots were measured after 10 days in treatment.

- Experiment 2 used seed sourced from 2004 field trials (2003 seed re-sown). The treatment solution and methods were identical to Experiment 1, but with four replications (tanks).
- Experiment 3 used seed sourced from 2005 field trials (2004 seed re-sown). The treatment solution contained 5mM $NaHCO_3$, 1mM Na_2CO_3, 5mM $CaCO_3$, 2.5µM $ZnSO_4.7H_2O$, 15µM H_3BO_3, adjusted with Na_2CO_3 to pH 9.1-9.2 daily. The experiment had four replications (tanks) and roots were measured after 10 days in treatment.
- Experiment 4 used seed sourced from glasshouse grown plants (original seed source). The treatment solution and methods were identical to Experiment 3, except with six replications (tanks).

The mean root length of each line in each experiment was determined and correlations (r) identified between experiments.

5.2.3 Results

Optimum pH

Root length measurements for each of the varieties recorded after 10 days growth in solution show a similar growth pattern when plotted against pH (Figure 5.01). Between pH 7.5 and 8.6 little difference exists for mean root length, although a slight reduction in mean root growth did occur at pH 8.0 (Table 5.01). At pH 8.0 and 8.8 the least tolerant varieties, Yallaroi and Molineux, had shorter roots than the other varieties. After pH 8.8 the mean root length of the varieties was reduced with increasing pH, but only at pH 9.0 did the mean root length reached a level of reduction that was much less than any of the mean root length values calculated for the pH range 7.5 to 8.8. From pH 8.8 to 9.0 many of the varieties, particularly the durum varieties such as, Tamaroi and Daki-Cyn, had a large decrease in root length, whereas the more tolerant bread wheats such as, Krichauff and Frame, also decreased, but to a lesser extent.

The standard deviation of varietal root length means indicated a high level of variation between varieties for all pH values, except pH 9.5. From pH 8.8, where mean root length begins to significantly decline, the standard deviation decreased due to severe reductions in

root length to all varieties. At pH 9 the mean root lengths were much less that the mean root lengths between 7.5 and 8.6, and large variations exists between varietal means to enable discrimination between levels of tolerance to bicarbonate.

Note: The data for root length across pH treatments were not transformed prior to analysis to account for major differences in the size of residuals, due to the loss of data. The test was not repeated, but presented as a simple line graph of means. Caution must therefore be taken in drawing significant conclusions from this data.

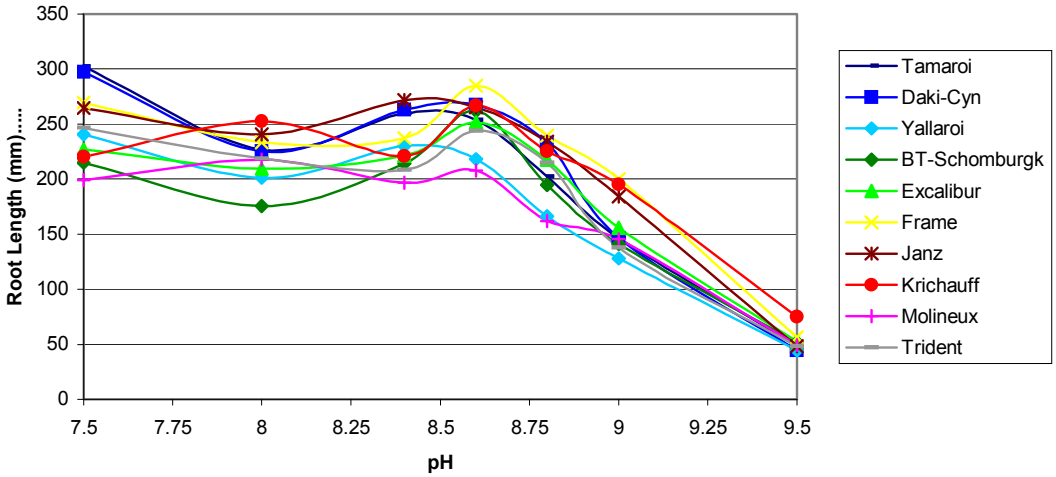


Figure 5.01: The mean root length of bread and durum wheat varieties after 10 days in bicarbonate treatment at varying alkaline pH.

Table 5.01: Average root length of 10 bread and durum wheat varieties, BT-Schomburgk (BT-S), Daki-Cyn (D-Cyn), Excalibur (Exc), Frame (Fra), Janz, Krichauff (Kri), Molineux (Mol), Tamaroi (Tam), Trident (Tri), Yallaroi (Yal), including mean of varieties and standard deviation, after 10 days in bicarbonate treatment at varying alkaline pH levels.

pH	Bt-S	D-Cyn	Exc	Fra	Janz	Kri	Mol	Tam	Tri	Yal	Mean	St.Dev.
7.5	214.9	297.4	226.9	269.4	264.8	220.3	199.3	302.5	246.6	240.7	248.3	33.0
8.0	175.8	225.2	209.7	233.6	240.7	252.8	217.7	226.9	218.7	201.3	220.2	20.4
8.4	214.4	263.3	221.7	237.4	271.7	221.1	197.1	259.9	208.5	230.2	232.5	23.8
8.6	261.8	267.6	251.4	284.8	265.5	267.1	208.0	253.8	243.7	217.9	252.1	22.3
8.8	194.8	233.1	217.4	239.3	234.3	225.4	161.6	202.3	215.1	166.0	208.9	26.1
9.0	141.1	146.3	155.6	199.9	184.3	195.5	145.7	146.5	137.9	128.1	158.1	24.2
9.5	51.9	44.1	52.7	56.5	48.2	74.9	49.4	48.5	48.3	44.2	51.9	8.4

l.s.d (0.05) of pH = 16.9

Days in treatment

The mean root length of the five varieties steadily increased up until day 9 before tapering off between day 10 and 15 (Figure 5.02). The test was stopped 3 days earlier than planned due to severe yellowing of the shoots and fungal growth on the seeds. From day 3 to day 10 the root growth rate remained linear and increased on average from 58mm to 174mm. The variation (standard deviation) also increased between varieties until day 10, before remaining steady with a standard deviation of approximately 20.5mm between day 10 and 15 (Table 5.02). Between day 7 and 10 the level of variation between the varieties was adequate to separate levels of tolerance to bicarbonate. At day seven the bicarbonate tolerant variety Krichauff had significantly longer roots than the intolerant varieties Tamaroi, Daki-cyn and Excalibur, but not the moderately tolerant line Frame. A similar difference in varieties also occurred at day 10, although a greater separation in root length was observed between the least tolerant varieties, with Daki-cyn having significantly shorter root lengths than the other four varieties. From day 10 onwards a greater separation of varieties occurred, however, due to the slowing of the growth rate, other factors, such as nutrient deficiency and low airflow around the bulk of the roots, were likely to be influencing the root length.

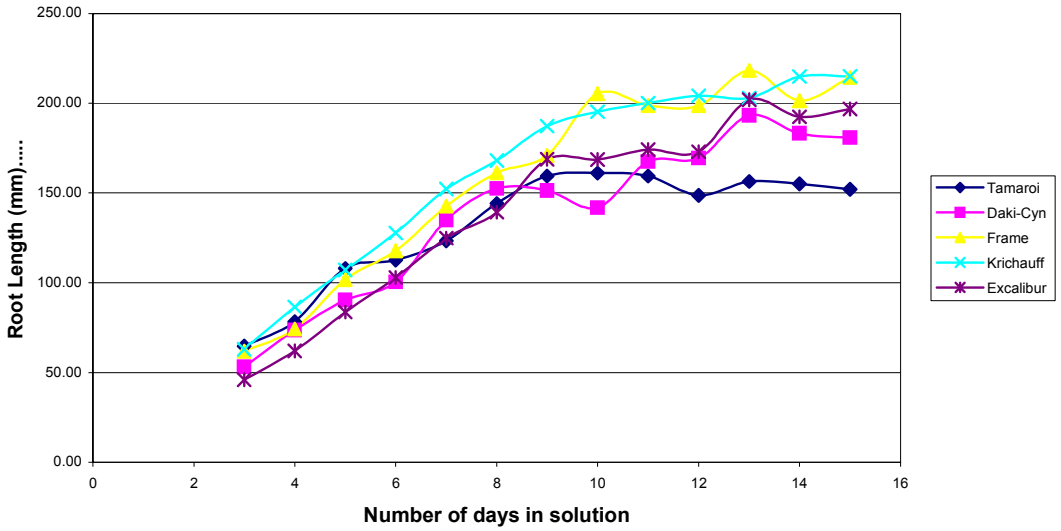


Figure 5.02: The mean root length of bread and durum wheat varieties from 3 to 15 days in bicarbonate treatment solution.

Table 5.02: Mean root length and standard deviation of 5 wheat varieties after 10 days in bicarbonate treatment at pH 8.8.

Days	Tamaroi	Daki-Cyn	Frame	Krichauff	Excalibur	Mean	St.Dev.
3	64.7	53.2	61.9	62.9	45.9	57.7	7.1
4	78.3	73.6	74.3	86.6	62.0	74.9	7.9
5	107.9	90.4	101.8	107.2	83.6	98.2	9.6
6	112.6	100.4	117.8	127.8	103.0	112.3	10.0
7	123.5	134.9	142.6	152.2	124.9	135.6	10.8
8	144.3	152.6	161.3	168.2	139.4	153.1	10.6
9	159.5	151.5	171.0	187.2	168.9	167.6	12.0
10	161.1	141.8	205.5	195.4	168.6	174.5	23.1
11	159.4	167.5	198.8	200.2	174.2	180.0	16.6
12	148.7	169.6	198.6	204.2	173.0	178.8	20.3
13	156.5	193.3	218.3	203.0	202.0	194.6	20.7
14	155.2	183.2	201.6	215.0	192.5	189.5	20.1
15	152.1	180.9	214.5	215.1	196.8	191.9	23.6

l.s.d (0.05) between days = 15.16

Nutrient supplements

No significant difference in the mean root length of the varieties was found between the Ca, Zn, B treatment and $CaCO_3$ treatment. However, the nil treatment had significantly shorter root lengths than both nutrient treatments (Table 5.03).

Table 5.03: The mean root length of 10 bread and durum wheat varieties in bicarbonate solution at pH 8.8 with three nutrient supplements.

	Nutrient supplement		
Variety	Zn, Ca, B	$CaCO_3$	Nil
Tamaroi	202.3	206.8	140.0
Yallaroi	166.0	175.9	134.2
Daki-Cyn	233.1	205.7	140.6
Frame	239.3	225.8	171.2
BT-Schomburgk	194.8	212.4	132.9
Trident	215.1	207.7	165.2
Krichauff	225.4	234.7	156.2
Janz	234.3	263.7	134.7
Excalibur	217.4	233.4	138.9
Molineux	161.6	193.1	142.6
Mean	208.9	215.9	142.6
St.Dev	27.6	24.5	17.4

l.s.d (0.05) between supplements = 26.5

Observing the daily pH measurements it was noted that the treatment with $CaCO_3$ had a lower and less rapid decline in pH with up to a 0.2 unit difference after 24hrs compared to the Ca, Zn, B and nil treatments and this may have influenced the final root lengths.

Bicarbonate or hydroxide

The mean root length of the bread wheat lines for the NaOH treatment at pH 9.2 was not significantly different (P>0.05) from the mean root length for the control treatment at pH 8.4. The relative root length (RRL) of NaOH indicated that on average NaOH reduced root length by only 3.3 percent (Table 5.04).

The mean root length for the HCO_3^-/CO_3^{2-} treatment at pH 9.2 (72.6mm) was significantly less than both the mean root length of the control treatment at pH 8.4 (177.9mm) and the NaOH treatment at pH 9.2 (170.6mm). The relative root length (RRL) of the HCO_3^-/CO_3^{2-} treatment was on average 58.5 percent of the control. All the individual varieties and the RAC875/Cascades population had a mean root length in bicarbonate significantly less than the control treatment, with significant variation between varieties in their level of tolerance.

Table 5.04: Mean root length (mm) and relative root length (RRL) of 10 bread and durum wheat lines and 30 double haploid lines from the RAC875/Cascades population, after 10 days in bicarbonate (pH 9.2), hydroxide (pH 9.2) or control treatments (pH 8.4).

	Solution Treatments					
Variety	HCO_3^-/CO_3^{2-}	control	RRL	NaOH	Control	RRL
AUS7890/13	82.2	226.6	36.3	202.2	226.6	89.3
AUS7823/1	56.8	172.6	32.9	135.6	172.6	78.6
Cascades	78.2	174.7	44.8	165.7	174.7	94.8
AUS8634/1	49.9	171.2	29.1	159.9	171.2	93.4
Krichauff	101.6	153.2	66.3	177.8	153.2	116.1
RAC875	60.2	172.9	34.8	176.9	172.9	102.3
R622Sh/Tm//WLYY9Tm2	67.3	170.1	39.6	167.2	170.1	98.3
AUS4897/19	84.1	237.2	35.5	212.7	237.2	89.6
AUS9893	78.9	152.8	51.6	167.2	152.8	109.5
C8MMDYk/BTWLYY9//Tm	89.2	162.6	54.9	160.0	162.6	98.4
RAC875/Cascades popul.	50.4	162.9	30.9	151.9	162.9	93.5
Mean	72.6	177.9	41.5	170.6	177.9	96.7

l.s.d (0.05) between treatments = 10.5, variety = 15.5, treatment x variety = 34.7.

Absolute root length or relative root length

The 42 commercial bread wheat varieties had a high level of variation for root length after 10 days in both the control and bicarbonate solutions (Section 5.3). A strong positive correlation was identified between RO and RL, and RL and RRL, but not between RO and RRL (Table 5.05). Therefore, the longer the roots in the control (RO), the longer the roots in bicarbonate (RL), but tolerance to bicarbonate (RRL) was not associated with early root vigour (root length in the RO). A similar response was identified for the 22 *T. dicoccoides*, which had very poor tolerance to bicarbonate, but tolerance to bicarbonate was not associated with early root vigour.

Table 5.05: Correlation between the mean root lengths of control (RO) and bicarbonate (RL) treatments, and relative root length (RRL), for varieties, landraces, double haploid and single-seed descent populations.

		Treatments		
Population	No. of entries	RL and RO	RRL and RO	RL and RRL
Varieties	42	0.447***	-0.003^{ns}	0.488***
Dicoccoides	22	0.241*	-0.015^{ns}	0.643***
RAC875/Cascades	88	0.152***	-0.095**	0.533***
Berkut/Krichauff	131	0.313***	-0.141***	0.305***
Kukri/Excalibur	231	0.224***	-0.227***	0.287***
Wk/TmWLYY9//WLYY9Tm	194	-0.001^{ns}	-0.256***	0.766***
Frame/Yarralinka//Pugsley	154	0.015^{ns}	-0.167***	0.728***
Meering/90072	29	0.133*	0.023^{ns}	0.949***
Kukri/RAC875	27	0.022^{ns}	-0.100^{ns}	0.790***
Meering/Yitpi	28	0.008^{ns}	-0.268**	0.639***
Stylet/Westonia	29	0.161*	-0.142**	0.475***

ns non-significant, *(P<0.05), **(P<0.01), ***(P<0.001)

The tolerance to bicarbonate in the Meering/90072 and Kukri/RAC875 population was not associated with early root vigour, and in the Kukri/RAC875 population there was also no correlation between the control and the bicarbonate treatment root length. For the RAC875/Cascades, Berkut/Krichauff, Kukri/Excalibur and Stylet/Westonia populations, positive correlations were identified between RO and RL, and RL and RRL, but there was a significant negative correlation between RO and RRL. Therefore, the lines with a longer or more vigorous root length in the control treatment (RO) were being significantly reduced at a greater percentage than the lines that had shorter roots in the control. A similar negative correlation between RO and RRL was also identified for the populations

Frame/Yarralinka//Pugsley, Meering/Yitpi and Wk/TmWLYY9//WLYY9Tm, although no correlation was found between RO and RL.

Seed source

The mean root length for bread and durum wheat varieties was similar for seed collected at the 13 sites (Table 5.06). Mean root lengths ranged from 216.7mm at Wunkar to 235.6mm at Paskerville, which was the only site found to be significantly different from the mean of the sites (223.7mm).

Table 5.06: Mean root length of five bread wheat and four durum wheat varieties after 10 days growth in bicarbonate solution at pH 8.8 for the thirteen field sites.

	Variety										
Site	Arrivato	Excalibur	Frame	Gunderoi	Janz	Krichauff	Machete	Tamaroi	Yallaroi	Mean	St.Dev
Booleroo	261.3	195.4	240.8	217.6	218.4	205.7	170.1	233.1	220.0	218.0	26.4
Cummins	271.7	218.5	252.0	224.0	224.3	226.6	202.2	193.7	247.7	229.0	24.6
Kimba	255.1	203.1	249.0	202.5	230.8	209.7	207.1	227.0	213.5	222.0	19.7
Lock	256.8	212.1	237.5	206.2	228.8	215.7	200.7	237.7	213.4	223.2	18.2
Minnipa	270.5	213.2	255.1	213.9	214.0	205.6	196.3	223.9	250.5	227.0	25.5
Paskeville	272.9	206.3	256.4	234.3	228.8	221.9	197.3	264.8	237.9	235.6	25.6
Spalding	242.2	218.2	241.3	203.4	235.2	239.0	216.2	222.2	218.0	226.2	13.7
Turretfield	251.4	208.4	224.6	217.3	234.5	198.1	192.9	242.3	210.9	220.0	19.9
Ungarra	239.4	214.2	235.0	220.4	240.7	212.5	201.5	208.7	219.2	221.3	14.0
Urania	263.3	193.0	238.9	224.2	219.5	215.5	203.0	251.6	235.8	227.2	22.6
Wonkura	263.1	215.3	225.7	221.2	220.3	185.8	186.6	207.0	234.9	217.8	23.8
Wunkar	252.3	194.3	240.6	218.4	221.8	196.1	189.3	212.7	225.0	216.7	21.3
Mean	258.4	209.0	240.6	215.8	227.1	211.6	197.0	227.1	227.1	223.7	
St.Dev	10.5	10.1	10.4	9.8	8.0	13.9	11.1	19.4	13.1		

l.s.d (0.05) site = 7.15, variety = 5.95, site x variety = 21.46

Reproducibility

Four separate bicarbonate solution screens for the RAC875/Cascades population conducted with seed collected over three consecutive years (Exp. 1-2003, Exp.2-2004, Exp.3-2005) from field trials and glasshouse grown seed (Exp.4-GH) from the original seed source (i.e. not contaminated by other genotypes) found all combinations to have a highly positive correlation (Table 5.07).

Table 5.07: Correlation between the root lengths of four separate bicarbonate solution tests of the RAC875/Cascades population with approximately 93 lines.

Data Set	r^2
Exper. 1 (2003) vs Exper. 2 (2004)	0.323***
Exper. 1 (2003) vs Exper. 3 (2005)	0.261***
Exper. 1 (2003) vs Exper. 4 (GH)	0.148***
Exper. 2 (2004) vs Exper. 3 (2005)	0.196***
Exper. 2 (2004) vs Exper. 4 (GH)	0.159***
Exper. 3 (2005) vs Exper. 4 (GH)	0.511***

***(P<0.001)

While some seed mixing would have occurred over three years of field trials at different locations, the bicarbonate test was still able to consistently identify the same tolerant lines.

5.2.4 Discussion

Optimum pH

The optimum pH for screening bicarbonate lines was found to be approximately pH 9.0 (Figure 5.01). At this pH value the mean root length of varieties was found to be significantly less than the mean root length between pH 7.5 – 8.6 and retained a considerable level of variation between varieties so as to be able to select tolerant lines. Using a pH greater than 9.2 was considered unsuitable since the very low root growth and minimal variation between varieties identified at pH 9.5 would prevent the discrimination between levels of bicarbonate tolerance.

The daily drop in treatment pH (~0.3 units) also needs to be considered when adjusting the solution pH. Adjusting the solution to an initial pH of 9.2 would result in solution pH declining to approximately pH 8.9 after 24hrs. The rate of decline decreased as time progressed (seedlings grew).

Days in treatment

After 15 days in solution root growth had stopped and severe yellowing of the seedlings had occurred. The stagnation in plant growth was mainly due to nutrient deficiency and a lack of oxygen around the roots as a result of the high root bulk that had developed in the treatment tank and inadequate airflow or water movement within the tank for roots. The rate of root growth had begun to rapidly decline by day 10 (Figure 5.02). From day 3 to

day 9, root length had a linear increase, with a corresponding increase in the variation between varieties. Levels of bicarbonate tolerant between varieties could be discriminated between days 5 and 10, but the variation between varieties was greatest between days 9 and 15. The testing of lines on laboratory benches exposed the seedlings to variable temperatures throughout the year, resulting in differing rates of growth, and the observation that growth often slowed prior to day 10 under warm conditions with rapid growth, but growth continued under cooler condition for longer than 12 days. The timing of root measurement in subsequent bicarbonate testing was therefore based on visual assessment of the general root length (approximately 200mm in control), the health of the plant, and the variation observed between lines.

Nutrient supplements

The significantly higher root growth in the Ca, Zn, B and the $CaCO_3$ treatments compared to the nil treatment, and the lack of significance between Ca, Zn, B and the $CaCO_3$ treatments (Figure 5.03) suggests that Ca may be essential for early function and growth of roots (Haynes and Robbins 1948, Chantachume *et. al.* 1995). However, since the $CaCO_3$ treatment maintained a higher treatment pH due to the buffering capacity of $CaCO_3$, a comparison between the $CaCO_3$ treatment and the Ca, Zn, B and nil treatments may be affected by the periods of lower pH in the latter treatment, with the expectation that root length would be longer. Based on the current test and with the aim of retaining a simplified procedure, the addition of 0.5M $CaCO_3$ would appear to be the more satisfactory inclusion to the bicarbonate treatment solution. Where seed is sourced from areas know to suffer nutrient deficiencies, however, the addition of 2.5µM $ZnSO_4$ and 15µM H_3BO_3 may be beneficial. No testing of other nutrients was conducted at this time due to the need to keep the test simple, but may be beneficial in the future to assess the influence complete nutrient additions and essential nutrients, such as P, have on root growth.

Bicarbonate or hydroxide

High pH alone (OH-) has no significant influence on the root length of bread and durum wheat seedlings at pH 9.2. However, the addition of $NaHCO_3$ and Na_2CO_3 to the treatment solution (pH 9.2) significantly reduced the root length (~58.5%) of bread and durum wheat seedlings compared to the NaOH and the control solution at pH 8.4 (Table 5.04). The

bicarbonate ion is therefore influencing root length beyond any affects of OH^- (high pH). The large difference between the bicarbonate and hydroxide response may have be partially due to the more rapid drop in pH over the 24 hour time interval for the hydroxide treatment between adjustments, resulting in up to a 0.2 unit difference in pH. However, this small difference in pH is unlikely to account for the average 58.5% reduction for the bicarbonate treatment as compared to only 3.3% for the NaOH treatment.

In contrast to the control, the bicarbonate and hydroxide solutions both included the addition of Na^+ from Na_2CO_3 and $NaHCO_3$, or NaOH. While a reduction in root length of the treatments could be hypothesised as being associated with Na^+ toxicity, this is unlikely at the concentrations added. Furthermore, Lui and Rathjen (1998) found no deleterious effect of Na^+ when comparing 5mM $NaHCO_3$ and 5mM $KHCO_3$ treatment solutions.

Absolute root length or relative root length

Significant variation was identified in the root length of seedlings in control and bicarbonate treatments when screening bread wheat varieties, *T. dicoccoides* and populations (Table 5.05). Correlations between the treatment root lengths for the varieties and *T. dicoccoides* found that tolerance to bicarbonate was not generally associated with longer more vigorous roots. For seven out of the nine populations, however, lines with longer, more vigorous root growth were reduced by bicarbonate relatively more than lines with shorter roots. The greater damage to the lines with longer roots in the control treatment (RO) may be due to a larger root surface area being exposed to the bicarbonate ions in the bicarbonate treatment (RL). Selection of plants based on high relative root lengths in high bicarbonate solutions to improve grain yield in the field under strongly alkaline conditions may lead to poor root growth and lower grain yields when grown in the field under low bicarbonate conditions. Breeding varieties that are capable of tolerating exposure to high concentrations of bicarbonate ions with resulting higher grain yield in the field would require the use of the absolute root length measurements from the bicarbonate treatment (RL), rather than the calculation of relative root length (RO/RL*100). However, for research purposes, the use of relative root length would be beneficial to isolate the bicarbonate effect from general root vigour.

Seed Source and reproducibility

The seed source had no significant influence on the root length of seedlings in bicarbonate treatment (Table 5.06). Grain could therefore be confidently used from a number of different field sources. Seed could also be reliably used from field trials over a number of years, provided minimal grain mixing had occurred.

General Summary

Based on the above results, the hydroponic screening technique adopted to select bicarbonate tolerant lines uses 5mM $NaHCO_3$, 1mM Na_2CO_3, 5mM $CaCO_3$, 15µM H_3BO_3, 2.5µM $ZnSO_4.7H_2O$, adjusted with Na_2CO_3 to pH 9.1-9.2 daily, with root length measured after 9 or 10 days. The control treatment contains 5mM $CaCO_3$, 15µM H_3BO_3, 2.5µM $ZnSO_4.7H_2O$, and is not adjusted, retaining a pH of approximately 8.4. Seed is sourced from various field sites and from plants grown in glasshouses.

*Note: The bicarbonate solution screening methods were developed throughout the course of the research and the following chapters use various versions of this bicarbonate test.

5.3 Determining the level of tolerance to bicarbonate toxicity in commercial bread and durum wheat varieties.

5.3.1 Introduction

In South Australia breeders and researchers often observe the superiority of some varieties on highly alkaline soils over several years, notably the bread wheat *cv.* Krichauff. Bicarbonate experiments developed by Lui and Rathjen (1998) confirmed that Krichuaff had a high level of tolerance, along with Kharchia and Olympic, from measuring root length in 5mM $NaHCO_3$. The least tolerant varieties included Matong, Silverstar and the durum wheat varieties Tamaroi and Yallaroi. Further screening of bread and durum wheats identified significant genetic variation between varieties, landraces and *Triticum* species (Lui and Rathejn 1998, Das and Cooper, unpublished). Many of the South Australian bred varieties were found to have a relatively high level of bicarbonate tolerance, most likely

resulting from decades of selection for higher yielding types in the field under high bicarbonate and high pH conditions (Rathjen *et. al.* 1999).

This study aims to evaluate further commercial wheat lines to determine the level of genotypic variation amongst bread and durum wheat varieties for tolerance to bicarbonate at high pH; to assess whether tolerance to bicarbonate has contributed to the dominance of particular varieties on alkaline soils; and to identify potential sources of bicarbonate tolerance to improve currently grown varieties.

5.3.2 Materials and Methods

Bread and durum wheat seed was sourced from the Australian Winter Cereal Collection (AWWC) at Tamworth and from the Durum Breeding group at the University of Adelaide.

General methods for bicarbonate solution screening are described in section 3.3.

Experiment 1: Seeds of forty-six bread wheat varieties and twenty durum wheat varieties were placed on trays in a completely randomised design with two replications. The varieties were chosen to represent a range of successful varieties from mainly southern Australia, across several decades. The durum varieties also included international varieties, since few durum varieties were widely grown in southern Australia.

Experiment 2: Seeds of forty bread wheat varieties and the durum wheat variety Kalka were placed on trays in a completely randomised design with four replications. The varieties chosen included many of the ancestors of the most tolerant varieties identified in Experiment 1.

The bicarbonate treatment solutions contained 5mM $NaHCO_3$, 1mM Na_2CO_3, 5mM $CaCO_3$, 15µM H_3BO_3 and 2.5µM $ZnSO_4.7H_2O$, adjusted with Na_2CO_3 to pH 9.1-9.2 daily. The control treatment solution contained 5mM $CaCO_3$, 15µM H_3BO_3 and 2.5µM $ZnSO_4.7H_2O$, with pH not adjusted, retaining a pH of 8.4. Root length of the wheat seedlings was measured after 10 days in the treatment solution. The mean root length of bicarbonate and control treatments, and the relative root length were determined for each variety.

5.3.3 Results

All bread and durum wheat varieties were severely reduced (>50% in Exp. 1 and >70% in Exp. 2) by 5mM $NaHCO_3$ and 1mM Na_2CO_3. Significant variation was found to exist between varieties of both bread and durum wheat. In Experiment 1 the relative root length of the bread wheats ranged from 47.1% for Krichuaff to 20% for Tatiara (Table 5.08). The durum wheats were on average less tolerant to bicarbonate than the bread wheats with relative root lengths ranging from 41.7% for Kalka to 15.3% for Yallaroi (Table 5.09).

The mean root length of varieties in bicarbonate showed a similar pattern, with bread wheats ranging from 83.3mm for Cascades (Krichauff 2nd longest at 73.1mm) to 39.5mm for Bindawarra (Tatiara 6th shortest at 45.8mm). Similarly, the durum wheats in bicarbonate had mean root length ranging from 79.0mm for Kalka to 33.2mm for Giorgio 298 (Yallaroi 5th shortest at 44.2mm). In the control treatments the mean root lengths varied significantly, although on average the durum wheats had longer root lengths than the bread wheats.

In Experiment 2 the relative root lengths and mean root length measurements for bread wheats in bicarbonate were higher than in Experiment 1 (Table 5.10). Methods were identical between the two experiments, however, since the experiments were conducted on laboratory benches, the seedling were exposed to different temperatures, day lengths and light intensities depending on the time of year. The relative root length of the bread wheats ranged from 71.0% for Krichauff to 38.7% for T/Inia 66. The mean root lengths of the varieties in bicarbonate showed a similar pattern, with root lengths ranging from 156.1mm for Krichauff to 73.4mm for Kloka (T/Inia 66 was 93.8mm). Experiments 1 and 2 had seventeen bread wheat varieties were in common, however, while the ranking of varieties changed considerably between these experiments (reflecting a lack of range in the varieties), Krichauff and Cascades remained at the top end of the scale. Many of the other varieties, such as, Spear, Machete and Halberd were lower ranked, and Schomburgk and Cranbrook higher ranked in the second test. High error existed in the testing procedure with standard deviations between the four replications for Experiment 2 for each variety ranging from 3.7 to 24.7mm with an average of 14.2mm.

Table 5.08: Root length (mm) of bread wheat varieties grown for 10 days in bicarbonate (HCO_3^- /CO_3^{2-}) or control treatment (Control), relative root length (RRL), pedigree, year of release, and geographical origin information.

Varieity	HCO_3^-/CO_3^{2-}	Control	RRL	Pedigree	Release	Origin
Krichauff	73.17	145.67	47.14	Wariquam//Kloka/Pitic62/3/Warimek/Halberd/4/ 3A G3Aroona	1996	SA
Dollarbird	59.83	143.33	42.33	Wren/Gaboto/2/Kalyansona/Bluebird	1987	NSW
Silverstar	62.00	172.50	37.68	Pavon S/TM56	1996	VIC
Westonia	65.17	156.67	37.23	C01190-203/84W127-501	1997	WA
Cascades	83.33	193.33	35.86	Aroona*3/(AusenVII-95)Tadorna.Inia66	1994	WA
Songlen	56.00	191.67	33.39	Lerma Rojo 64/Sonora 64A/2/Timgalen	1975	NSW
Kite	48.17	135.00	33.33	Norin 10/Brevor 14/2/4*Eureka 2/3/T-A/3* Falcon/4/T-A/4*Falcon/5/T-A/5*Falcon	1973	NSW
BT-Schomburgk	61.17	158.33	32.63	Halberd/Aroona//3*Schomburgk	1992	SA
Spear	50.50	201.67	31.40	Sabre/Mec 3/2/Insignia	1984	SA
Kukri	54.50	152.00	30.92	DPR((FN-58XN10B/GB55)NA160)/(TOB-CNO S XTOB-8156/CAL X BB-CNO)/2/MDN/6*RAC177	1999	SA
Yitpi	58.67	203.33	30.66	(Chamlein*8156)*(Mengavi*Siete ceros)(Chamein* 8156)*Heron)*(Mengavi*Siete)*Frame	1999	SA
Machete	54.33	165.00	30.51	Sonora 64/2/TZPP/Yaqui 54/3/*Gabo/4/Madden	1985	SA
Camm	48.67	162.50	30.15	VMP1/5*Cook//3*Spear	1998	WA
Dagger	55.92	213.33	29.77	Sabre/Mec 3/2/Insignia	1984	SA
Babbler	51.17	171.67	29.51	Janz/Lark	2000	NSW
Worrakatta	57.83	211.67	29.29	Wariquam//Kloka/Pitic62/3/Warimek/Halberd/4/ 3A G3Aroona	1997	SA
Aroona	55.33	181.67	29.17	WW-15/Raven	1981	SA
Condor	52.67	165.00	29.09	Penjamo 62/4*Gabo 56/2/TZPP/Nainari 60/4/ 2*Lerma Rojo/2/Norin10/Brevor14/3/3*Andes	1973	NSW
Halberd	59.17	175.00	28.76	Scimitar/Kenya C 6042/Bobin/2/Insignia 49	1969	SA
Meering	49.50	180.00	28.70	Condor selection	1984	VIC
Bayonet	50.33	181.67	28.62	Pitic 'S'/Glaive	1984	SA
Wyalkatchem	54.67	170.00	28.43	Machete/W84.129*504	2001	WA
Frame	60.83	191.67	28.35	Molinuex/Dagger#3	1994	SA
Yarralinka	54.00	186.67	28.04	Mengavi/Siete Cerros/3/Mengavi/Siete Cerros// Crim/4/Combination III/2*Warigal	1994	SA
Festiguay	45.67	186.67	27.68	Festival/Uruguay C10837	1963	NSW
Janz	58.33	205.00	26.99	3AG3/4*Condor//Cook	1989	QLD
Cook	52.33	201.67	26.45	Timgalen/Condor sib//Condor	1977	QLD
Mitre	52.83	208.33	26.40	Janz/Beulah	2000	VIC
RAC655	60.75	215.00	25.58	CHA*Meng/CNO 'S' Gallo//BZ2/3/*3095	-	SA
Molineux	63.50	210.00	24.60	Pitic62/Festiquay//2*Warigal	1988	SA
Stylet	44.33	178.33	24.49	-	2003	SA
Trident	53.17	165.00	24.44	VMP1/Cook//4*Spear	1993	SA
Pugsley	49.50	190.00	24.21	Frame/Corrigin//Frame/3/Trident	2003	SA
Buckley	48.42	196.67	24.15	Pitic 62/Festiguay//Warimba/3/Mexico 120/ Quadrat/2/Kloka/Pitic 62//Bayonet/4/3*Tatiara	1997	SA
Bindawarra	39.50	150.00	24.00	Mexico 120/Koda/2/Raven	1980	SA
Cranbrook	44.50	180.00	23.89	WE/2/Ciano S/Noroeste 66/3/Zamezi	1985	WA
H45	49.83	193.33	23.62	B1814//WW-15/QT7605	1998	NSW
Schomburgk	61.67	206.67	23.06	W3589/Oxley/2/2*Warigal/3/2*Aroona	1986	SA
Anlace	51.50	211.67	22.05	Amigo/4*Tatiara	1999	SA
Lance	47.00	223.33	21.49	Collafen/Raven	1978	SA
Chara	44.00	165.00	21.41	BD225 (Cook*2/Millewa/TM56)*(Pacon S/Condor)	1999	VIC
RAC875	49.33	215.00	21.40	RAC655/(Sr21/4*Lance//4*Bayonet)	-	SA
Excalibur	41.50	183.33	20.00	RAC177(Sr26)/Uniculm492//RAC311S	1991	SA
Tatiara	45.83	205.00	20.00	Mexico 120/Koda/2/Raven/3/Mengavi/Siete Cerros	1987	SA
Durum Wheat	56.34±12.17	217.61±27.57	25.71±7.36			

l.s.d (0.05) between varieties for HCO_3^-/CO_2^{3-} = 8.32, control = 29.70, RRL = 7.59

Table 5.09: Root length (mm) of durum wheat varieties grown for 10 days in bicarbonate or control treatment, relative root length (RRL), pedigree, year of release, and geographical origin information.

Varieity	Bicarbonate	Control	RRL	Pedigree	Release	Origin
Kalka	79.00	193.33	41.72	Wollaroi*(Linghzi*Yallaroi#)*RH88009	2003	SA
Kalti 4	60.17	191.67	34.43	-	-	Mexico
(Kalka#4*Na49)/85	66.83	211.67	34.17	-	-	SA
LYTam/4	64.25	196.67	34.07	Linghzi/Yallaroi/Tamaroi/4	-	SA
Biodur	74.50	226.67	31.91	-	-	Germany
Arrivato	53.17	175.00	31.43	-	-	NZ
(Kalka#4*Na49)/95	67.33	210.00	31.11	-	-	SA
Kamillaroi	60.83	205.00	30.08	Durati/Leeds	1983	NSW
Helidur	74.83	235.00	30.07	132/Pandur/3/Pandur/Valgerardo//	-	Austria
Bellaroi	64.67	225.00	29.48		2003	NSW
Renville	61.67	220.00	27.58	Rollete/Vic	-	USA
Omruf-3	59.50	248.33	24.97	OM Rabi 5/Tu	-	Syria
Shag 26	52.50	195.00	24.96	-	-	Mexico
Gundaroi	50.50	212.50	22.59		1999	NSW
Wollaroi	51.50	225.00	20.59	Tam1B-17/Kamillaroi sib//Rokel seln/Kamillaroi sib	1993	NSW
Aconchi 89	49.50	255.00	20.52	-	-	Mexico
Kyle	42.83	250.00	19.33	Wakooma/DT 322//Wakooma/DT320	-	Canada
AC Avonlea	59.33	281.67	19.05	-	-	Canada
Tamaroi	42.67	212.50	18.20	Altar 84/4/Tam1B-17/Kamillaroi/3/Wells/56111// Guillemont	1998	SA
Altar 84	42.00	257.50	17.48	CD22344 Ruff S/FG S/2/Mexii 75/3/Shaw S	-	Mexico
Giorgio 298	33.25	170.00	16.76	-	-	Italy
Kronos	40.75	195.00	15.64	-	-	USA
Yallaroi	44.25	212.50	15.29	Guillemon 3/Kamilaroi sib	1987	NSW
Bread wheat	54.10±8.20	184.08±22.45	28.34±5.67			

l.s.d (0.05) between varieties for bicarbonate = 7.84, control = 45.66, RRL = 8.46.

No significant trends were observed between the level of bicarbonate tolerance and the year of release for the bread and durum varieties tested. The year of release ranged from Bobin in 1925 to Pugsley and Stylet in 2003, yet over this 78 year period no trend toward greater or reduced tolerance could be identified, including lines selected on alkaline soils in SA. The state of origin had little effect on the level of bicarbonate tolerance.

Common ancestral links were identified in the tolerant bread and in the durum wheat lines. Krichuaff exceed all other varieties tested for bicarbonate tolerance, but no clear source of bicarbonate tolerance could be identified in its parents, and may represent a transgressive segregant at low probability. Pitic 62 and Aroona both had moderate bicarbonate tolerance, while Halberd and Kloka also had low to moderate tolerance. The tolerance of Aroona was observed to some extent in that varieties progeny, including Cascades and Schomburgk,

Table 5.10: Root length (mm) of bread wheat varieties grown for 10 days in bicarbonate or control treatment, plus relative root length, pedigree, year of release, and origin information.

Variety	Bicarbonate	Control	RRL	Pedigree	Release	Origin
Krichauff	156.08	219.88	70.99	Wariquam//Kloka/Pitic62/3/Warimek/Halberd/4/ 3A G3Aroona	1996	SA
Cascades	144.62	212.91	67.92	Aroona*3/(Ausenvii-95)Tadorna.Inia66	1994	WA
Ciano 'S'	87.17	135.24	64.45	Pitic 62/Chris S/2/Sonora 64	-	Mexico
Combination III	104.67	164.71	63.55	Vernstein (W 3196)/Cl 12632 (W 1656)	-	AUS
Scimitar	91.13	145.56	62.61	Nabawa/Egyptian 4	1930	SA
Pitic 62	114.42	199.42	57.38	Yaktana 54/Norin 10 Brevor	-	Mexico
Noroeste 66	118.83	215.42	55.16	Lerma Rojo 64/Sonora 64	-	Mexico
Aroona	111.38	202.63	54.97	WW-15/Raven WW-15= (Lerma Rojo/2/Norin 10/Brevor 14/3/3*Andes)	1981	SA
Condor	112.58	205.21	54.86	Penjamo 62/4*Gabo 56/2/TZPP/Nainari 60/4/ 2*Lerma Rojo/2/Norin10/Brevor14/3/3*Andes	1973	NSW
Cranbrook	105.34	193.04	54.57	WE/2/Ciano S/Noroeste 66/3/Zamezi	1985	WA
Bayonet	126.71	233.54	54.26	Pitic 'S'/Glaive	1984	SA
Kloka	73.38	138.33	53.04	Seln from Weihenstephanes 43/48	-	Germany
Schomburgk	135.67	257.33	52.72	W3589/Oxley/2/2*Warigal/3/2*Aroona	1986	SA
Kalka	126.17	239.97	52.58	Wollaroi*(Linghzi*Yallaroi#)*RH88009	2003	SA
Frame	98.68	187.75	52.56	Molinuex/Dagger#3	1994	SA
Corrigin	90.00	171.25	52.55	Tincurrin*2//Gamenya/Iassul	1989	WA
Siete Cerros	110.67	210.80	52.50	Penjamo 62 Sib/Gabo 55		Mexico
Lance	124.54	239.05	52.10	Collafen/Raven	1978	SA
Pugsley	102.63	198.21	51.78	Frame/Corrigin//Frame/3/Trident	2003	SA
Oxley	103.17	200.79	51.38	Penjamo 62/4*Gabo 56/2/TZPP/Nainari 60/4/ 2*Lerma Rojo/2/Norin10/Brevor14/3/3*Andes	1974	NSW
Crim	105.54	207.04	50.98	Thatcher/3/Thatcher*3/Klein Titan/2/Kenya 58/ NewThatcher	-	USA
Insignia 49	80.96	159.18	50.86	Gabo/4*(Ghurka/Ranee)	1951	SA
Janz	106.25	209.58	50.70	3AG/4*Condor//Cook	1989	QLD
Halberd	88.96	177.96	49.99	Scimitar/Kenya C 6042/Bobin/2/Insignia 49	1969	SA
Warimek	122.04	246.65	49.48	Mexico 120/Koda 106-54		
Yarralinka	103.68	211.30	49.07	Mengavi/Siete Cerros/3/Mengavi/Siete Cerros// Crim/4/Combination III/2*Warigal	1991	SA
Warigal	97.33	202.58	48.05	WW-15/Raven	1978	SA
RAC875	103.13	219.25	47.04	RAC655/(Sr21/4*Lance//4*Bayonet)	-	SA
Machete	79.42	169.01	46.99	Sonora 64/2/TZPP/Yaqui 54/3/*Gabo/4/Madden	1985	SA
Wariquam	91.58	196.92	46.51	Mexico 120/Quadrat –14-46		
Mengavi	80.30	172.80	46.47	Eureka/Cl 122362/2/2*Gabo/3/Mentana/6*Gabo	1960	
Molinuex	93.50	201.92	46.31	Pitic62/Festiquay//2*Warigal	1988	SA
Spear	96.79	209.83	46.13	Sabre/Mec 3/2/Insignia	1984	SA
Zambezi	86.16	187.37	45.99	-	-	Zimbabwe
Kenya C6042	76.48	168.55	45.37	Introduction	-	Kenya
Festiquay	85.46	199.76	42.78	Festival/Uruguay C10837	1963	NSW
Bobin	75.82	178.83	42.40	Thew/Steinwede	1925	NSW
Cook	90.25	215.79	41.82	Timgalen/Condor sib//Condor	1977	QLD
Trident	86.25	206.34	41.80	VMP1/5*Cook//4*Spear	1993	SA
Dagger	88.67	212.54	41.72	Sabre/Mec 3/2/Insignia	1984	SA
T/Inia 66	93.79	245.15	38.26	Tadorna/India 66	-	USA

l.s.d (0.05) between varieties for bicarbonate = 14.57, control = 23.31, RRL = 9.50

while the tolerance in Pitic 62 may have also been passed on to Bayonet or Cranbrook and Songlen via Ciano 'S'. Both Aroona and Pitic 62 share two common parents, Norin 10 and Brevor 14, although Aroona is via the parent WW-15. Norin 10 and Brevor 14 were also

parents to the moderately tolerant variety Songlen, both directly and via Ciano 'S'. Tolerance to bicarbonate may have also originated from the varieties Lerma Rojo and Sonora 64, which were both parents to the variety Songlen and the moderately tolerant Noroesete 66 (Cranbrook parent). Sonora 64 was also a parent to Ciano 'S', while Lerma Rojo was a parent for WW-15 (Aroona ancestry). All moderately tolerant lines could therefore be linked to the varieties Sonora 64, Lerma Rojo, Norin 10 and Brevor 14, none of which were tested, but in view of the transgressive segregation, would be expected to include at least two separate sources of bicarbonate tolerance. Interestingly, Brevor and Norin 10 are both parental lines for Sonora 64, along with Yakatana 54, which was a parent for Pitic 62. Furthermore, Norin 10 and Brevor 14 both have a common parent Turkey Red.

Only low to moderate bicarbonate tolerance was observed in the Insignia and Condor families. Insignia, Halberd, Spear, Dagger, Trident, Frame, Yitpi and Pugsley all had relative root lengths in the middle of the range and tended to change ranking between tests. Frame, Yitpi and Pugsley have ancestral links with both Pitic 62 and Aroona via Molineux, but the tolerance observed in Pitic 62 and Aroona did not appear to be passed onto Molineux, since Molineux had very poor root growth in the bicarbonate treatment. Condor and the Condor family (Cook, Janz) also had ancestral links to Aroona via the WW-15 line, although only low to moderate tolerance was observed in their progeny.

The origins of bicarbonate tolerance in the durum varieties was difficult to ascertain due to the lack of pedigree information and restricted testing of ancestral lines. The tolerance of Kalka does not appear to have originated via Yallaroi or Wollaroi, but from the boron tolerant line Linghzi or possibly RH88009 (Yallaroi//Tam 1B-17/Kamillaroi sib). The line Linghzi is also likely responsible for the low to moderate tolerance in the breeder's line LYTam/4, since Yallaroi and Tamaroi have very low tolerance to bicarbonate. The tolerance in Kalka appears to be due to multiple genes since both of the derived backcross lines, (Kalka#4*Na49)/85 and /95 did not reach the tolerance level of Kalka.

5.3.4 Discussion

The screening of bread and durum wheat seedlings for bicarbonate tolerance identified significant variation in root length and relative root length. A difference of up to 30% in

relative root length between the commercial bread wheat varieties indicates a level of genetic variation for bicarbonate tolerance that could be exploited in the breeding of more bicarbonate tolerant varieties. The severe reduction in root length at pH 9.2 with 5mM $NaHCO_3$, 1mM Na_2CO_3 and 5mM $CaCO_3$, compared to pH 8.4 with 5mM $CaCO_3$ indicates a major potential effect of bicarbonate toxicity under highly alkaline field conditions, with most bread and durum wheat varieties showing only moderate levels of tolerance as best.

The difference in results between Experiment 1 and Experiment 2 were associated with a higher growth rate in Experiment 2 from differing laboratory conditions during that test. The increased root growth was observed in both the control and bicarbonate treatments, although this did not explain the inconsistencies between varieties observed in the relative root lengths. On closer inspection of the varieties in common between experiments it was found that the increase in root length from Experiment 1 to 2 was minor for the control treatment but almost double for the bicarbonate treatment. The differences were therefore likely due to the use of different batches of stock $NaHCO_3$ and Na_2CO_3 solutions, since only pH in the tank was measured and not the actual concentrations of $NaHCO_3$ and Na_2CO_3 throughout the experiment. To ensure consistency between experiments, without measuring actual $NaHCO_3$ and Na_2CO_3 concentrations, indicator varieties would need to be used as a basis for comparison.

A greater concern was the change in ranking of varieties for bicarbonate tolerance between the two experiments. The moderately tolerant and low tolerance lines remained in similar positions, however, many of the low-moderate lines changed positions. An increase in the number of replications from two to four may have resulted in increased accuracy in Experiment 2. The difference between the two experiments in root length, relative root length and rankings, and the high standard deviations between replications, highlights the sensitivity of wheat lines to the bicarbonate treatment and the need to retain consistency between aeration, light and temperature across the experimental trays and tanks, and for a high number of replications to be included with standard varieties of known levels of bicarbonate tolerance.

In the bread wheat varieties the South Australian line Krichauff was identified as the most tolerant variety, as previously found by Lui and Rathjen (1998). Of the durum wheat

varieties, Kalka, also a South Australian bred line, was found to be the most tolerant, although it had significantly shorter roots than Krichauff. The general superiority of the commercial bread wheat varieties over the durum varieties is likely due to the affect of the D-genome, but may also be associated with bread wheats longer breeding history in southern Australia, although no solid evidence was found to support the theory, since the year of release had no relationship to the level of bicarbonate tolerance. The area under which the selection was performed, however, may have had some influence on the difference observed between the bread and durum varieties, particularly in reference to the Australian varieties. The durum varieties have mainly originated from and been bred to suit deep, heavy soils, such as northern New South Wales, with a lower alkaline selection pressure. The lack of alkaline selection pressure may also be reflected in durum wheats higher incidence of trace element deficiency and high Na uptake, problems also commonly associated with alkaline soils. The bread wheat lines, however, mainly represent lines that have been bred and released in southern Australia on alkaline soils.

Moderate bicarbonate tolerance was identified in wheat varieties from South Australia, Western Australia, New South Wales and Victoria, but this was not generally associated with the dominance of any of the varieties. In South Australia varieties such as Insignia, Halberd, Condor, Kite, Warigal, Spear, Machete, Frame and Yitpi have dominated wheat production throughout the last 50 years, but measured only low to moderate levels of bicarbonate tolerance. The Condor and Janz group were especially suited to the red brown earth (Chromosol) type soils, while the Insignia, Spear, Frame group were widely grown in the Murray Mallee area (Calcarosol). The selection of Krichauff was influenced by breeding work at Rudall on the Eyre Peninsula on high $CaCO_3$ soil, where a high level of bicarbonate tolerance would have been advantageous. However, the adoption of Krichauff into the farming system was restricted due to a poor quality classification. Both the lack of improvement in the level of bicarbonate tolerance in the last half century and the dominance of varieties with low to moderate levels of tolerance may be a result of bicarbonate toxicity only representing a major selection factor under some environmental conditions (e.g. subsoil waterlogging).

The moderate level of tolerance to bicarbonate found in Australian bread wheat lines appears genetically complex with several genes of minor effect originating from all families. Common Australian varieties with the highest relative root length, such as,

Krichauff, Cascades, Songlen, Condor, Cranbrook, Bayonet and Schomburgk could be traced back to the moderately tolerant lines Aroona (WW-15*Raven), Pitic 62 and Noroeste 66. These three lines shared similar parentage, including the Mexican line Lerma Rojo and the related lines Norin 10 and Brevor 14, originating from Japan and the United States of America, respectively. The level of bicarbonate tolerance was not assessed in these latter lines, and it is unknown if one or more sources of tolerance have provided the tolerance observed in many of Australia's wheat varieties. The superiority of Krichauff above the parental lines, Aroona, Pitic 62, Halberd, Kloka, Warimek and Wariquam suggests the presence of several genes and transgressive segregation.

Almost all Australian durum wheat lines have very low tolerance to bicarbonate. Kalka appears to be the exception, although it only reaches a level of moderate bicarbonate tolerance. From the minimal pedigree information available, several genes of minor effect currently explain the available data. Further bicarbonate testing of ancestral lines would be required for any conclusions to be reached on the source of tolerance. Kalka represents the first durum variety both bred and selected for under South Australian conditions in medium to low rainfall environments with mainly alkaline soils. For bicarbonate tolerance Kalka far exceeds other durum varieties grown in South Australia, such as Tamaroi and Yallaroi. Tolerance to bicarbonate and alkaline soils is almost certainly a significant factor controlling the general adaptation of durum varieties to South Australia. The continued prevalence of Tamaroi, however, suggest that bicarbonate tolerance is only one factor in a suite of problems, such as, crown rot, heat induced sterility, boron and salinity, which influence grain yield and adoption of varieties in local areas of South Australia.

The potential for improving the level of bicarbonate tolerance for South Australian adapted varieties seems limited. While significant genetic variation was identified in both bread and durum wheats, no lines were found to exceed either Krichauff or Kalka and no major alternative source of tolerance to bicarbonate could be identified in ancestral varieties. However, transgressive segregation between some lines may potentially increase the level of bicarbonate tolerance. The identification of tolerance originating from many of the older introduced varieties, suggests that international germplasm collections may provide further levels of tolerance.

5.4 Identifying tolerance to bicarbonate toxicity in durum landraces and advanced durum breeding material.

5.4.1 Introduction

Durum wheat is generally poorly adaptated to calcareous, sodic-alkaline, and siliceous sand soils, producing grain yields well below bread wheat in many areas in South Australia (Table 4.02, 4.07, and 4.11). The poor adaptation to alkaline soils in Australia's durum wheat lines is likely associated with short breeding history for durum in South Australia and the use of genetic material from northern NSW, where tolerance to alkaline soils was not a selection pressure. Currently, durum production, as a high value alternative to bread wheat, is mainly restricted to the higher rainfall areas of the northern Mt Lofty Ranges. For the durum industry to expand, the level of production and reliability of annual tonnage needs to be improved to cater for the expected local domestic requirements (>200k t) and provide a stable export market. To reach these goals in South Australia production needs to increase in traditional growing areas, but also expand into new production areas, such as the low rainfall, highly alkaline areas.

The durum variety Kalka, a recently released (2003) durum variety in South Australia, has been identified as having the greatest tolerance to bicarbonate of Australia's durum varieties. However, Kalka falls well below the most tolerant bread wheat varieties, and its cultivation remains restricted to higher rainfall areas. To improve further the tolerance to durum wheat to alkaline soils and to increase the feasibility of production in lower rainfall areas, other sources of bicarbonate tolerance needed to be identified, which equal or surpass the level of tolerance in bread wheat.

In previous bicarbonate screens of durum wheat at the Waite Campus by Lui, Das and Cooper (pers.comm.) a number of landrace lines, including Palestine7 (AUS5174), Karpaz (AUS8330), Menshia54, sourced from the AWCC were identified as having greater tolerance that the current durum varieties. These lines were subsequently used as parents in the Durum breeding program, University of Adelaide, with advanced lines from Karpaz currently in multiplication for potential release. Furthermore, Ma et al (2003) and Hewitt (pers. comm.) selected durum lines, both advanced lines and landraces, on the basis of root growth at high pH (bicarbonate) and low pH for high Al, with some of these lines expected

to have increased tolerance to bicarbonate toxicity through selection for the maintenance of root growth in high stress conditions under field conditions. Minimal bicarbonate testing of these lines followed the initial screening, but many lines were advanced through the Durum breeding program on the basis of high grain yield across several trial sites. The re-testing of these advanced lines may define a source of bicarbonate tolerance in relatively adapted lines that can be readily used as a parental varieties, either in backcrossing or progeny breeding.

Landrace lines from international collections have often been used for the sourcing of genetic material that may not currently be available within the Australian germplasm pool. The Australian Winter Cereal Collection in Tamworth was a source of landrace lines, storing thousands of *Triticum* accessions from throughout the world. With little information on the regions most likely to provide a high level of bicarbonate tolerance, the aim was to select a broad range of landraces from different areas, which may have evolved on alkaline soils. The purpose was to determine if a greater level of tolerance could be identified above that of the commercial durum varieties and advanced breeding lines that could be used to improve the level of bicarbonate tolerance currently in the Durum breeding germplasm.

5.4.2 Materials and Methods

Experiment 1: Advanced breeding lines

Durum wheat seed was sourced from 2004 glasshouse grown material from the Durum breeding group at the University of Adelaide. The lines in tests A and B had previously been selected for tolerance to aluminium under low pH conditions. Test C included advanced lines originating from crosses between bicarbonate tolerant landraces (Palestine7, Karpaz and Menshia 54) and Kalka types (LY) that had undergone yield selection. The standard lines, Krichauff, Frame, Tamaroi and Yallaroi, were used as check varieties across the trays.

The bicarbonate treatment solutions contained 5mM $NaHCO_3$, 1mM Na_2CO_3, 5mM $CaCO_3$, 15µM H_3BO_3 and 2.5µM $ZnSO_4.7H_2O$ adjusted with Na_2CO_3 to pH 9.1-9.2 daily. The control treatment solution contained 5mM $CaCO_3$, 15µM H_3BO_3 and 2.5µM

$ZnSO_4.7H_2O$, with pH not adjusted, retaining a pH of 8.4. Root length of seedlings was measured after 10 days in the treatment solution. The mean root length of bicarbonate and control treatments, and the relative root length were determined for each variety.

Test A: Seeds of thirty F_6 or F_7 durum wheat lines and four standard varieties were placed on trays in a completely randomised design with two replications.

Test B: Seeds of fifteen F_5 or F_6 durum wheat lines and four standard varieties were placed on trays in a completely randomised design with two replications.

Test C: Seeds of thirteen F_7 durum wheat lines and five standard varieties were placed on trays in a completely randomised design with two replications.

Experiment 2: Landrace lines

Test A: Bread and durum wheat seed was sourced from the Australian Winter Cereal Collection (AWCC) at Tamworth and from the Durum Breeding group at the University of Adelaide from field trials in 2003, to include several geographical areas. Seeds of 484 durum wheat landraces and 12 durum and bread wheat varieties were placed on trays in a completely randomised design with two replications.

Test B: *T.dicoccoides* seed was sourced from Dr Yusef Genc, University of Adelaide from 2005 glasshouse grown material. Seeds of twenty-four *T. dicoccoides* lines and five bread and durum wheat varieties were placed on trays in a completely randomised design with two replications.

5.4.3 Results

Experiment 1: Advanced breeding lines

The advanced durum lines in Test A and B originated from lines selected from aluminium hydroponic screens by Hewitt (*pers. comm*.) at low pH. In test A the mean root lengths ranged from 97.5mm to 27.2mm after 10 days in bicarbonate solution (Table 5.11). No advanced durum lines were identified with a level of bicarbonate tolerance greater than the bread wheat variety Krichuaff, although most were more tolerant than the dominant South

Table 5.11: Test A - Root length of advanced durum lines (F_6 or F_7) originating from lines selected from aluminium hydroponic screens by Hewitt (pers. comm.) at low pH after, grown in bicarbonate solution at pH 9.2 for 10 days.

Advance durum line	Root Length (mm)	St.Dev
(Bezbazak*LY#Tm)*Na49LY#4/1	97.50	10.2
(R622SLYTmLY*LY)*Na49LY#4/1	88.88	10.5
(LYTmNa49LY#4/1)*LY#Tm	88.17	12.8
(LYTm*LY#Tm)*Na49LY#4/1	85.88	10.7
(LYTm*LY)Na49LY#4/1	82.75	15.0
(LYTm*LY)*LY#Tm	80.25	20.5
(T#LYLY#WY#3K*LY)*Na49LY#4/1)	78.75	13.4
(T#LYLY#WY#3K*Na49LY#4/1)*Na49LY#4/1	77.83	5.2
(Bezbazak*LY)*LY#Tm	75.79	12.3
(T#LYLY#WY#3K*Na49LY#4/1)/6/2	74.75	8.9
(LYTm*LY#Tm)*LY#Tm	73.50	8.0
(Bezbazak*LY)*Na49LY#4/1	70.75	9.8
(Bezbazak*LY#Tm)/10/2	70.67	15.3
(Bezbazak*LY#Tm)*LY#Tm	69.88	12.5
(LYTm*LY#Tm)3/3	69.38	13.7
(R622SLYTmLY*LY)*LY#Tm	68.63	10.5
(R622SLYTmLY*LY)/1/1	68.00	11.7
(T#LYLY#WY#3K*LY)*LY#Tm)	68.00	15.8
(Menemen*LY#Tm)*LY#Tm	67.79	11.4
(Menemen*LY#Tm)*Na49LY#4/1	67.38	19.2
(R622SLYTmLY*LY)/1/3	66.75	10.1
(T#LYLY#WY#3K*Na49LY#4/1)/6/1	65.13	10.7
(LYTm*LY)/2/1	63.17	10.3
(R622SLYTmLY*LY)/1/2	63.00	9.4
(AUS11478*LY)/12/2	57.92	10.3
(T#LYLY#WY#3K*Na49LY#4/1)*LY#Tm	55.38	3.3
(R622SLYTmLY*LY)/1/5	54.58	6.7
(T#LYLY#WY#3K*Na49LY#4/1)/6/3	50.00	11.5
(AUS11478*LY#Tm)/11/3	46.75	6.3
(Bezbazak*LY)/9/1	27.25	8.2
Krichauff	100.54	15.7
Frame	67.38	11.6
Tamaroi	48.71	7.4
Yallaroi	40.79	13.2

l.s.d (0.05) between varieties = 12.52

Australian durum variety Tamaroi. Kalka was not included in this test, however, as previous variety tests found that Kalka ranks between Frame and Krichauff, and it would therefore be assumed that the longest mean root lengths for the advanced lines measured in test A would exceed Kalka.

In Test B the mean root lengths ranged from 138.0mm to 80.6mm after 10 days in bicarbonate solution (Table 5.12). Five advanced durum lines were identified with a level of bicarbonate tolerance greater than the bread wheat variety Krichuaff and all were more tolerant than the durum variety Tamaroi. These lines were different selections of Kalka (WLYY9) and Tamaroi (Tm) crossed with the bread wheats R622Sh (RAC655/Schomburgk) or C8MMDYk (Siete Cerros, Mengavi derivative/Yarralinka). Since some of these lines had a level of bicarbonate tolerance greater that Krichauff, it would be expected that the bread wheats were providing additional sources of tolerance above that of the durum varieties Kalka and Tamaroi.

Table 5.12: Test B - Root length of advanced durum lines (F_5 or F_6) originating from lines selected from aluminium hydroponic screens by Hewitt (pers. comm.) at low pH after, grown in bicarbonate solution at pH 9.2 for 10 days.

Advance durum line	Root Length (mm)	St.Dev
(R622Sh*Tm)*WLYY9Tm2/4/5	138.00	15.0
(R622Sh*Tm)*WLYY9Tm2/4/4	135.67	10.9
(C8MMDk*BTWLYY9)Tm/2/2	132.44	11.4
(C8MMDk*BTWLYY9)Tm/3/2	121.78	14.1
(R622Sh*Tm)*WLYY9Tm2/4/1	121.50	20.3
(R875a*R1)*WLYY9Tma/1/2	117.89	19.4
(R622Sh*Tm)*WLYY9Tm2/4/3	117.33	15.2
(C8MMDk*BTWLYY9)Tm/3/4	107.33	18.5
(R875a*R1)*WLYY9Tma/1/3	102.56	8.5
(R875a*R1)*WLYY9Tma/2/2	97.44	13.4
(C8MMDk*BTWLYY9)Tm/2/1	95.44	13.1
(C8MMDk*R1)*WLYY9Tm2/1/1	91.17	7.7
(C8MMDk*R1)*WLYY9Tm2/4/2	88.44	16.5
(C8MMDk*R1)*WLYY9Tm2/2/3	80.61	8.6
Krichauff	120.78	14.0
Frame	85.78	7.2
Tamaroi	70.00	13.7
Yallaroi	83.22	15.1

l.s.d (0.05) between varieties = 17.04

The advanced durum lines in Test C originated from lines selected from bicarbonate hydroponic screens by Das and Cooper (*pers. comm*.) at high pH. In test C the relative root lengths ranged from 87.7mm to 58.4mm after 10 days in bicarbonate solution (Table 5.13). All the selections tested were more tolerant than the durum variety Tamaroi and half of the lines were more tolerant than Kalka, indicating improvements had been made in the durum breeding material for bicarbonate tolerance. The advanced lines were durum wheat landraces Pal7 (Palestine 7), Kpz (Karpaz) and M54 (Menshia54) backcrossed to a Kalka type (LY). Four of the lines also had longer roots than the bread wheat variety Krichuaff.

Table 5.13: Test C – Mean bicarbonate treatment root length (pH 9.2), mean control treatment root length (pH 8.4) and relative root length (RRL) of advanced durum lines (F_7) originating from lines selected from bicarbonate hydroponic screens at pH 8.8, after 10 days.

	Root Length (mm)		
Advance durum line	Bicarbonate	Control	RRL
(Pal7 * Ly#)/2/2	93.00	106.00	87.74
(Kpz * Ly#)/1/3/4	80.00	94.33	84.81
(m54 * Ly#)/6/2	105.25	130.08	80.91
(Kpz * Ly#)/1/3/1	143.50	179.58	79.91
(Iraq * Ly#)/1/2/1	128.00	169.92	75.33
(m54 * Ly#)/6/3	90.58	128.25	70.63
(Iraq * Ly#)/1/2/4	106.67	156.17	68.30
(m54 * Ly#)/6/1	104.50	156.00	66.90
(Iraq * Ly#)/1/2/2	105.50	158.00	66.77
(Kpz * Ly#)/1/3/3	64.50	96.75	66.67
(m54 * Ly#)/6/4	115.50	173.83	66.44
(Iraq * Ly#)/1/2/3	121.33	189.00	64.20
(Pal7 * Ly#)/2/3	80.83	138.33	58.53
Krichauff	125.00	159.92	78.17
Frame	77.33	186.83	41.39
Kalka	115.92	161.33	71.85
Tamaroi	74.83	181.33	41.27
Yallaroi	59.00	147.50	40.00

l.s.d (0.05) between lines for Bicarbonate = 16.18, Control = 58.72, RRL = 33.16

Experiment 2: Landrace lines

The mean root length was measured for 484 durum landraces from 30 countries mainly in west and south Asia, Europe and North Africa. Significant variation was identified in the durum landraces, which ranged from 44.3mm to 125.7mm (Appendix 4). The majority of landraces (88%) had mean root lengths that fell between the range of 99 to 55mm in the moderate to moderately intolerant category, with similar lengths to most of the standards (Table 5.14). Thirty-nine landraces (8.1%) were found to be equal or had longer roots than the durum variety Kalka, while thirteen landraces (2.7%) were found to be equal or had longer roots than the bread wheat variety Krichauff. The landrace line AUS9893 had the greatest mean root length with a measurement of 171.2mm, but subsequent chromosome staining identified AUS9893 as hexaploid wheat with 21 chromosome pairs.

Table 5.14: Test A - Distribution of tolerance to bicarbonate based on the mean root length across geographical regions or countries, including Afganistan (Afg), Pakistan (Pak), North Africa (Afr), Spain (Spa), Eastern Europe (E Eur), Turkey (Tur), Azerbaijan (Aze), Palestine (Pal), Lebanon (Leb), Ethiopia (Eth), Former Soviet Union (FSU), Turkmenistan (Turkm), India (Ind), Iran, Iraq and Syria.

	Root Length (mm)						
	>115	114-100	99-85	84-70	69-55	<54	Total
Afg, Pak	3	5	7	16	10	0	41
Afr, Spa	0	1	19	32	14	4	71
E Eur	2	3	6	16	12	0	39
Tur, Aze	1	4	14	33	15	4	71
Pal, Leb	0	1	9	17	7	1	35
Eth	0	0	4	12	9	4	29
FSU, Turkm	0	3	15	7	4	0	29
Ind	2	2	16	18	6	3	47
Iran	2	4	7	17	9	1	40
Iraq	3	3	8	21	3	0	38
Syria	0	0	8	29	5	2	44
TOTAL	13	26	113	218	94	19	484
Varieties	Krichauff	Kalka	Frame Janz Kukri	Excalibur Stylet Tamaroi Wollaroi Yallaroi	Machete Gunderoi		

The thirty countries were divided into eleven geographical regions for comparison (Figure 5.03). Mean root lengths in the high range of >115 were mainly from Afganistan (Afg), Iran, Iraq and India (Ind), with two also identified from material originating from Greece (E Eur) and Turkey (Tur). India, Former Soviet Union (FSU), Turkmenistan (Turkm) and Kazakhstan (Kaz) had a high proportion in the moderate to high range, while Ethiopia (Eth) and Syria had a large proportion of landraces in the lower ranges.

The relative root length of *T. dicoccoides* lines were found to vary from 52.8% to 36.1%, with only half the lines equal or longer than the durum variety Tamaroi, and no lines more tolerant than Kalka (Table 5.15). *T. dicoccoides* (AB) were ancestors of the pasta wheat *T. turgidum ssp.* durum (AB), but appear not to have the level of adaptation of the more modern cultivated durum species, offering little potential for use in improving bicarbonate tolerance in current durum varieties.

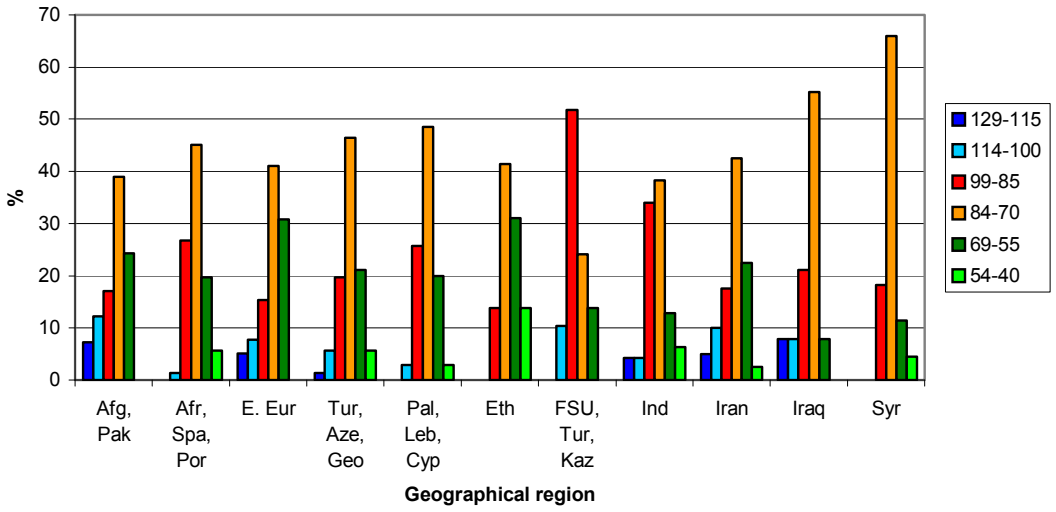


Figure 5.03: The percentage of landraces in each of the six categories of root length under bicarbonate treatment for the eleven geographical areas, which included Afganistan (Afg), Pakistan (Pak), North Africa (Afr), Spain (Spa), Portugal (Por), Eastern Europe (E Eur), Turkey (Tur), Azerbaigan (Aze), Georgia (Geo), Palestine (Pal), Lebanon (Leb), Cyprus (Cyp), Ethiopia (Eth), Former Soviet Union (FSU), Turkmenistan (Turkm), Kazakhstan (Kaz), India (Ind), Iran, Iraq and Syria (Syr).

Table 5.15: Test B – Mean bicarbonate treatment root length (pH 9.2), mean control treatment root length (pH 8.4) and relative root length (RRL) of *T. dicoccoides* after 10 days in treatment solution.

	Root Length (mm)		
AUS no.	Bicarbonate	Control	RRL
15826	101.83	192.67	52.85
3717	95.83	186.50	51.39
3734	84.33	172.00	49.03
10559	92.67	192.67	48.10
22293	92.17	192.17	47.96
17643	73.83	157.50	46.88
3804	80.17	171.50	46.74
22290	87.83	189.83	46.27
3735	78.83	178.83	44.08
17641	89.17	205.17	43.46
3731	71.17	164.67	43.22
19385	81.17	193.17	42.02
11491	75.67	182.17	41.54
17968	65.83	160.50	41.02
22297	76.75	188.83	40.64
22291	76.33	192.83	39.59
22296	85.33	215.83	39.54
21758	68.17	178.00	38.30
19592	71.00	187.67	37.83
22292	70.67	187.00	37.79
22286	75.17	198.92	37.79
3738	78.33	211.33	37.07
3740	64.00	174.67	36.64
22287	61.50	170.17	36.14
Chacan	78.00	175.75	44.38
Lagost	47.00	135.83	34.60
Krichauff	125.00	159.92	78.17
Frame	77.33	186.83	41.39
Kalka	115.92	161.33	71.85
Tamaroi	74.83	181.33	41.27
Yallaroi	59.00	147.50	40.00

l.s.d (0.05) between varieties for Bicarbonate=18.44, Control= 27.36, RRL=9.94.

5.4.4 Discussion

Experiment 1: Advanced breeding lines

In Test A no advance durum lines were identified that surpassed the bread wheat variety Krichauff, although some of the more tolerant lines would have been around the level of tolerance generally observed in the durum variety Kalka. The top five lines have parentage that includes the B tolerant durum landrace Lingzhi (L), the Na excluding durum genotype (Na49), and the durum varieties Yallaroi (Y) and Tamaroi (Tm). These lines are closely related to each other and to Kalka (WLYY9) and similar sources of tolerance to bicarbonate may be present. The highest line also descended from the durum landrace Bezbazak, which may contain an alternate source of bicarbonate tolerance, although it was also a parent in some of the poor performing crosses. Similarly, the bread wheat R622S (RAC622/Schomburgk) may have provided an alternate source of tolerance from the LY types.

In Test B, the durum derived from the bread wheat line R622Sh crossed with Kalka and Tamaroi also provided a level of bicarbonate tolerance greater than Kalka, and also greater than the bread wheat variety Krichauff. Similarly, the bread wheat parent C8MMDk in the durum wheat lines (Table 5.12) provided a level of tolerance greater than both Kalka and Krichuaff. The level of bicarbonate tolerance in the R622Sh and C8MMDk parents is unknown and therefore it cannot be determined if these advanced lines represent transgressive segregation between the bread wheat parents and Kalka, or if the lines are equal to the bread wheat parents and the bread and durum wheats have similar genetics for tolerance. Previously it had been stated that the superiority of bread wheat over durum wheat was probably associated with the D-genome, however the tolerance from bread wheat here is likely coming from the A and B genomes, as the likelihood of D-genome transformations is low. The ability to improve the tolerance of durum wheats (AB) from the transfer of genetic material from bread wheat lines (ABD) offers the potential for tolerant bread wheats, such as Krichauff, to be used for the improvement of bicarbonate tolerance in durum wheats.

The initial selection of the advanced lines in Test A and B for aluminium tolerance at low pH appears to have also unknowing led the selection of more bicarbonate tolerant lines.

The relationship between greater tolerance to Al at low pH and higher tolerance to bicarbonate toxicity at high pH in the lines tested may be associated with similar root morphological or physiological characteristics. In both tests the wheat roots were measured as average root length in bicarbonate solution and not relative root length, which may have resulted in different root vigour influencing the results. Alternatively, tolerance to both Al and bicarbonate may be a general tolerance response of wheat roots to toxic concentrations of ions (i.e. malic acid secretion at the root tip). The most likely explanation is that following testing and selection of Al tolerance at low pH, the lines were progressed through the Waite Durum breeding program on the basis of high grain yield under mainly alkaline field conditions with secondary selection for bicarbonate tolerance in high pH soils.

In Test C the parental landrace lines were specifically selected for on the basis of root length under bicarbonate toxicity. These lines were subsequently backcrossed to the moderately tolerant Kalka type line and progressed through the Waite Durum Breeding program on the basis of selection for high grain yield in the field. The improved tolerance achieved in the backcross lines in comparison to Kalka and Krichauff imply that tolerance to bicarbonate can be improved through crosses with more tolerant durum landraces and that tolerance to bicarbonate in durums can be improved beyond the levels observed in the most tolerant commercial wheat varieties. Furthermore, the retention of a proportion of the bicarbonate tolerant lines in the Durum Breeding program on the merits of grain yield with no further selection for bicarbonate tolerance indicates that bicarbonate tolerance may be a significant adaptational character for field selection.

Experiment 2: Landrace lines

In sourcing landraces for further identification of genes for bicarbonate tolerance many were selected from the west Asia region in an attempt to find other lines with similar or greater tolerance than Palestine 7, Karpaz and Menshia 54. Landraces were also selected from material originating from the traditional durum growing areas of southern and eastern Europe, and north Africa. Measurements of root length from the different geological regions identified a wide range of tolerance levels in the landraces. Comparison between standard commercial durum and bread wheat varieties indicated that the median root length

value of the durum landraces was at the Tamaroi level and a proportion (8%) of lines exceeded Kalka to reach root lengths equal to or longer than the bread wheat Krichauff.

The tolerant bicarbonate types mostly originated from the central Asia region of Iran, Afganistan, Iraq and India, and to a lesser extent countries of the Former Soviet Union, such as, Kazakhstan and Turkmenistan. The diverse range of geographical and climatic conditions from mountain ranges or plateaus to valleys or lowlands with arid to subtropical climates, combined with a lack of specific collection locations prevents associations being made with certain soil types. However, previous landrace selection for both boron and salinity tolerance in landrace collections has identified the Asia regions of India and Iraq as sources of tolerant germplasm (Moody *et. al.* 1988, Paull *et. al.* 1992). The combination of boron, salinity and alkalinity and similarities between South Australian and the semi-arid regions of west and south Asia, makes the region a potential target for sourcing adaptational characters that may be useful in South Australian durum breeding programs.

The higher level of tolerance identified in several of the durum landraces compared to the current commercial durum lines, provides additional germplasm to improve the tolerance of durum varieties to be equal or greater than Krichauff. Previously the crossing and selection of durum lines with both bread wheats (R622Sh and C8MMDYk) and the moderately tolerant landraces (Pal7, Kzp and M54) had led to a significant improvement over the recurrent parent Kalka (or Kalka derivative 'LY'), implying bicarbonate tolerance was heritable. The multiple sources of bicarbonate tolerance in bread wheat lines, durum landraces and possibly bread wheat landraces (AUS9893) needs further evaluation to determine the mode of inheritance, presence of major genes and mechanisms of tolerance to identify how to best utilise the germplasm in a breeding program.

Chapter 6

Genetic evaluation of bicarbonate tolerance in durum and bread wheat populations.

6.1 Introduction

Large variation exists between genotypes in response to alkaline soils for lupins (Tang *et. al.* 1996), rice, rye (Hajiboland *et. al.* 2003), soybean (Coulombe *et. al.* 1984), and wheat (Lui and Rathjen 1998). However, no genetic studies have been conducted in wheat or any other crop to determine the mode of inheritance and number of genes involved, except in soybeans, were tolerance was related to a single recessive gene and many additive genes (Coulombe *et. al.* 1984). In many species the genetic mechanisms of bicarbonate tolerance are difficult to isolate due to numerous interacting factors involved in the bicarbonate response and no direct assessment of the level of bicarbonate toxicity. The response to bicarbonate toxicity in most species has generally been measured as a reduction in root or shoot growth, which may be confounded by other genetic mechanisms, such as root vigour, root morphology, Zn or Fe efficiency (Hajiboland *et. al.* 2003).

The current screening method for bicarbonate tolerance only assesses root length in comparison to a mildly alkaline calcareous control, with minimal nutrients. The bicarbonate tolerance response recorded may therefore involve a number of tolerance mechanisms either directly (HCO_3^- toxicity) or indirectly (Zn or Fe efficiency). However, under field conditions it is expected that numerous interacting factors would be responsible for tolerance to alkaline soils, so the effects of OH^- toxicity and nutrient deficiency have been minimised in the methods of bicarbonate screening, but not removed entirely.

The aims here are to develop a suitable durum mapping population for genetic studies of bicarbonate tolerance, conduct an initial assessment of the genetic variation in the durum populations, establish a set of isogenic durum lines with differing levels of bicarbonate tolerance for field testing, and to evaluate existing bread and durum wheat populations for variation to bicarbonate toxicity to determine the number of genes involved and their mode of inheritance for the bicarbonate tolerance response.

6.2 Evaluation of F_2 seedlings from crosses between durum landraces, advanced lines and commercial varieties.

6.2.1 Introduction

The poor adaptation of durum wheats to South Australia's alkaline soils has confined current production to the higher rainfall, red-brown earths of the Northern Mt Lofty ranges. Durum wheats have previously been identified as having significantly lower tolerance to bicarbonate toxicity than bread wheats and it has been hypothesised that a lack of bicarbonate tolerance is partly responsible for the lack of adaptation across South Australia. To increase production, durum wheats need to be competitive with bread wheats, through increased yields and reliability, particularly in more arid areas of the Murray Mallee, Yorke Peninsula and Eyre Peninsula. Improving bicarbonate tolerance in durum wheats to a level equal or greater than currently available commercial bread wheats may be a step towards achieving greater production in alkaline areas.

Significant variation has been identified in commercial varieties, advanced breeding lines and durum landraces for root length in bicarbonate treatments (Chapter 5). The aim here is to use this variation to develop a suitable durum mapping population for both field and genetic studies of bicarbonate tolerance and conduct an initial evaluation on the genetic variation for bicarbonate tolerance in the F_2s of the durum wheat populations.

6.2.2 Materials and Methods

Three landraces, three advanced durum lines and two commercial durum lines were used in eight crosses;

1. AUS4897 x Tamaroi
2. AUS4897 x Kalka
3. AUS4897 x Na49/Kalka#4
4. AUS7890 x Tamaroi
5. AUS7890 x Na49/Kalka#4
6. R622Sh/Tm//WLYY9///Tm x Na49/Kalka#4
7. C8MMDYk/BTWLYY9//Tm x Na49/Kalka#4
8. Na49/Kalka#4 x Tamaroi

The landrace and advanced lines were originally selected for use in population development because of their long root length when grown in bicarbonate solution. These lines had a mean root length at least 50% longer than Tamaroi (Table 6.01)

Table 6.01: Mean root length (mm), percent root length longer that Tamaroi, and description of the seven parental lines used to develop durum populations.

Line	Root Length (mm)	%>Tam	Description
AUS4897	125.67	77.78	Landrace – Iraq
AUS7890	122.57	73.39	Landrace – Iran
Tamaroi	70.69	-	NSW bred, SA selected
Kalka	122.72	74.10	WLYY9, SA bred
Na49/Kalka#4	111.53	57.78	Na excluding backcross derivative of Kalka
R622Sh/Tm//WLYY9///Tm	138.00	95.22	Durum line with BW parent (R622Sh)
C8MMDYk/BTWLYY9//Tm	134.67	90.51	Durum line with BW parent (C8MMDYk)

Seed for parental lines was sourced from the Waite Durum breeding program from glasshouse grown seed. The landrace lines had previously been multiplied over two generations prior to bicarbonate testing and use as parentals. No bicarbonate testing was performed on the individual plants before undergoing crossing. Plants were grown in 12" black plastic pots (7 plants/pot) in Palmer sand in a glasshouse. Pots were fertilised with 10g granulated Nitrophoska® as required. Parental lines were crossed in October 2003 and harvested at maturity. F_1 seeds (5 –10 seeds) were replanted in January 2004 and bulk harvested within three months at maturity.

Poor grain set resulting from high temperatures produced few F_1 and F_2 seeds. All F_1 seed was replanted to produce F_2 seed, with approximately 100 to 180 F_2 seeds obtained from each of the durum crosses. The F_2 seed was re-sown to provide 100 to 120 F_2 derived F_3 families. The remainder of the F_2 seed was bicarbonate tested.

Two treatments were used (bicarbonate and control) with 18 to 45 F_2 and 30 to 24 parental seeds screened for six of the durum crosses. The bicarbonate screening method followed the procedures outlined in section 3.3. The bicarbonate treatment contained 5mM $NaHCO_3$, 1mM Na_2CO_3, 5mM $CaCO_3$, 15µM H_3BO_3, and 2.5µM $ZnSO_4.7H_2O$, adjusted with Na_2CO_3 to pH 9.1-9.2, daily. The control contained 5mM $CaCO_3$, 15µM H_3BO_3, and 2.5µM $ZnSO_4.7H_2O$, was not adjusted, maintaining a pH of 8.4. Root length was measured after 10 days in the treatment solutions.

The mean, standard deviation, variance and genetic models were calculated, and frequency distributions graphed. One and two gene models compared the observed variance of the segregating populations to the expected variances in the models to determine the number of genes controlling the response. The quantitative distributions were fitted to additive-dominance genetic models, with the assumption of no epistasis and no linkage (Jamjod 1996, Mather and Jinks 1977).

One gene segregation

$V_{F2} = \frac{1}{2}d^2 + \frac{1}{4}h^2 + E$

Two gene segregation

$V_{F2} = \frac{1}{4}d^2 + \frac{1}{8}h^2 + E$

where,

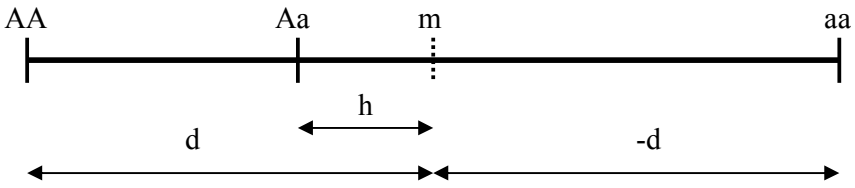
V_{F2} = variance of the F_2 population

d = departure from the mid-point (m) of the mean of each homozygous genotype (additive).

h = departure from the mid-point of the heterozygous genotypes (dominance).

E = environmental variance, and since no F_1 seeds were available for testing,

$E = \frac{1}{2}V_{P1} + \frac{1}{2}V_{P2}$



The confidence intervals of the observed variances for the F_2 populations were calculated as,

$(V_{F2} \times df)/\chi^2_a \leq \text{Confidence Interval} \geq (V_{F2} \times df)/\chi^2_b$

where,

V_{F2} = observed variance of the F_2 population.

df = degrees of freedom, n-1.

n = number of individuals in the F_2 population.

χ^2_a and χ^2_b = upper and lower level chi-square values at P = 0.95.

The one and two gene models were considered to fit the observed data when the expected variance was within the confidence interval of the observed variance for the F_2 populations (Jamjod 1996, D.G Pederson, *pers comm.*).

6.2.3 Results

Seed was obtained from 7 of the crosses, since the cross AUS7890 x Tamaroi had high sterility, yielding little seed of poor quality, i.e. was subsequently removed from further testing. The AUS7890 landrace also failed to produced adequate quantities of seeds for the AUS7890 x Na49/Kalka#4 cross with all available seed re-planted to produce F_3 seeds. Similarly, all F_2 seeds from the AUS4897 x Tamaroi cross were re-planted.

Landrace and advanced lines were originally selected for the development of durum populations because of their long mean root length when grown in bicarbonate solution when compared to Tamaroi and Kalka. Later bicarbonate testing of the parental lines with larger quantities of seeds prior to screening the populations using the pH 8.4 $CaCO_3$ control, and calculating tolerance on the basis of relative root length, the parental lines were not nearly as superior as previously identified (Table 6.01), with only one line (AUS4897) having a greater RRL than Kalka, although all remained more tolerant than Tamaroi (Table 6.02).

Table 6.02: Average root length measurements for the treatments bicarbonate and control, with relative root length (RRL) for each of the parental lines.

	Root Length (mm)		
Parental line	Bicarbonate	Control	RRL
AUS4897	139.72	166.40	83.97
AUS7890	135.61	194.13	69.86
R622Sh/Tm//WLYY9///Tm	122.50	199.17	61.51
C8MMDYk/BTWLYY9//Tm	120.17	166.50	72.17
Na49/Kalka#4	118.08	179.20	65.89
Kalka	115.92	161.33	71.85
Tamaroi	74.83	181.33	41.27

l.s.d (0.05) between line for Bicarbonate = 11.96, Control = 27.64, RRL = 8.38.

Similarly, parental lines tested with the F_2s failed to identify significant differences between the mean root lengths (Table 6.03). The F_2 populations of the six crosses were

screened consecutively in separate tests and therefore different solutions and environmental conditions influenced each cross, preventing accurate comparison between crosses and the root lengths of individual parental lines have varied between crosses. For instance AUS4897 had mean values of 59.8, 82.6 and 93.8mm in the test of its three derived populations (Table 6.03).

Table 6.03: The number of seedlings tested, mean root length, and standard deviation of the bicarbonate and control treatments for parental and F_2 lines of each cross.

	Treatment												
	Bicarbonate						Control						
Line	No.	Mean	StDev	Co-eff	St Dev of Diff.		No.	Mean	StDev	Co-eff	St Dev of Diff.		RRL
					P1	P2					P1	P2	
AUS4897	43	59.8	16.5	27.6			40	195.7	39.2	20.0			30.6
Tamaroi	40	58.2	12.5	21.4	3.12^{ns}		41	248.3	35.8	14.4	8.34***		23.4
F_2		No seed available											
AUS4897	45	82.6	12.4	14.8			45	250.5	29.4	11.7			33.0
Kalka	36	78.4	10.6	13.5	2.54^{ns}		37	272.1	44.5	16.4	8.53*		28.8
F_2	30	77.3	19.2	24.8	3.95^{ns}	3.92^{ns}	29	234.7	49.1	20.9	10.12^{ns}	11.69**	32.9
AUS4897	42	93.8	13.9	14.8			40	239.3	22.8	9.5			39.2
Na49/Kalka#4	36	89.9	9.5	10.5	2.66^{ns}		41	228.0	27.2	11.9	5.57*		39.4
F_2	43	92.5	16.8	18.1	3.34^{ns}	3.00^{ns}	27	236.9	28.5	12.0	6.56^{ns}	6.93^{ns}	39.0
AUS7890													
Na49/Kalka#4		No seed available											
F_2													
R622Sh/Tm//WLYY9///Tm	43	73.8	13.0	17.6			42	235.5	29.0	12.3			31.3
Na49/Kalka#4	40	72.3	13.2	18.3	2.88^{ns}		35	202.3	34.2	16.9	7.31***		37.7
F_2	43	74.0	14.6	19.8	2.99^{ns}	3.06^{ns}	42	212.6	34.9	16.4	7.00**	7.90^{ns}	34.8
C8MMDYk/BTWLYY9//Tm	37	102.5	19.7	19.2			35	241.9	26.5	11.0			42.4
Na49/Kalka#4	39	93.6	16.8	18.0	4.50^{ns}		30	228.0	34.4	15.1	7.71^{ns}		41.0
F_2	42	93.8	15.9	17.0	4.07^{ns}	3.97^{ns}	38	244.5	35.7	14.6	7.33^{ns}	8.54^{ns}	38.4
Na49/Kalka#4	30	90.5	13.9	15.3			40	239.7	40.8	17.0			37.8
Tamaroi	29	69.3	12.6	18.1	3.91***		36	263.1	47.8	18.2	10.25**		26.3
F_2	18	75.3	10.8	14.3	3.59***	1.57^{ns}	27	250.7	38.9	15.5	9.88^{ns}	10.93^{ns}	30.0

ns – not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

The root length distributions under bicarbonate treatment of the durum landrace AUS4897 were not significantly different from Tamaroi, Kalka, or Na49/Kalka#4. The root length of Tamaroi was expected to be approximately 50% shorter than AUS4897, and Kalka and Na49/Kalka#4 approximately 15% shorter than AUS4897. The root length distribution of

the advanced lines R622Sh/Tm//WLYY9///Tm and C8MMDYk/BTWLYY9//Tm was not significantly different from Na49/Kalka#4, although the advanced lines also failed to show significant difference to Na49/Kalka#4 in the previous bicarbonate test (Table 6.02). The cross between the advance breeding line Na49/Kalka#4 and the commercial line Tamaroi was the only cross with a significant difference between root length distribution, although the distance between parental means had narrowed from 40.8mm (Table 6.01) and 43.3mm (Table 6.02) in the previous tests, to 21.2mm in the current bicarbonate test (Table 6.03).

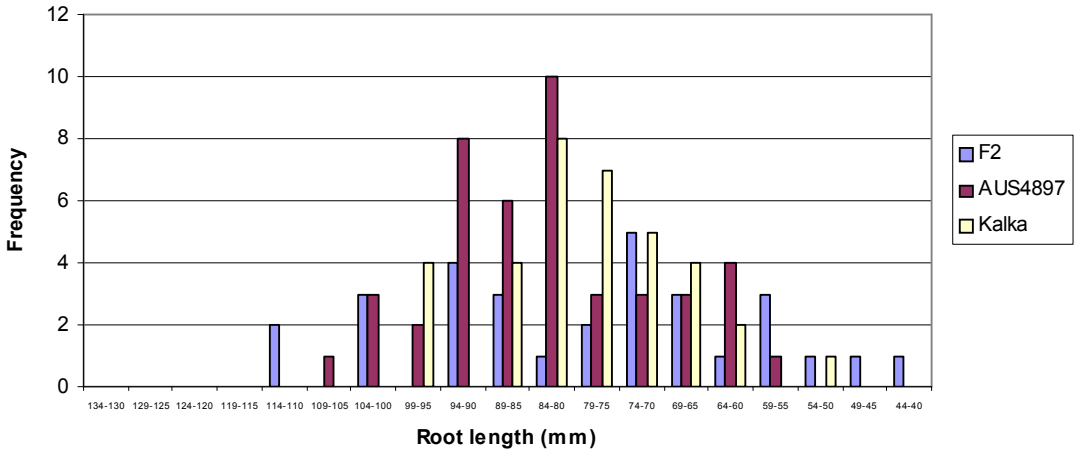
The mean root lengths of parentals measured in the control treatment at pH 8.4 were all significantly different, except for the C8MMDYk/BTWLYY9//Tm x Na49/Kalka#4 cross (Table 6.03). The results were similar to previous tests (Table 6.02), where Tamaroi had a significantly longer mean root length than AUS4897, and to a lesser extent Na49/Kalka#4. R622Sh/Tm//WLYY9///Tm was significantly greater than Na49/Kalka#4, while C8MMDYk/BTWLYY9//Tm was not significantly different from Na49/Kalka#4. The only discrepancy from the previous control test was with AUS4897, where previously the line was found not to be significantly different from either Kalka or Na49/Kalka#4, however, in the current test AUS4897 was found to be significantly shorter than Kalka (0.05 level), but significantly longer than Na49/Kalka#4 (0.05 level).

AUS4897 x Kalka

The mean root length in bicarbonate treatment was not significantly different between the F_2 population and parents, with frequency distributions overlapping (Figure 6.01). The variance of the F_2 was greater than the variance of the parents and outside of the range of either AUS4897 or Kalka, suggesting possible transgressive segregation. The observed variance was higher than the expected variance for both the one and two gene models (Table 6.04).

The mean root length in the control treatment was significantly different between AUS4897 and Kalka, and Kalka and the F_2 population. However, the expected variances for both the one and two gene models were within the range of the confidence interval for the observed F_2 variance (Table 6.05).

(a) AUS4897 x Kalka



(b) AUS4897 x Na49Kalka#4

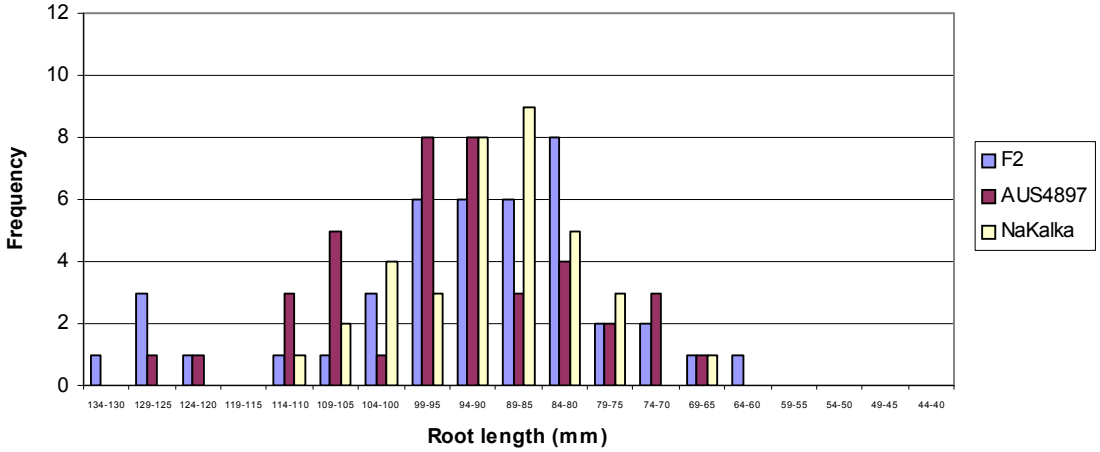


Figure 6.01: Frequency distribution of F_2 progeny and parentals for root length in bicarbonate solution. Durum landrace AUS4897 crossed with (a) Kalka and (b) Na49/Kalka#4.

AUS4897 x Na49/Kalka#4

Similarly, the distributions of the AUS4897 x Na49/Kalka#4 cross had overlapping parental distributions in bicarbonate treatment, with possible transgressive segregation, and an observed variance that was higher than the expected variance for both the one and two gene models (Figure 6.01, Table 6.04).

The mean root lengths were significantly different between AUS4897 and Na49/Kalka#4, but not between either parent and the F_2 in the control, with the expected variances for both

the one and two gene models within the range of the confidence interval for the observed F_2 variance (Table 6.05).

Table 6.04: Observed variance of parents and F_2 populations and the expected variance of the F_2 populations for one and two gene models from measurements of root length in bicarbonate treatment. CI = Confidence interval.

Cross			Observed variance				Estimated parameters				Expected variance		heritability
P_1		P_2	V_{P1}	V_{P2}	V_{F2}	CI	E	m	d	H	1 gene	2 gene	H^2
AUS4897	x	Tamaroi	*	*	*	*	*	*	*	*	*	*	*
AUS4897	x	Kalka	149.9	112.2	368.0	250.8-602.7	131.1	80.5	2.1	6.4	143.5	137.3	64.4
AUS4897	x	NaKalka	192.6	89.4	281.5	203.4-420.1	141.0	91.8	1.9	1.4	143.3	142.2	49.9
AUS7890	x	NaKalka	*	*	*	*	*	*	*	*	*	*	*
R622Sh/Tm//K//Tm	x	NaKalka	169.3	174.6	214.4	154.9-319.9	172.0	73.1	0.8	1.8	173.1	172.6	19.8
C8MMDYk/BTK//Tm	x	NaKalka	389.0	282.9	253.2	182.3-380.0	335.9	98.0	4.5	8.4	363.5	349.7	-32.7
NaKalka	x	Tamaroi	192.9	159.1	116.3	72.6-223.2	176.0	80.2	10.3	9.8	253.2	214.6	-51.3

Table 6.05: Observed variance of parents and F_2 populations and the expected variance of the F_2 populations for one and two gene models from measurements of root length in control treatment.

Cross			Observed variance				Estimated parameters				Expected variance		heritability
P_1		P_2	V_{P1}	V_{P2}	V_{F2}	CI	E	m	d	H	1 gene	2 gene	H^2
AUS4897	x	Tamaroi	*	*	*	*	*	*	*	*	*	*	*
AUS4897	x	Kalka	864.6	1978.4	2412.3	1634.0-3990.1	1421.5	261.3	10.8	53.2	2187.4	1804.4	41.1
AUS4897	x	NaKalka	520.4	737.7	810.5	545.6-1354.9	629.0	233.9	5.9	6.2	655.6	642.3	22.4
AUS7890	x	NaKalka	*	*	*	*	*	*	*	*	*	*	*
R622Sh/Tm//K//Tm	x	NaKalka	841.0	1171.0	1218.0	877.0-1827.5	1006.0	218.9	16.6	12.6	1183.5	1094.7	17.4
C8MMDYk/BTK//Tm	x	NaKalka	702.6	1180.1	1277.1	905.4-1962.8	941.4	234.9	7.0	19.1	1056.5	998.9	26.3
NaKalka	x	Tamaroi	1665.9	2284.8	1512.1	1017.8-2527.7	1975.3	251.4	11.7	1.4	2044.5	2009.9	-30.6

R622Sh/Tm//WLYY9///Tm x Na49/Kalka#4

The mean root length of R622Sh/Tm//WLYY9///Tm x Na49/Kalka#4 in bicarbonate treatment was not significantly different between the F_2 population and parents, with frequency distributions overlapping and a high level of environmental variation (Figure 6.02). The expected variances for both the one and two gene models were within the range

of the confidence interval for the observed F_2 variance (Table 6.04), but as the V_{F2} was not much larger than V_{P1} and V_{P2}, it is likely that any gene(s) were of minor effect.

The mean root length in the control treatment was significantly different between R622Sh/Tm//WLYY9///Tm and Na49/Kalka#4, and between R622Sh/Tm//WLYY9///Tm and the F_2 population. However, the expected variances for both the one and two gene models were within the range of the confidence interval for the observed F_2 variance (Table 6.05).

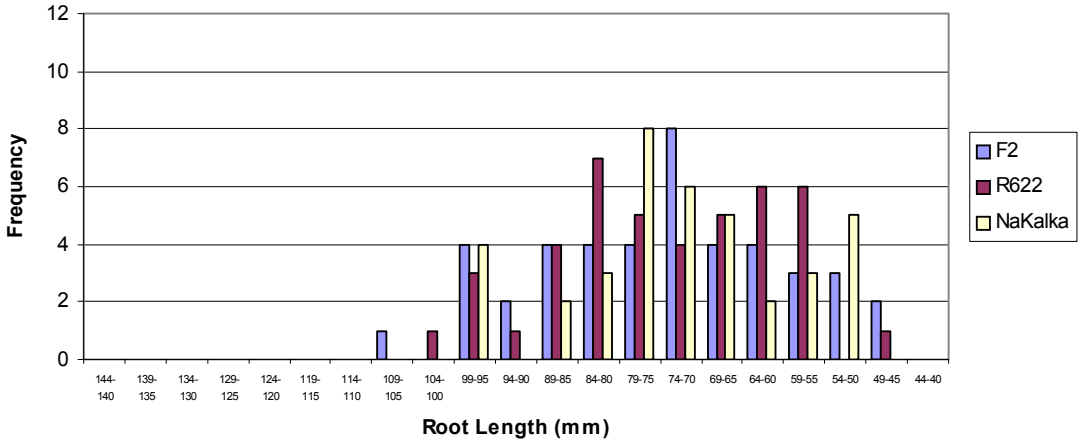


Figure 6.02: Frequency distribution of F_2 progeny and parentals for root length in bicarbonate solution of the durum advance line R622Sh/Tm//WLYY9///Tm crossed with Na49/Kalka#4.

C8MMDYk/BTWLYY9//Tm x Na49/Kalka#4

Again, the mean root length in bicarbonate treatment was not significantly different between the F_2 population and parents, with frequency distributions overlapping (Figure 6.03). The observed variance of the parental lines was greater than the observed variance of the F_2 population, suggesting no segregation was occurring in the C8MMDYk/BTWLYY9//Tm x Na49/Kalka#4 cross.

The mean root length in the control was not significantly different between the F_2 population and parents. The observed variation of the F_2 was only marginally higher than the environmental variation, with the expected variance for both the one and two gene models fitting within the confidence interval (Table 6.05), again suggesting the absence of any genetic variation.

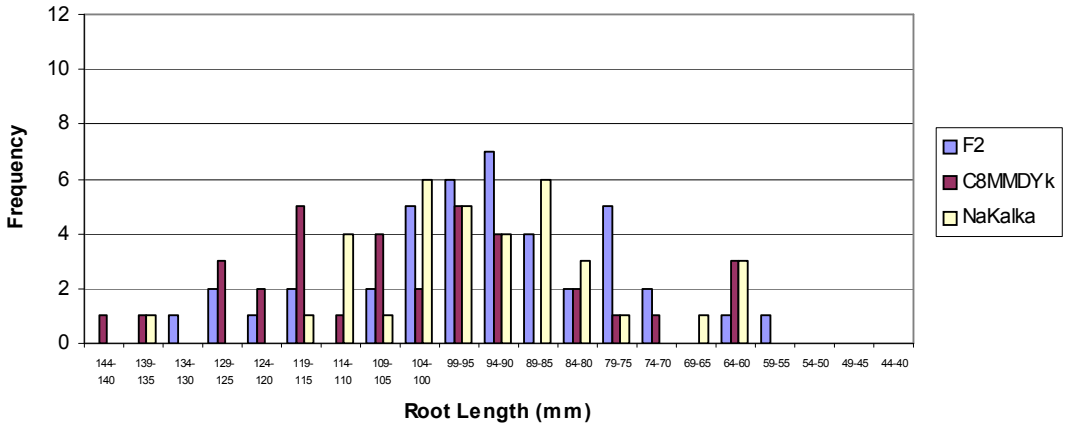


Figure 6.03: Frequency distribution of F_2 progeny and parentals for root length in bicarbonate solution of the durum advance line C8MMDYk/BTWLYY9//Tm crossed with Na49/Kalka#4.

Na49/Kalka#4 x Tamaroi

A significant difference between Na49/Kalka#4 and Tamaroi, and Na49/Kalka#4 and the F_2 population was identified for the mean root length in the bicarbonate treatment for the Na49/Kalka#4 x Tamaroi population. The frequency distributions indicate a partial overlapping between the parents, with no transgressive segregation in the F_2 (Figure 6.04). However, the environmental variation is greater than the observed variance of the F_2 (Table 6.04), suggesting a lack of genetic diversity between the parents.

Similarly, the environmental variation for the control treatment is greater than the observed variance for root length of the F_2 (Table 6.05).

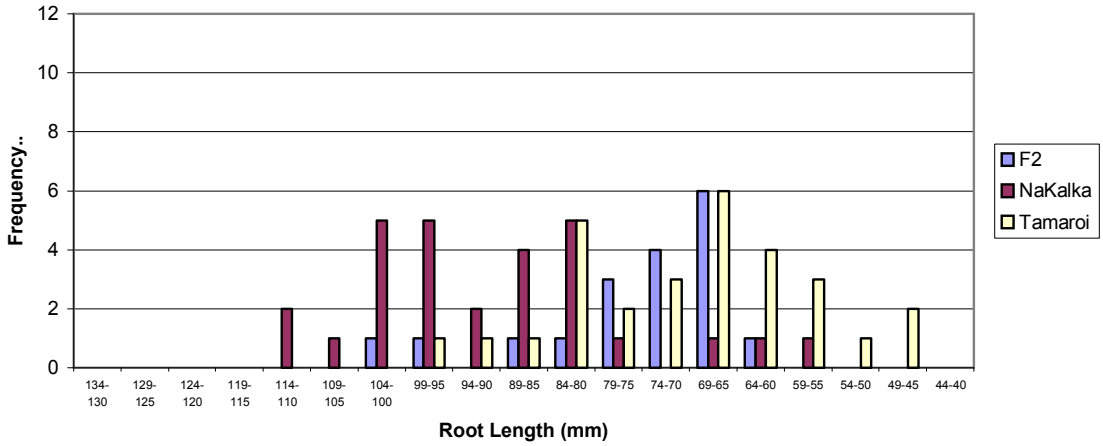


Figure 6.04: Frequency distribution of F_2 progeny and parentals for root length in bicarbonate solution. Durum advance line NaKalka crossed with the commercial durum line Tamaroi.

6.2.4 Discussion

The lack of difference between the mean and distribution of parental root lengths and high environmental variation were major contributing factor towards the inability to identify genetic segregation in the crosses. In previous bicarbonate testing parental lines recorded a greater mean difference between root length values, but a similar level of difference was not identified in the F_2 screening. These discrepancies in results may have occurred through the use of non-fixed lines (landraces and advanced lines). Several plants of a single line were grown in 12'' pots without prior screening of individual plants for bicarbonate tolerance, one plant within the pot was used for crossing, and the remaining plants bulked for use as parental seed. If segregation was occurring in the landrace and advanced lines, the line used for the cross may not have represented the mean of the parental population. In the landrace AUS4897 crosses particularly, the variation was significantly higher than the fixed lines Kalka or Na49/Kalka#4, and this may have been associated with AUS4897 being much taller in plant height, and therefore root length, than the varieties Tamaroi, Kalka, and Na49/Kalka#4.

Alternatively, the lack of a parental difference may have been associated with the screening procedure. A slightly stronger stock bicarbonate or carbonate solution added initially to the treatment solution maintains a slightly higher treatment pH (0.1 –0.2 units) for the first 2 to 3 days. As indicated in Figure 5.01, even a slight increase above the screening level of pH 9.1 can significantly decrease the differential in response between lines. The higher screening pH was evident in the low RRL values. Additionally, as the screening was undertaken in the winter with lower temperatures, the growth rate of the roots was reduced, which decreases the separation of varieties.

The similarity between the parental and F_2 distributions resulted in the failure to identify segregation for root length for the bicarbonate treatment. In the control treatment significant differences were identified between parental and F_2 distributions for the populations AUS4897 x Kalka and R622Sh/Tm//WLYY9///Tm x Na49/Kalka#4. However, the mean of the F_2 for AUS4897 x Kalka was found to lie outside of the mean of the parents, indicating over-dominance and/or epistasis, since $h > d$.

In the bicarbonate treatment, with low additive (d) and dominance (h) values, the expected variance models for one and two genes reflected the environmental (E) component, and were both lower than the confidence interval for the observed variance of the F_2 population. In the control treatments the expected variance for both the one and two gene models were within the confidence interval, which was a consequence of low d and h values, but also high environmental values.

The high E value occurred for all crosses, particularly C8MMDYk/BTWLYY9//Tm x NaKalka and NaKalka x Tamaroi, where the E component was greater that the variance of the F_2. In chapter 4 the bicarbonate testing procedure was found to produce variable results due to seed quality, timing of germination and tray placement effects, however, the variation could be reduced with the screening of higher numbers of individuals and a greater number of replications. In the F_2 screen only 18 to 43 parentals or F_2 individuals were tested per cross with no replication, which was insufficient to assess accurately a quantitative character under variable conditions that have led to a high environmental variation value. Few conclusions could be drawn from the screening of the F_2 populations and with potential errors in the screening procedure, no further genetic models were fitted.

6.3 Evaluation of populations at F_3 from crosses between durum landraces, advanced lines and commercial varieties.

6.3.1 Introduction

In the previous section, as a result of the testing of inadequate numbers of individuals within a population, and high environmental variation, no segregation could be identified in any of the F_2 populations. Consequently, the one and two gene models generally did not fit the observed variance of the F_2 populations. Inconsistencies also occurred between successive bicarbonate tests for the landrace and advanced breeding parental lines confounding the accurate assessment of populations. The aim in this section was to assess the F_3 generation of the eight durum crosses, with 24 F_3 seeds for 100 F_2 derived lines for each cross, which was expected to reduce the experimental error.

6.3.2 Materials and Methods

One hundred and twenty F_2 seeds were sown from each of the seven crosses (Section 6.2.2). One hundred F_2 derived F_3 families were selected randomly from each cross, with twenty-four seeds per family used for bicarbonate testing. The seeds were arranged into four completely randomised replications (trays), with three seeds for each F_3 family per tray, and two treatments (bicarbonate and control). Parentals and standard varieties were also included in the test.

The bicarbonate screening method followed the procedures outlined in section 5.2.

The correlations, mean, standard deviation, variance and genetic models were calculated, and frequency distributions graphed, using Microsoft Excel®. One and two gene models were used to compare the observed variance of the segregating populations to expected variances to determine the number of genes controlling the response to bicarbonate toxicity. The quantitative distributions were fitted to additive-dominance genetic models, with the assumption of no epistasis and no linkage (Jamjod 1996, Mather and Jinks 1977).

One gene segregation $V_{F3} = 3/4d^2 + 3/16h^2 + E$

Two gene segregation $V_{F3} = 3/8d^2 + 3/32h^2 + E$

where,

V_{F3} = variance of the F_3 population

d = departure from the mid-point (m) of the mean of each homozygous genotype (additive).

h = departure from the mid-point of the heterozygous genotypes (dominance).

E = environmental variance, and since F_1 seed was not available, $E = ½V_{P1} + ½V_{P2}$

For two loci, the above equation assumes that there are two independent loci of equal effect. If there are two independent loci of unequal effect,

$$V_{F3} = 3/16(1+k^2)d^2 + 3/64(1+k^2)h^2 + E$$

where,

k = a constant. If k=1, then same expected variance as equation with loci of equal effects. If k=2, then one locus has 2 times the effect of the other locus, and so on.

The confidence intervals of the observed variances for the F_3 populations were calculated as,

$$(V_{F3} \text{ x df})/\chi^2_a \leq \text{Confidence Interval} \geq (V_{F3} \text{ x df})/\chi^2_b$$

where, V_{F3} = observed variance of the F_2 population.

df = degrees of freedom, n-1.

n = number of individuals in the F_2 population.

χ^2_a and χ^2_b = upper and lower level chi-square values at P = 0.95.

The one and two gene models were accepted as fitting the observed data when the expected variance was within the confidence interval of the observed variance for the F_3 populations (Jamjod 1996, D.G Pederson, *pers comm.*).

6.3.3 Results

The mean root length of each of the 100 F_3 families per cross was correlated between the four replications (tanks) and found to have a high level of significance for each of the seven crosses, providing confidence in the reliability in the testing procedure (Appendix 5).

Correlations were also calculated between the mean root length measurements of the 100 F_3 families for the bicarbonate and control treatments for the seven populations (Table 6.06). For each population it was found that those with longer root lengths in the control (RO) had longer root lengths in the carbonate solution (RL), but were proportionally more affected by carbonate (RRL).

Table 6.06: Correlations between the mean root lengths in bicarbonate (RL) and control (RO) treatment, and relative root length (RRL), of 100 F_2 derived F_3 families of seven durum populations.

	RL and RO	RRL and RO	RL and RRL
AUS4897 xTamaroi	0.134***	-0.419***	0.210***
AUS4897 x Kalka	0.289***	-0.343***	0.127***
AUS4897 x Na49/Kalka#4	0.113**	-0.321***	0.330***
AUS7890 x Na49/Kalka#4	0.382***	-0.132***	0.246***
R622Sh/Tm//WLYY9///Tm x Na49/Kalka#4	0.199***	-0.221***	0.332***
C8MMDYk/BTWLYY9//Tm x Na49/Kalka#4	0.218***	-0.086***	0.500***
Na49/Kalka#4 x Tamaroi	0.109**	-0.187***	0.497***

ns – not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

Similar results for the mean root length of parentals and populations were found as for the previous section. The mean root length of the landrace and advanced lines were below the expected length in comparison to Tamaroi, Kalka and Na49/Kalka#4 (Table 6.07).

A significant difference in the means for the bicarbonate treatment distributions between parentals for the crosses AUS4897 x Kalka, AUS4897 x Na49/Kalka#4, and C8MMDYk/BTWLYY9//Tm x Na49/Kalka#4 was identified, however, in each case AUS4897 or C8MMDYk/BTWLYY9//Tm had a shorter mean root length than the reciprocal parent. The Na49/Kalka#4 x Tamaroi cross again had significantly different parental means with a smaller difference of 18.6mm between the mean root lengths.

Table 6:07: The number of seedlings tested, mean root length, and standard deviation of the bicarbonate and control treatments for parental and F_3 lines (100 families) of each cross. (Co-eff = Co-efficient of variation, St Dev of Diff = Standard deviation of difference).

	Bicarbonate						Control						
Line	No.	Mean	StDev	Co-eff	St Dev of Diff.		No.	Mean	StDev	Co-eff	St Dev of Diff.		RRL
					P1	P2					P1	P2	
AUS4897	57	71.6	8.5	11.9			53	265.4	24.3	9.2			27.0
Tamaroi	63	70.6	11.6	16.4	1.84^{ns}		62	298.7	31.7	10.6	5.23***		23.6
F_3	821	73.5	13.9	18.9	1.22^{ns}	1.53^{ns}	806	256.2	40.0	15.6	3.63*	4.26***	28.7
AUS4897	32	76.8	12.5	16.3			35	221.4	22.3	10.1			34.7
Kalka	23	89.7	12.8	14.3	3.47**		28	219.1	16.1	7.3	4.84^{ns}		41.0
F_3	941	95.5	17.9	18.8	2.29***	2.74*	941	208.5	32.7	15.7	3.92***	3.22***	45.8
AUS4897	57	80.0	11.7	14.7			56	292.4	30.4	10.4			27.4
Na49/Kalka#4	54	89.3	10.1	13.3	2.07***		54	306.9	28.9	9.4	5.65**		29.1
F_3	1019	88.7	18.2	20.5	1.65***	1.49^{ns}	1026	279.6	43.5	15.5	4.29***	4.16***	31.7
AUS7890	36	120.8	13.4	11.1			35	263.0	28.3	10.8			45.9
Na49/Kalka#4	34	115.5	16.6	10.1	3.00^{ns}		31	242.4	27.5	11.4	6.68**		47.7
F_3	929	126.2	29.5	23.4	2.44*	2.22***	913	239.5	44.9	18.7	5.01***	5.16^{ns}	52.7
R622Sh/Tm//WLYY9///Tm	23	110.6	13.7	12.3			24	295.4	34.6	11.7			37.5
Na49/Kalka#4	24	104.3	11.5	11.0	3.69^{ns}		23	263.6	35.3	13.4	10.19**		39.6
F_3	1165	105.6	17.8	16.9	2.90^{ns}	2.40^{ns}	1131	281.2	38.6	13.7	7.16^{ns}	7.44*	37.5
C8MMDYk/BTWLYY9//Tm	11	65.1	7.1	10.8			12	188.2	15.6	8.3			34.6
Na49/Kalka#4	24	70.9	6.1	8.6	2.47*		24	201.5	18.0	8.9	5.81*		35.2
F_3	1059	79.8	12.9	16.2	2.16***	1.31***	1008	200.7	22.1	11.0	4.57**	3.73^{ns}	39.7
Na49/Kalka#4	37	103.5	11.9	11.5			35	273.2	31.3	11.4			37.9
Tamaroi	36	84.9	16.1	18.9	3.31***		36	303.1	37.5	12.4	8.19**		28.0
F_3	1167	101.5	17.7	17.5	2.02^{ns}	2.73***	1123	294.7	38.9	13.2	5.41**	6.36^{ns}	34.4

ns – not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

In the control treatment at pH 8.4 the mean roots lengths of the parentals measured were all significantly different, except for AUS4897 x Kalka (Table 6.07). Tamaroi and Na49/Kalka#4 had significantly longer mean root lengths than AUS4897, although in previous tests the AUS4897 had a longer mean root length than Na49/Kalka#4. R622Sh/Tm//WLYY9///Tm was again found to be significantly longer than Na49/Kalka#4, and Na49/Kalka#4 significantly less Tamaroi, while C8MMDYk/BTWLYY9//Tm was found to be marginally shorter than Na49/Kalka#4.

AUS4897 x Tamaroi, Kalka or Na49/Kalka#4

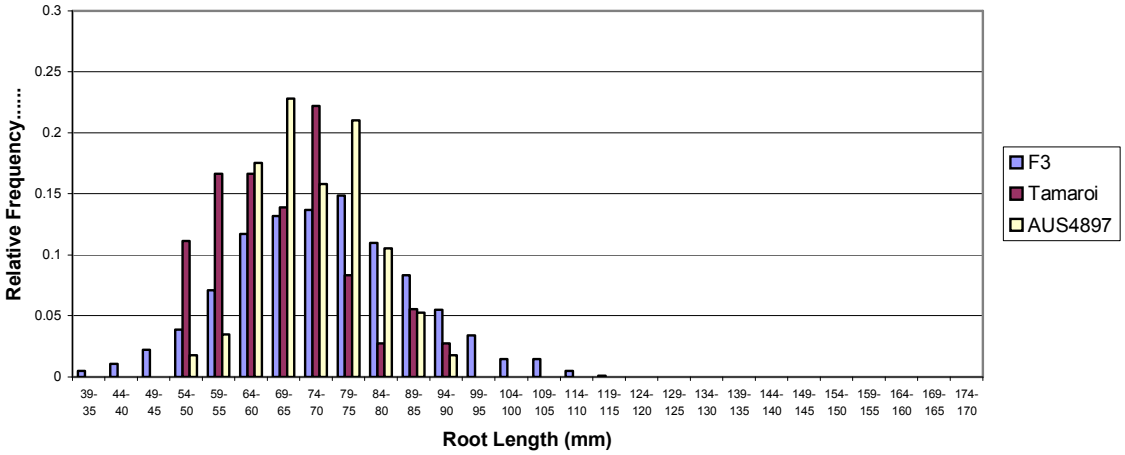
The AUS4897 and Tamaroi, Kalka or Na49/Kalka#4 F_3 and parental frequency distributions for the mean root length in bicarbonate were all overlapping (Figure 6.05). Significant differences in the mean root length between the F_3 population and parents were identified for the AUS4897 x Kalka and AUS4879 x Na49/Kalka#4 cross (Table 6.07). For all the crosses, AUS4897 x Tamaroi, AUS4897 x Kalka and AUS4897 x Na49/Kalka#4, the variance of the F_3 was greater than the variance of the parents (Table 6.08) and outside the distribution of both parents (Figure 6.05), suggesting transgressive segregation. With transgressive segregation, more than two genes would be segregating in the populations, resulting in the observed variance being higher than the expected variance of both the one and two gene models (Table 6.08). For the AUS4897 x Kalka cross, the mean of the F_3 distribution was significantly less than the mean root length of both parents (Table 6.07), suggesting over-dominance may be occurring in the progeny, or a mix up in seed tested.

In the control treatment the parental mean root lengths for the crosses AUS4897 and Tamaroi, Kalka or Na49/Kalka#4 were found to significantly differ from the F_3 population (Table 6.07). However, for all crosses, the mean of the F_3 distribution was significantly less than the mean root length of both parents, but with the observed variance of the F_3 much greater than the parents, transgressive segregation would be occurring in the populations (Table 6.09).

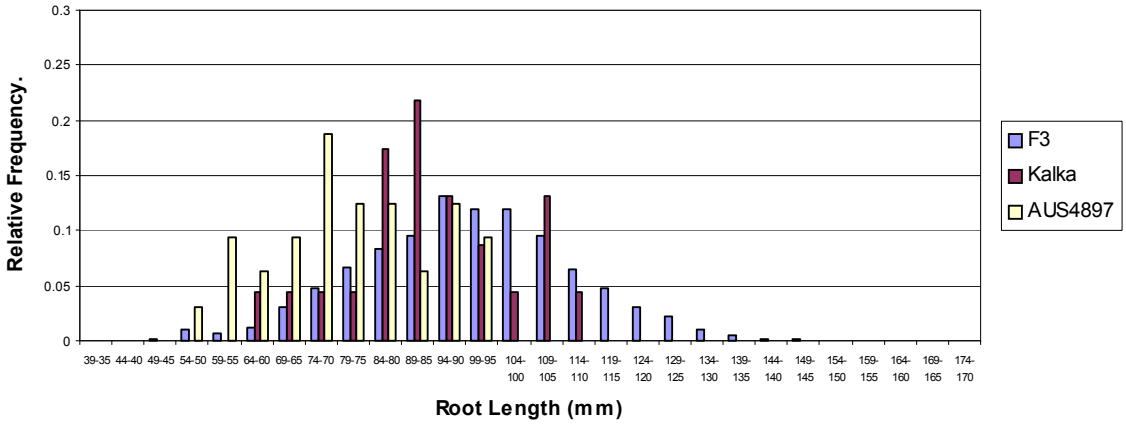
AUS7890 x Na49/Kalka#4

The parentals and the F_3 also had overlapping mean root length distributions in the AUS7890 x Na49/Kalka#4 cross, with highly probable transgressive segregation for bicarbonate tolerance (Figure 6.06). With more than two genes assumed segregating in the

(a) AUS4897 x Tamaroi



(b) AUS4897 x Kalka



(c) AUS4897 x Na49/Kalka#4

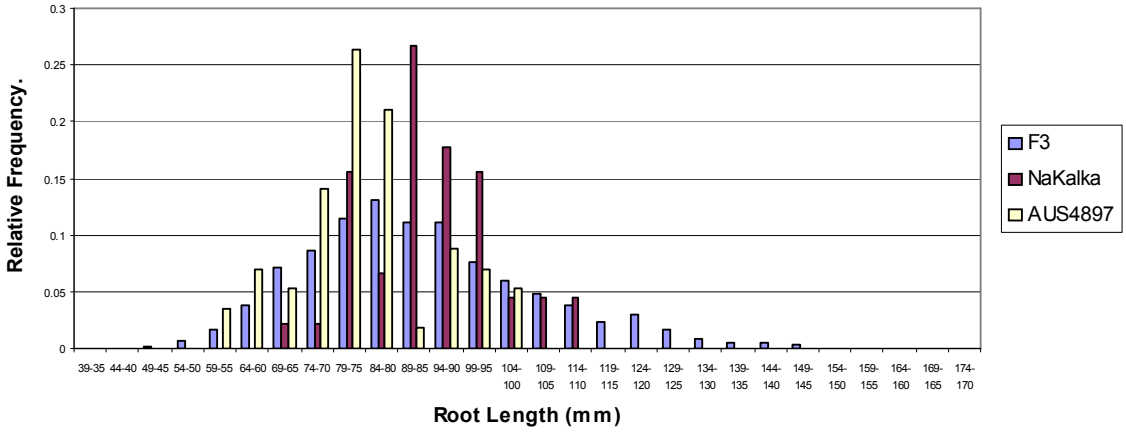


Figure 6:05: Frequency distribution of F_3 progeny and parentals for root length in bicarbonate solution. Durum landrace AUS4897 crossed with (a) Tamaroi, (b) Kalka and (c) Na49/Kalka#4.

F_3, the observed variance of the F_3 was higher than the expected variance for both the one and two gene models (Table 6.08).

Similarity, for the AUS4897 x Na49/Kalka#4 cross, the observed variance of the F_3 in the control treatment was greater than the variance of both parents, and higher than expected variance of both the one and two gene models (Table 6.09), indicating that transgressive segregation for root length was occurring in the population.

Table 6:08: Observed variance of parents and F_3 populations and the expected variance of the F_3 populations for one and two gene models from measurements of root length in bicarbonate treatment. CI = confidence interval.

Cross			Observed variance			Estimated parameters					Expected variance		heritability
P_1		P_2	V_{P1}	V_{P2}	V_{F3}	CI	E	m	d	h	1 gene	2 gene	H^2
AUS4897	x	Tamaroi	72.1	133.4	191.3	178.2-209.9	102.7	71.1	0.5	9.2	118.7	110.7	46.3
		Kalka	157.2	164.4	273.7	255.6-297.9	160.8	83.2	6.5	47.6	617.8	389.3	41.2
		NaKalka	119.8	101.9	313.5	295.3-338.5	110.9	84.3	5.0	16.3	178.7	144.8	64.6
AUS7890	x	Tamaroi	*	*	*	*	*	*	*	*	*	*	*
		AUS4896	*	*	*	*	*	*	*	*	*	*	*
		NaKalka	180.2	136.1	798.4	743.3-866.2	158.1	118.2	2.7	28.5	315.3	236.7	80.2
R622Sh/Tm//K//Tm	x	NaKalka	125.8	131.9	308.0	298.0-342.5	128.9	105.7	1.4	1.0	130.5	129.7	58.1
C8MMDYk/BTK//Tm	x	NaKalka	49.7	37.6	161.9	154.9-179.1	43.6	68.0	2.9	46.7	459.2	251.4	73.1
NaKalka	x	Tamaroi	141.0	198.3	305.4	285.2-327.9	169.7	93.7	9.8	30.7	419.2	294.5	44.4

Table 6:09: Observed variance of parents and F_3 populations and the expected variance of the F_3 populations for one and two gene models from measurements of root length in control treatment.

Cross			Observed variance			Estimated parameters					Expected variance		heritability
P_1		P_2	V_{P1}	V_{P2}	V_{F3}	CI	E	m	d	h	1 gene	2 gene	H^2
AUS4897	x	Tamaroi	591.8	1001.8	1596.3	1492.0-1757.1	796.9	282.1	16.6	103.5	3012.5	1904.7	50.1
		Kalka	498.1	257.7	1071.9	985.1-1145.6	377.9	220.3	1.2	47.2	796.7	587.3	64.7
		NaKalka	925.8	833.1	1888.6	1758.9-2034.2	879.5	299.6	7.3	80.1	2122.4	1500.9	53.4
AUS7890	x	Tamaroi	*	*	*	*	*	*	*	*	*	*	*
		AUS4896	*	*	*	*	*	*	*	*	*	*	*
		NaKalka	801.4	756.3	2013.4	1867.3-2178.5	778.8	252.7	10.3	52.6	1377.4	1078.1	61.3
R622Sh/Tm//K//Tm	x	NaKalka	1197.9	1244.3	1486.8	1402.5-1611.8	1221.1	279.3	16.1	7.8	1426.2	1323.7	17.9
C8MMDYk/BTK//Tm	x	NaKalka	244.3	323.0	487.7	453.9-525.6	283.6	194.9	6.7	23.6	421.4	352.5	41.8
NaKalka	x	Tamaroi	976.9	1407.2	1509.1	1409.7-1620.1	1192.1	288.1	14.9	26.5	1490.9	1341.5	21.0

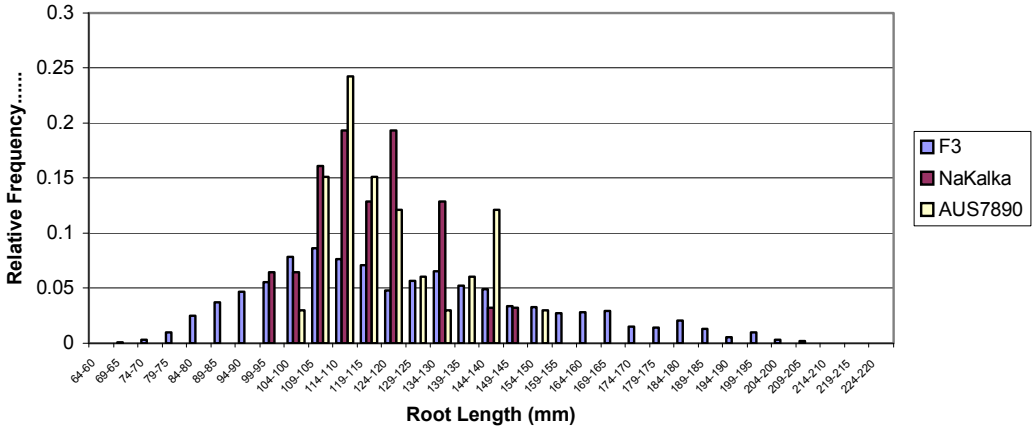


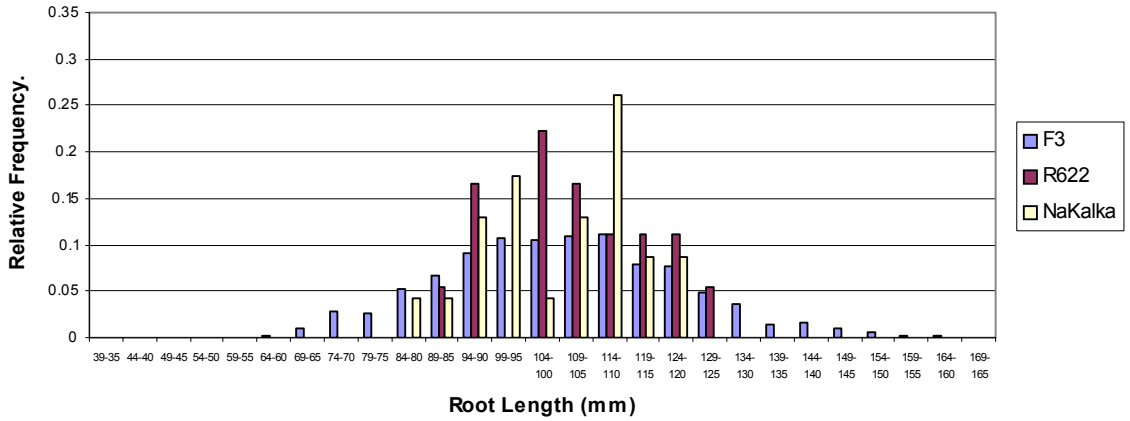
Figure 6.06: Frequency distribution of F_3 progeny and parentals for root length in bicarbonate solution. Durum landrace AUS7890 crossed with Na49/Kalka#4.

R622Sh/Tm//WLYY9///Tm or C8MMDYk/BTWLYY9//Tm x Na49/Kalka#4

In the crosses with the advanced breeding lines R622Sh/Tm//WLYY9///Tm and C8MMDYk/BTWLYY9//Tm with Na49/Kalka#4, a significantly different mean root length in bicarbonate between the F_3 population and parents was only found for the C8MMDYk/BTWLYY9//Tm x Na49/Kalka#4 cross (Table 6.07). However, only the R622Sh/Tm//WLYY9///Tm x Na49/Kalka#4 population had an observed variance of the F_3 higher than the variance of the parents, indicating transgressive segregation was occurring in the population (Figure 6.07, Table 6.08).

In the control treatment for the crosses R622Sh/Tm//WLYY9///Tm or C8MMDYk/BTWLYY9//Tm with Na49/Kalka#4 the mean root lengths were found to be significantly different between the parents and the F_3 (Table 6.07). The C8MMDYk/BTWLYY9//Tm x Na49/Kalka#4 population had an observed F_3 variance higher than the variance of both parents (Figure 6.07a) and greater than the expected variance of the one and two gene models, indicating that more than two genes were likely segregating in the population. The R622Sh/Tm//WLYY9///Tm x Na49/Kalka#4 population was found to have an expected variance within the range of the confidence interval for the observed F_3 variance of the one gene model (Table 6.09, Figure 6.07b).

(a) R622Sh/Tm//WLYY9///Tm x Na49/Kalka#4



(b) C8MMDYk/BTWLYY9//Tm x Na49/Kalka#4

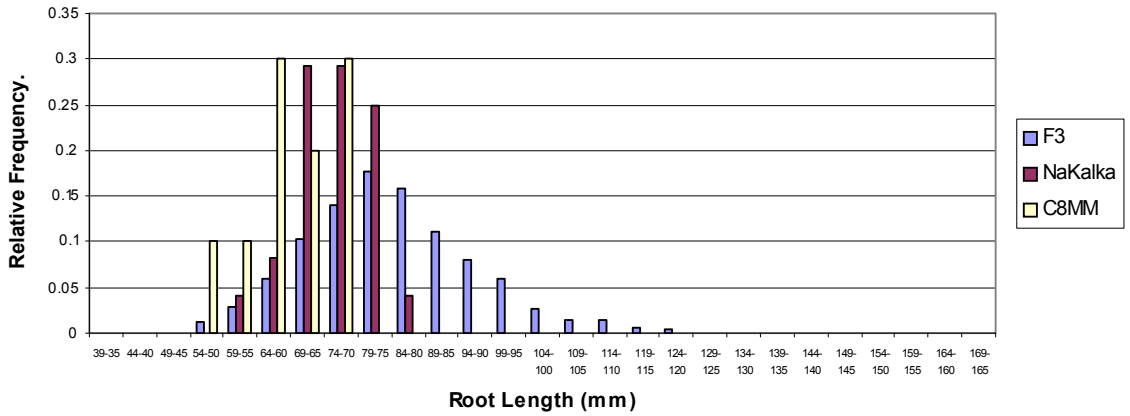


Figure 6.07: Frequency distribution of F_3 progeny and parentals for root length in bicarbonate solution. Durum advanced lines (a) R622Sh/Tm//WLYY9///Tm and (b) C8MMDYk/BTWLYY9//Tm, crossed with Na49/Kalka#4.

Na49/Kalka#4 x Tamaroi

The lines Na49/Kalka#4 and Tamaroi were found to have a significantly different mean root lengths in bicarbonate treatment and from the F_3 population. Similar to the F_2 population testing, the frequency distributions indicate a partial overlapping between the parents, although the F_3 population also shows transgressive segregation (Figure 6.08). The observed variance of the F_3 population was found to fit the expected variance of the two genes model (Table 6.08). The model of two independent loci were found to fit within the confidence interval when loci were of equal effect, but also when one locus had 2 or 3 times the effect of the other.

In the control the mean root length was significantly different between Na49/Kalka#4 and Tamaroi, and Na49/Kalka#4 and the F_3 population. The observed variance of the F_3 population was found to fit the expected variance of the one gene model (Table 6.09).

(a)

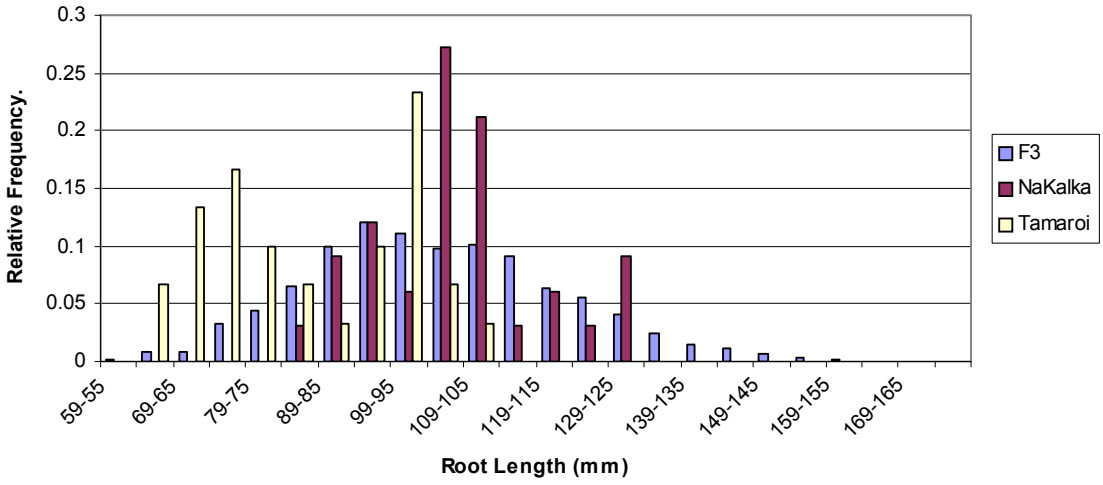


Figure 6.08: Frequency distribution of F_3 progeny and parentals for root length in bicarbonate solution. Durum advanced line Na49/Kalka#4 crossed with Tamaroi.

6.3.4 Discussion

The bicarbonate screening of F_2 derived F_3 populations found similar results as for the F_2 screening. The landraces and advanced lines had lower than expected root lengths, which were mostly lower or not significantly different from Tamaroi, Kalka or Na49/Kalka#4. However, in all the populations, except R622Sh/Tm//WLYY9///Tm x Na49/Kalka#4 and Na49/Kalka#4 x Tamaroi, transgressive segregation was observed in the F_3 progeny.

The AUS4897 parental had a similar root length in the bicarbonate treatment to Tamaroi, which was significantly less than Kalka and Na49/Kalka#4. Previously, AUS4897 had been reported to have superior root growth under bicarbonate toxicity, however, the results of the F_2 and F_3 screening support the notion that the parental seed bulked for screening was not representative of the seed used in previous testing or in the crossing for the production of the F_3 populations. In the AUS4897 populations the mean root length of the F_3 was greater than the parents AUS4897, Tamaroi and Kalka, and not significantly

different from Na49/Kalka#4. Again, in the control treatment the mean root length of the F_3 was less than the parents AUS4897, Tamaroi, Kalka and Na/Kalka#4 (Table 6.07).

The landrace AUS7890 had previously been reported to have 10% superiority over Na49/Kalka#4, however, in the F_3 bicarbonate treatment the parentals were not significantly different. Furthermore, the mean root length of the F_3 in bicarbonate was found to be significantly greater than both parentals, and significantly less than the parentals in the control treatment (Table 6.07).

The advanced line R622Sh/Tm//WLYY9///Tm had previously produced a 24% longer root length in bicarbonate than Na49/Kalka#4, but as with the F_2 screen, the mean root lengths of the parentals and the F_3 were not significantly different (Table 6.07). The higher observed variance of the F_3 in comparison to the parents and the expected variance of the models did, however, indicate transgressive segregation was occurring in the population. In the control treatment the observed F_3 variance was marginally greater than the parental variances, with the expected variance of the one gene model fitting within the confidence interval of the observed F_3 variance. The high level of environmental variation in both the F_3 and F_2 tests, and the extensive overlap of parental distributions, however, casts some doubt over the validity of the 1 locus result. Only a fraction of the variation observed in the F_3 was due to genetic variation.

The advance line C8MMDYk/BTWLYY9//Tm had very inconsistent results across tests. In initial bicarbonate tests C8MMDYk/BTWLYY9//Tm had a 21% longer root length than Na49/Kalka#4. In the F_2 bicarbonate screen the mean root length of the parentals and F_2 progeny were not significantly different, with no segregation and an environmental variation greater than the observed variation of the F_2 (Section 6.2) In the F_3 bicarbonate screen C8MMDYk/BTWLYY9//Tm had a mean root length significantly lower than Na49/Kalka#4, and both parentals had a mean root length significantly lower than the F_3 progeny (Table 6.07). The unavailability and poor quality of the parental seed at the time of testing led to only 11 seedlings of C8MMDYk/BTWLYY9//Tm and 24 seedlings of the Na49/Kalka#4 parents being measured, resulting in an unreliable mean root length value, a low observed variance for the parentals, and therefore a low value for the environmental variation (Table 6.08). If a parental variance similar to the other populations were obtained then, as with the F_2 results, the environmental variation would have almost entirely

accounted for the low observed F_3 variation. For instance, Na49/Kalka#4 had previously attained an observed variance of 101.9, 136.1 and 131.9, compared to a variance of 37.6 for the C8MMDYk/BTWLYY9//Tm x Na49/Kalka#4 cross. However, in figure 6.07b, the distribution of the F_3 progeny for root length in bicarbonate treatment extends beyond the distribution of both parents. Further testing with a larger number of parental lines would be necessary to confirm if transgressive segregation was occurring in the C8MMDYk/ BTWLYY9//Tm x Na49/Kalka#4 population for bicarbonate tolerance.

In the F_2 screen for the cross between Na49/Kalka#4 and Tamaroi, significant variation was identified between the parentals and the F_2 progeny for mean root length in bicarbonate treatment, however, the low number of F_2 seeds tested and the very high environmental variation prevented the accurate fitting of genetic models for both the bicarbonate and control treatments (Section 6.2). The testing of much larger numbers of F_3 seeds provided an improved comparison of the F_3 to the parents, with the genetic variation accounting for 44 and 21% of the observed F_3 variation for the bicarbonate and control treatments, respectively. Two loci were identified as segregating for root length in the bicarbonate treatment, and one locus was segregating in the control treatment (Table 6.08 and 6.09).

The Na49/Kalka#4 x Tamaroi population was the only population where the parents were both not positively contributing to the bicarbonate tolerance observed in the F_3 progeny, with tolerance to bicarbonate derived from the Na49/Kalka#4 parent. In contrast, the locus identified for root length in the control treatment was associated with the Tamaroi parent, which had significantly longer roots than Na49/Kalka#4. The identification of two loci in the Na49/Kalka#4 population for bicarbonate tolerance, as compared to greater than two for the landrace and advanced breeding line populations, is likely associated with the closer relationship between Na49/Kalka#4 and Tamaroi (i.e. Kalka and Tamaroi) and simpler genetic situation than the other populations.

The transgressive segregation in the populations AUS4897 x Tamaroi, AUS4897 x Na49/Kalka, AUS7890 x Na49/Kalka#4 and R622Sh/Tm//WLYY9///Tm x Na49/Kalka#4, and two loci identified in the Na49/Kalka#4 x Tamaroi population, provide the potential for the populations to be used further in genetic analysis or field studies, although the parentage would need some clarification.

6.4 Evaluation of bicarbonate tolerance in bread and durum wheat mapping populations.

6.4.1 Introduction

To further evaluate the genetic mechanisms of the bicarbonate tolerance response and to overcome the difficulties associated with high environmental variation in screening methods, several inbred populations were sourced. Populations were chosen whose parents had significantly different levels of bicarbonate tolerance as identified in section 5.3. The inbred populations were developed through either single seed decent or by doubling haploids, with large numbers of seeds for each of the homozygous lines in the population available, allowing for multiple replications. The aim was to evaluate bread and durum wheat populations for variation to bicarbonate toxicity to determine the number of genes involved and their mode of inheritance in the bicarbonate tolerance response.

6.4.2 Materials and Methods

Eight bread wheat and one durum wheat population were screened in bicarbonate following the procedures outlined in section 3.3 and 5.2.

Seed Source

Seed for the RAC875/Cascades (I) population was sourced from 2004 field grown plots of the Durum Breeding group, University of Adelaide. The population of ninety-three lines was developed by doubling haploids (DH) and was originally obtained by the Durum Breeding group from Hugh Wallwork, SARDI, in 2002. A second set of the same RAC875/Cascades DH lines (II) was sourced directly from Hugh Wallwork, SARDI, from 2006 glasshouse grown seed, along with 156 lines of the Berkut/Krichauff DH population.

The seed for populations Wk/TmWLYY9//WLYY9Tm and Frame/Yarralinka//Pugsley was sourced from 2004 and 2005 field grown plots, respectively, from the Durum Breeding Group. The Wk/TmWLYY9//WLYY9Tm population was developed by crossing the bread wheat Worrakatta with the durum wheat TmWLYY9, followed by the crossing the F_1 plant with the durum wheat WLYY9Tm (Topcross). The Frame/Yarralinka//Pugsley

population was developed from the crossing of the inbred lines Frame/Yarralinka/23/1 and Pugsley. Both populations underwent single-seed descent to produced 350 and 193 lines, respectively. However, only 167 single-seed descent (SSD) lines from the Wk/TmWLYY9//WLYY9Tm population were used in bicarbonate testing.

Thirty lines each of the populations Meering/Yitpi, Meering/90072, Kukri/RAC875, Kukri/Excalibur and Stylet/Westonia were selected for use in bicarbonate testing. Seed of Meering/Yitpi and Meering/90072 was developed by single seed descent and sourced from the Durum Breeding group from glasshouse grown seed. Seed for Kukri/RAC875, Kukri/Excalibur and Stylet/Westonia was developed by double haploid technology and sourced from the ACPFG (Thorsten Schnurbusch) and the Molecular Marker group, MPBCRC (Hugh Wallwork).

Experimental procedure

Twenty-four bread wheat seeds of each of the DH or SSD lines were used for bicarbonate testing. Seeds were arranged into four completely randomised replications (trays), with three seeds for each DH/SSD line per tray, and two treatments (bicarbonate and control). The exception was the RAC875/Cascades (II) population where thirty-six seeds of each of the ninety-three double haploid (DH) lines were arranged into six replications (trays).

Bicarbonate and control treatments

The bicarbonate treatment and controls were conducted as described in section 5.2, except the treatments for RAC875/Cascades (I) did not contain the nutrients $ZnSO_4.7H_2O$ or H_3BO_3.

Genetic models

The correlations, mean, standard deviation, variance and genetic models were calculated, and frequency distributions graphed. One and two gene models were used to compare the observed variance of the segregating populations to expected variances to determine the number of genes controlling the response to bicarbonate toxicity. The quantitative distributions were fitted to an additive genetic model, with the assumption of no epistasis

and no linkage (Jamjod 1996, Mather and Jinks 1977). Dominance could not be calculated in this model due to the absence of heterozygotes in the double haploid or single seed decent populations.

One gene segregation	*Two gene segregation*
$V_{PR} = d^2 + E$	$V_{PR} = 1/2d^2 + E$

where,

V_{PR} = variance of the SSD or DH population

d = departure from the mid-point (m) of the mean of each homozygous genotype (additive).

E = environmental variance, and since no F_1, $E = ½V_{P1} + ½V_{P2}$

The confidence intervals of the observed variances for the F_3 populations were calculated as,

$$(V_{PR} \text{ x df})/\chi^2_a \leq \text{Confidence Interval} \geq (V_{PR} \text{ x df})/\chi^2_b$$

where,

V_{PR} = observed variance of the population.

df = degrees of freedom, n-1.

n = number of individuals in the F_2 population.

χ^2_a and χ^2_b = upper and lower level chi-square values at P = 0.95.

The variance calculated from one and two gene models were compared with the observed data and the models, the model which provided an estimate within the confidence interval of the observed variance, was accepted as the appropriate model (Jamjod 1996, D.G Pederson, *pers comm.*).

Quantitative trait analyses were performed by the Molecular Marker Group, MPBCRC, on the DH populations RAC875/Cascades and Berkut/Krichauff. Bicarbonate data was compared to existing marker maps using Map Manager QTX to generate Likelihood Ratio Statistics (LRS), which were graphed by fitting the data to an additive regression coefficient model to produce an interval map (Manley *et. al.* 2001).

6.4.3 Results

To provide confidence in the reliability of the results for each population, the mean root length of the lines was compared between replications (trays and tanks) and found to

correlate for most of the replications (Appendix 6). Only the bicarbonate treatment replications for the population Stylet/Westonia were found to have a high number of non-significant correlations between its replications. In the control treatments the populations Frame/Yarralinka//Pugsley and Meering/90072 were also found to have a high number of non-significant correlations between their four replications.

Correlations were also calculated between the mean root length measurements of the SSD or DH lines for the bicarbonate and control treatments for the nine populations (Table 6.10). For the populations RAC875/Cascades, Berkut/Krichauff, Kukri/RAC875, Meering/90072 and Stylet/Westonia it was found that those with longer root lengths in the control (RO), had longer root lengths in the bicarbonate solution (RL), but were proportionally more affected by bicarbonate (RRL). The populations Wk/TmWLYY9//WLYY9Tm, Frame/Yarralinka//Pugsley, Kukri/ Excalibur and Meering/ Yitpi had no significant correlation between the root length in the control and the bicarbonate solution, but again, the longer the root lengths in the control the greater the apparent affect of bicarbonate toxicity (RRL).

Table 6.10: Correlations between the mean root lengths in bicarbonate (RL) and control (RO) treatment, and relative root length (RRL), for lines in bread and durum wheat populations.

	RL and RO	RRL and RO	RL and RRL
RAC875 x Cascades I	0.533***	-0.028^{ns}	0.829***
RAC875 x Cascades II	0.412***	-0.293**	0.748***
Worrakatta/TmWLYY9 x WLYY9Tm	-0.035^{ns}	-0.506***	0.875***
Frame/Yarralinka/23/1 x Pugsley	0.126^{ns}	-0.410***	0.851***
Berkut x Krichauff	0.559***	-0.375***	0.553***
Kukri x RAC875	0.471**	-0.030^{ns}	0.865***
Kukri x Excalibur	0.059^{ns}	-0.403*	0.887***
Meering x Yitpi	0.151^{ns}	-0.462**	0.800***
Meering x 90072	0.394*	0.192^{ns}	0.976***
Stylet x Westonia	0.528**	-0.104^{ns}	0.786***

ns – not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

A significant difference in the means of the parental distributions and the SSD/DH lines were identified for all populations in the bicarbonate and control treatments (Table 6.11).

Table 6.11: The number of seedlings tested, mean root length, and standard deviation of the bicarbonate and control treatments for parental and Double Haploid (DH) or Single Seed Decent (SSD) lines of each cross.

	Bicarbonate							Control							
Line	No.	Mean	StDev	Co-eff	St Dev of Diff.			No.	Mean	StDev	Co-eff	St Dev of Diff.			RRL
					P1	P2	P3					P1	P2	P3	
RAC875	22	68.9	18.6	27.1				11	219.5	11.2	5.1				31.4
Cascades	22	86.4	18.0	20.9	1.29***			11	200.5	10.6	5.3	1.41***			43.1
DH	976	73.5	20.8	28.3	0.93***	0.92***		491	203.6	26.5	13.0	1.03***	1.01**		36.1
RAC875	30	73.7	17.1	23.2				32	251.7	29.4	11.7				29.3
Cascades	32	87.8	20.4	23.3	1.10***			31	245.2	29.3	12.0	1.37***			35.8
DH	1425	80.7	22.9	28.4	0.76***	0.81***		1447	247.7	42.3	17.1	0.97***	0.99*		32.6
Worrakatta	83	96.1	12.9	13.5				75	177.8	30.2	17.0				54.0
Tamaroii	73	87.4	13.8	15.8	0.63***			78	288.5	30.5	10.6	0.87***			30.3
WLYY9	82	102.9	17.6	17.1	0.57***	0.64***		78	245.2	27.9	11.4	0.89***	0.86***		42.0
SSD	1963	90.4	20.7	22.9	0.41***	0.50***	0.42***	1891	262.4	41.5	15.8	0.65***	0.62***	0.64***	34.5
Fr/Yk/23/1	62	110.7	16.5	14.9				68	275.5	40.0	14.5				40.2
Pugsley	68	82.1	12.8	15.5	0.67***			68	280.3	36.8	13.1	1.06***			29.3
SSD	1609	97.8	20.2	20.7	0.53***	0.45***		1558	266.2	37.5	14.1	0.78***	0.75***		36.7
Berkut	180	87.3	12.9	14.8				176	228.9	29.5	12.9				38.1
Krichauff	202	119.8	15.5	12.9	0.39***			192	198.5	29.3	14.8	0.56***			60.4
DH	1899	96.8	19.0	19.7	0.29***	0.29***		1825	205.2	35.3	17.2	0.43***	0.40***		47.2
Kukri	10	98.1	16.8	17.1				12	281.6	28.9	10.3				34.8
RAC875	10	63.5	11.8	18.5	1.69***			11	249.6	27.1	10.9	2.21***			25.4
DH	353	69.7	21.4	30.6	1.32***	1.11***		356	252.6	35.9	14.2	1.58***	1.60ns		27.6
Kukri	10	84.9	21.1	24.9				12	270.5	26.7	9.9				31.4
Excalibur	12	76.3	16.3	21.4	1.86***			10	263.3	15.6	5.9	1.95**			29.0
DH	343	90.0	21.7	24.1	1.48**	1.19***		356	254.5	29.0	11.4	1.52***	1.28***		35.4
Meering	10	57.1	11.4	19.9				10	176.0	13.8	7.8				32.4
Yitpi	10	82.2	20.8	25.3	1.79***			10	262.1	24.8	9.5	1.97***			31.4
SSD	343	70.9	21.7	30.6	1.09***	1.46***		352	257.8	36.0	14.0	1.22***	1.61*		27.5
Meering	12	57.1	11.4	19.9				12	176.0	13.8	7.8				32.4
90072	12	94.4	14.6	15.5	1.47***			12	268.2	28.5	10.6	1.88***			35.2
SSD	356	60.2	21.2	35.1	1.00**	1.13***		356	270.4	32.8	12.1	1.11***	1.57ns		22.3
Stylet	12	53.2	12.0	22.5				12	185.6	19.2	10.3				28.7
Westonia	12	90.1	11.6	12.8	1.40***			12	240.8	27.5	11.4	2.08***			37.4
DH	341	86.9	25.9	29.8	1.04***	1.02**		353	238.6	37.3	15.6	1.30***	1.69ns		36.4

ns – not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

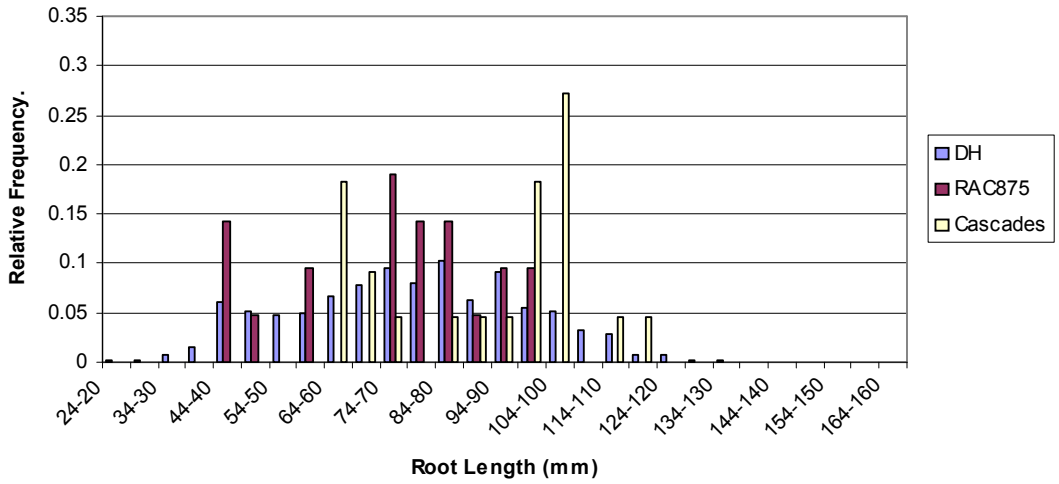
RAC875/Cascades (I)

The frequency distributions of the parents overlapped and indicated possible transgressive segregation (Figure 6.09). A high level of variance occurred in both the parents leading to a high environmental variance and low genetic variation. The observed variance of the double haploid population was found to fit the expected variance of the one gene model, with a narrow departure of the DHs (d value) from the mean of the parents (m value) (Table 6.12). A smaller parental variance was observed for the control treatment producing a lower environmental variance, however, poor germination led to only eleven seedling roots of each of the parents being measured and a lower than expected environmental value. The low environmental value and low d value led to the observed variance of the DHs being higher than the expected variance for both the one and two gene models (Table 6.13).

Quantitative trait analysis using Map Manager QTX identified several molecular markers significantly linked to a QTL for RRL on chromosome 7A associated with Cascades. Interval mapping indicates that the highly significant ($P<0.05$) LRS peaked between the markers ACTCAT42 and BCD873ADra, representing the most likely QTL position (Figure 6.10). The markers ACTCAT42 (LRS=16.4) and BCD873ADra (LRS=18.9) flanking the QTL explained 17 and 20% of the trait variation, respectively. Markers on chromosome 4A (Cascades) and 7D (RAC875) were also weakly linked to the RRL with an LRS of 11.7 (LOD=2.5) and 11.5(LOD=2.5), respectively, and each explaining 13% of trait variation (Table 6.14).

Analysis of the data for the mean root length in bicarbonate treatment (RL) of each line identified markers identical to the RRL markers on chromosome 7A. Markers on chromosomes 7A had a highly significant linkage with the RL with an LRS of 18.1 (LOD=3.9), explaining 19% of the trait variation. Similarly, markers for RL on chromosome 4A were identical to the RRL markers, but with a greater level of significance. Markers on chromosome 4A were significantly linked to the RL with an LRS of 16.0 (LOD=3.4), accounting for 17% of trait variation. A dwarfing gene (Rht1) was also identified as being significantly linked to the RL trait. The Rht1 marker on chromosome 4B was significantly linked to the RL with an LRS of 17.1 (LOD=3.7), explaining 18% of the variation for RL.

(a) RAC875/Cascades I



(b) RAC875/Cascades II

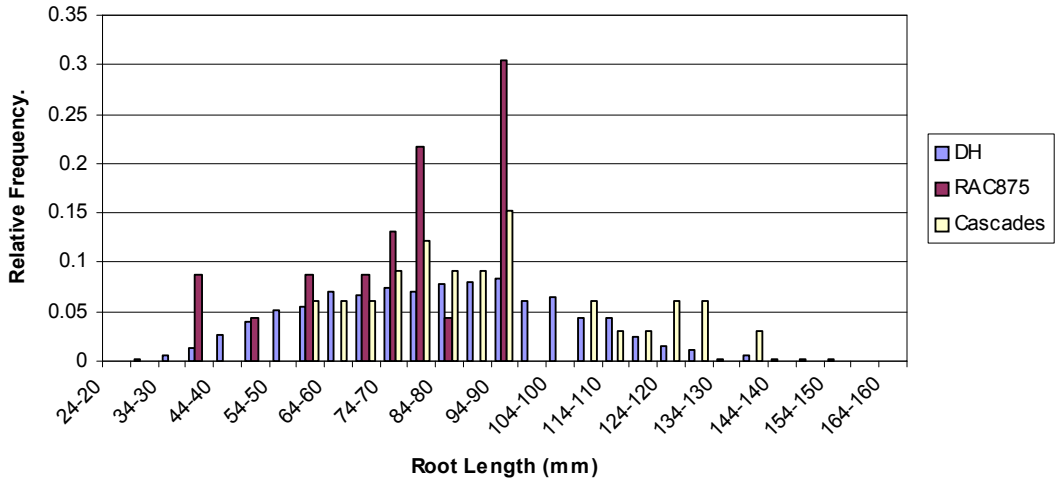


Figure 6:09: Frequency distribution of DH progeny and parentals from the cross RAC875/Cascades (a) I and (b) II for root length in bicarbonate solution.

The data for the mean root length in control treatment (RO) of each line was also found to have a highly significant linkage to the Rht1 marker associated with a dwarfing gene in RAC875. The Rht1 marker on chromosome 4B was significantly linked to the RO with an LRS of 22.3 (LOD=4.8), explaining 23% of the trait variation. A highly significant linkage was also identified between markers on chromosome 6D (RAC875) and the RO, with an LRS of 16.3 (LOD=3.5), explaining 17% of the variation for the RO. Two weakly linked markers for RO, associated with Cascades, were identified on chromosomes 1A and 3A, with an LRS of 12.2 (LOD=2.6) and 10.0 (LOD=2.2), explaining 14 and 11% of the trait variation, respectively.

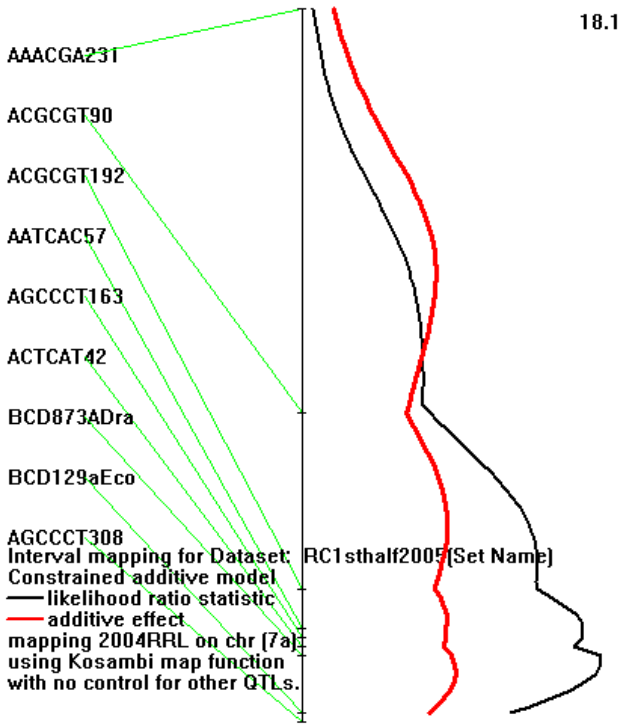


Figure 6.10: Interval map of chromosome 7A for relative root length in bicarbonate for the RAC875/Cascades (I) bread wheat population.

Table 6:12: Observed variance of parents and DH/SSD populations and the expected variance of the populations for one and two gene models from measurements of root length in bicarbonate treatment.

Cross			Observed variance				Estimated parameters			Expected variance		heritability
P_1		P_2	V_{P1}	V_{P2}	V_{PR}	CI	E	M	d	1 gene	2 gene	H^2
RAC875	x	Cascades I	347.5	324.2	431.2	400.9 - 465.3	335.9	77.6	8.8	412.4	374.1	22.1
		Cascades II	291.2	416.4	524.1	493.6 - 557.7	353.8	80.7	7.1	403.5	378.7	32.5
Worrakatta	x	Tam/Kal	167.2	190.0	428.1	-	-	-	-	-	-	-
Frame/Yarralinka/23/1	x	Pugsley	272.0	162.8	409.2	385.6 - 435.4	217.4	96.4	14.3	422.3	319.8	46.9
Berkut	x	Krichauff	167.5	240.4	362.1	343.1 - 385.3	204.0	103.5	16.3	467.9	335.9	43.7
Kukri	x	RAC875	282.1	138.7	456.1	404.7 - 518.7	210.4	80.8	17.3	510.5	360.4	53.9
		Excalibur	446.5	266.8	472.5	418.5 - 538.3	356.7	80.6	4.3	375.0	365.8	24.5
Meering	x	Yitpi	128.8	431.9	471.7	417.8 - 537.48	280.4	69.7	12.6	438.2	359.3	40.6
		90072	128.8	213.4	447.6	397.3 - 508.7	171.1	75.7	18.6	518.5	344.8	61.8
Stylet	x	Westonia	143.0	133.4	668.6	592.0 - 762.2	138.2	71.7	18.5	478.8	308.5	79.3

Table 6:13: Observed variance of parents and DH/SSD populations and the expected variance of the populations for one and two gene models from measurements of root length in control treatment.

Cross			Observed variance				Estimated parameters			Expected variance		heritability
P_1		P_2	V_{P1}	V_{P2}	V_{PR}	CI	E	M	d	1 gene	2 gene	H^2
RAC875	x	Cascades I	124.7	112.3	699.8	650.6 - 766.3	118.5	210.0	9.5	209.2	163.8	83.1
		Cascades II	865.5	860.1	1791.8	1687.5 - 1906.7	862.8	248.4	3.2	873.2	868.0	51.8
Worrakatta	x	Tam/Kal	912.8	929.5	1721.3	-	-	-	-	-	-	-
Frame/Yarralinka/23/1	x	Pugsley	1600.8	1351.8	1407.6	1326.3 - 1497.8	1476.3	277.9	2.4	1481.9	1479.1	-4.9
Berkut	x	Krichauff	868.1	857.1	1244.5	1179.2 - 1324.3	862.6	213.7	15.2	1095.3	978.4	30.7
Kukri	x	RAC875	834.3	735.6	1289.0	1143.6 - 1465.9	784.9	265.6	16.0	1040.1	912.5	39.1
		Excalibur	712.4	242.6	841.2	745.1 - 958.5	478.0	266.9	3.6	491.0	484.5	43.2
Meering	x	Yitpi	189.9	615.5	1296.9	1148.7 - 1477.8	402.7	219.1	43.1	2257.8	1330.3	68.9
		90072	189.9	811.4	1073.3	952.7 - 1219.8	500.6	222.1	46.1	2625.0	1562.8	53.4
Stylet	x	Westonia	367.1	753.8	1389.1	1229.9 - 1583.4	560.5	213.2	27.6	1323.1	941.8	59.7

RAC875/Cascades (II)

The second set of the same RAC875/Cascades double haploids produced similar results as the previous population with a highly significant correlation between the two populations for root length values (r=0.516***). In bicarbonate the mean root length of the double haploid population was between the parents, which were strongly overlapping with probable transgressive segregation (Figure 6.09). Parental variance and therefore the environmental variation estimate was again high, however, with a low d value, the observed variance of the DHs were higher than the expected variance for both the one and two gene models (Table 6.12). The control treatment had a parental variance more in line with the anticipated variance when compared to the previous DH population. However, the very low d value again led to the observed variance of the DHs being higher than the expected variance for both the one and two gene models (Table 6.13).

Quantitative trait analysis again identified several molecular markers significantly (P<0.63) linked to a QTL for RRL on chromosome 7A. Similar to the RAC875/Cascades (I) data, the LRS peaked between the markers ACTCAT42 and BCD873ADra, representing the

most likely QTL position (Figure 6.11). The markers ACTCAT42 (LRS=11.4) and BCD873ADra (LRS=11.8) flanking the QTL each explained 12% of the trait variation.

The marker BCD873Adra was again the location of the peak LRS for the bicarbonate data (RL). The BCD873Adra marker on chromosome 7A had a highly significant linkage to the RL with an LRS of 16.9 (LOD=3.6), explaining 17% of the trait variation. No other markers were found significantly linked to the RL data or the control data (RO).

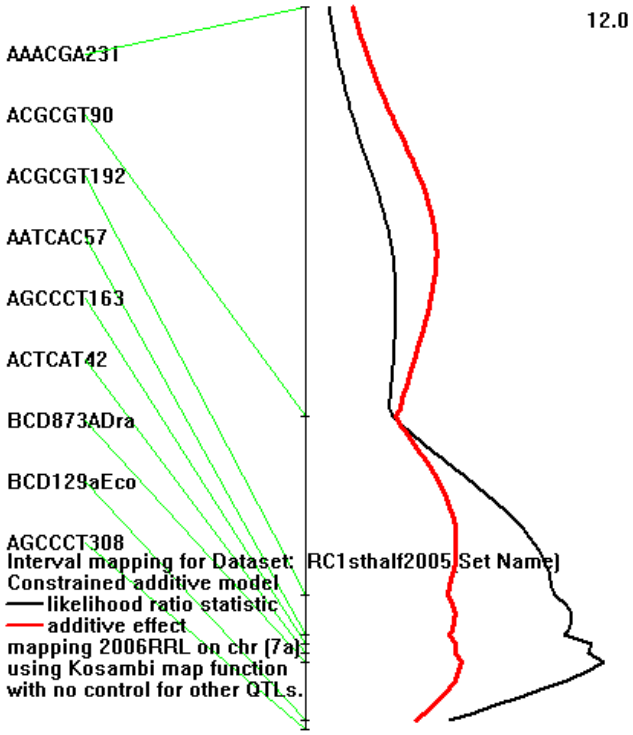


Figure 6.11: Interval map of chromosome 7A for relative root length in bicarbonate for the RAC875/Cascades (II) bread wheat population.

Wk/TmWLYY9//WLYY9Tm

The intermediate durum parentals TmWLYY9 and WLYY9Tm were not available for bicarbonate testing, preventing any genetic models from being fitted. Observations were made on the SSD population and the three commercial lines Worrakatta (25%), Kalka (WLYY9) (37.5%) and Tamaroi (37.5%), which genetically compose the population. The frequency distributions of the parentals and the SSD lines for the bicarbonate data

overlapped and indicated transgressive segregation (Figure 6.12). The observed variance of the population for both the bicarbonate and control treatments was twice the observed variance of the parentals, suggesting segregation for root length was occurring in the SSD population (Table 6.12 and 6.13).

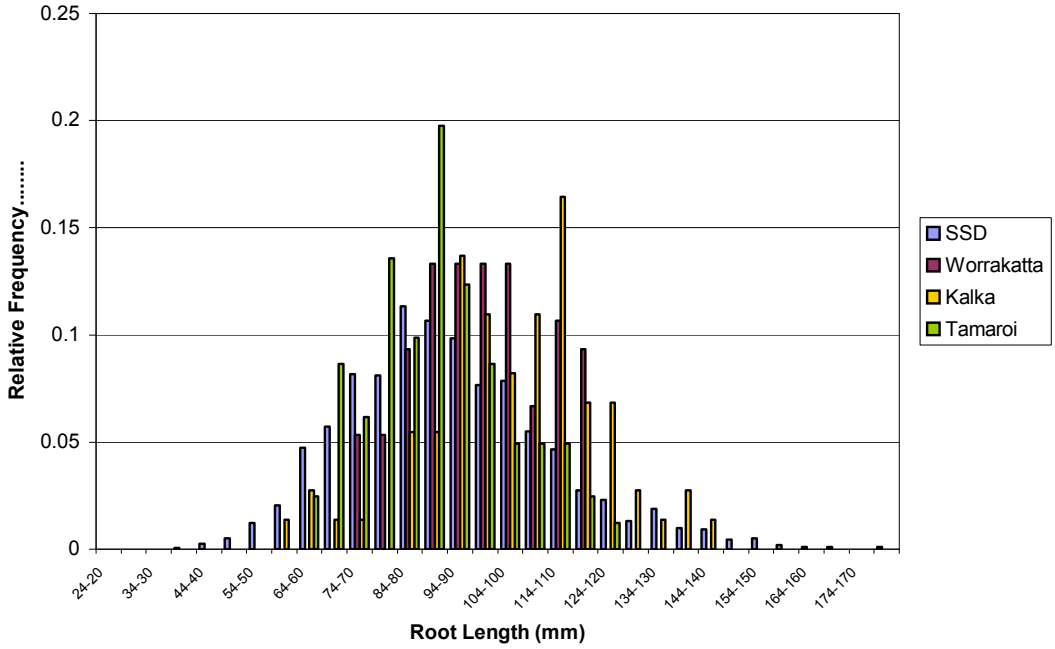


Figure 6:12: Frequency distribution of SSD progeny and parentals from the cross Wk/TmWLYY9//WLYY9Tm for root length in bicarbonate solution.

A frequency distribution for the relative root length (RRL) of each line indicated that two to three genes might be responsible for the bicarbonate tolerance response in the Wk/TmWLYY9//WLYY9Tm population (Figure 6.13). No lines were found to have a RRL greater than Worrakatta, Worrakatta appeared to have another gene compared to Kalka, and both were more tolerant than Tamaroi. No further analysis was conducted on the SSD population due to the inability to accurately separate the tolerance classes and the lack of parental information.

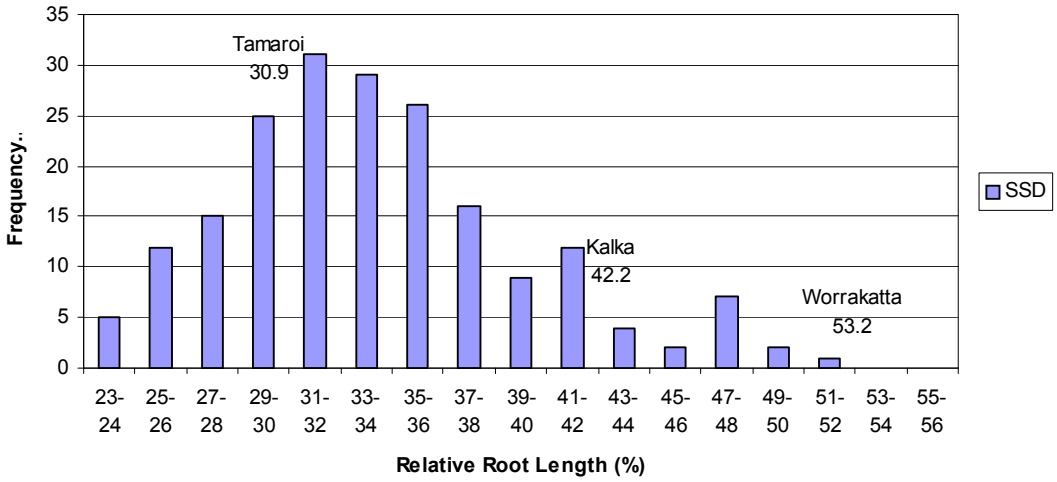


Figure 6:13: Frequency distribution of SSD progeny and parental means from the cross Wk/TmWLYY9//WLYY9Tm for relative root length (%) in bicarbonate solution.

Frame/Yarralinka//Pugsley

In bicarbonate treatment the frequency distributions of the parents, Frame/Yarralinka/23/1 and Pugsley, were partly overlapped and indicated possible minor transgressive segregation (Figure 6.14).

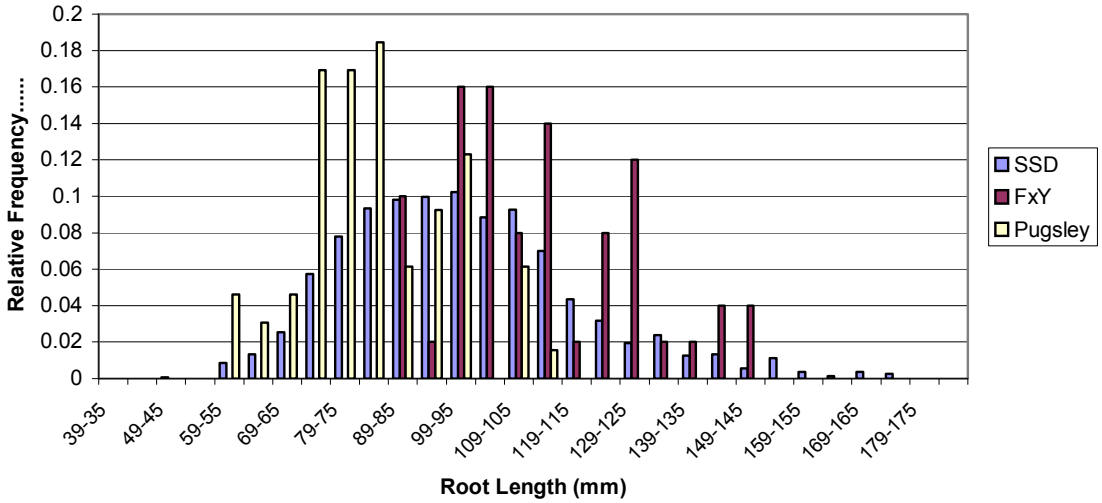


Figure 6:14: Frequency distribution of SSD progeny and parentals from the cross Frame/Yarralinka//Pugsley for root length in bicarbonate solution.

The observed parental variance was much lower than the observed variation of the SSD population and with a moderate separation of parents (d = 14.3), the observed variance was found to fit the expected variance of the one gene model (Table 6.12). The parental variance in the control treatment, however, was similar to the observed variance of the SSD population, and with a very small d value, no genetic segregation for root length was found to be occurring (Table 6.13). No molecular information was available on the Frame/Yarralinka//Pugsley SSD population.

Berkut/Krichauff

The frequency distributions for the bicarbonate data of the parents, Berkut and Krichauff, were partially overlapped and this indicated possible minor transgressive segregation (Figure 6.15). The parental variance was lower than the observed variation of the DH population, with a moderate separation of parents (d = 16.3), however, the observed variance was found to be between the expected variance of the one and two gene models (Table 6.12). The parental variance in the control treatment was also lower than the observed variance of the DH population, but with a low d value, the observed variance of the DHs were higher than the expected variance for both the one and two gene models (Table 6.13).

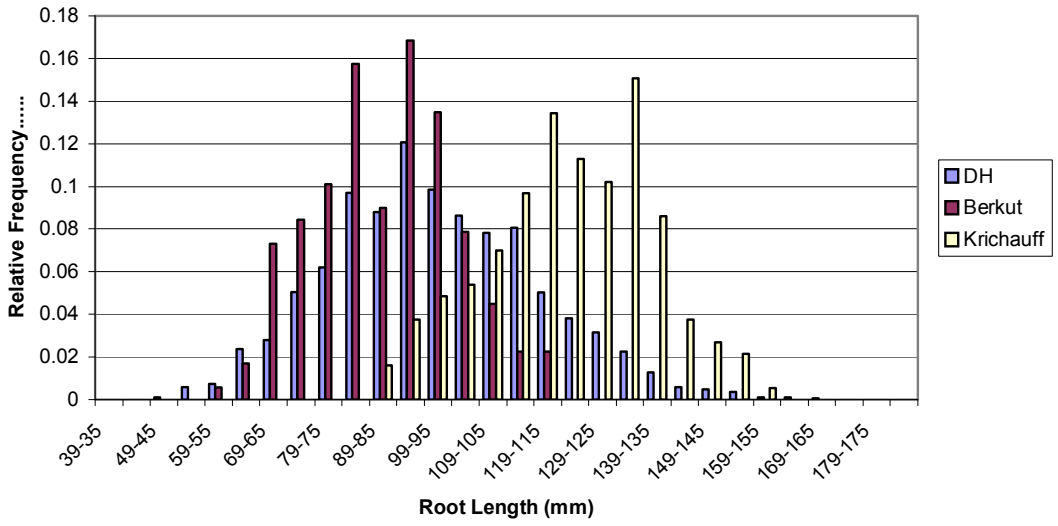


Figure 6:15: Frequency distribution of DH progeny and parentals from the cross Berkut/Krichauff for root length in bicarbonate solution.

The relative root length (RRL) was determined for each of the DH lines, with the mean RRL of the population found to be significantly different from both the parents (Table 6.14). The frequency distribution indicated that Krichauff and Berkut represent the upper and lower extremes of the RRL distribution, respectively (Figure 6.16). The tolerance to bicarbonate in the Berkut/Krichauff population appears to originate entirely from Krichauff.

Table 6.14: The number of lines tested, mean root length, and standard deviation of the RRL for parental and Double Haploids (DH) for the cross Berkut/Krichauff.

	Relative Root Length					
Line	No.	Mean	St Dev	Co-effic.	St Dev of Difference	
					P1	P2
Berkut	14	38.4	1.9	5.0		
Krichauff	14	59.0	4.0	6.8	0.65***	
DH	133	47.2	5.2	11.0	0.42***	0.57***

ns – not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

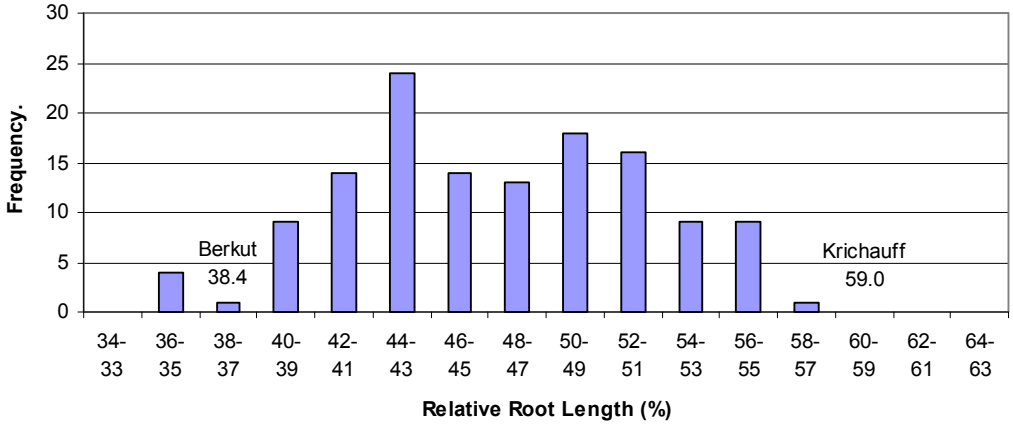


Figure 6:16: Frequency distribution of DH progeny and parental means from the cross Berkut/Krichauff for relative root length (%) in bicarbonate solution.

Quantitative trait analysis further supports the notion that the bicarbonate tolerance in the Berkut/Krichauff population is associated with the bicarbonate tolerant Krichauff parent. Several molecular markers were linked to a QTL for RRL on chromosome 7A. Interval mapping indicates that the highly significant (P<0.05) LRS peaked between the markers ksm019 and gwm292b, representing the most likely QTL position (Figure 6.17). The markers ksm019 (LRS = 22.3) and gwm292b (LSD = 18.7) flanking the QTL explained 15

and 14% of the trait variation, respectively. Markers on chromosome 2B were also weakly linked to the RRL with an LRS of 11.5 (LOD = 2.5), which explained 8% of trait variation.

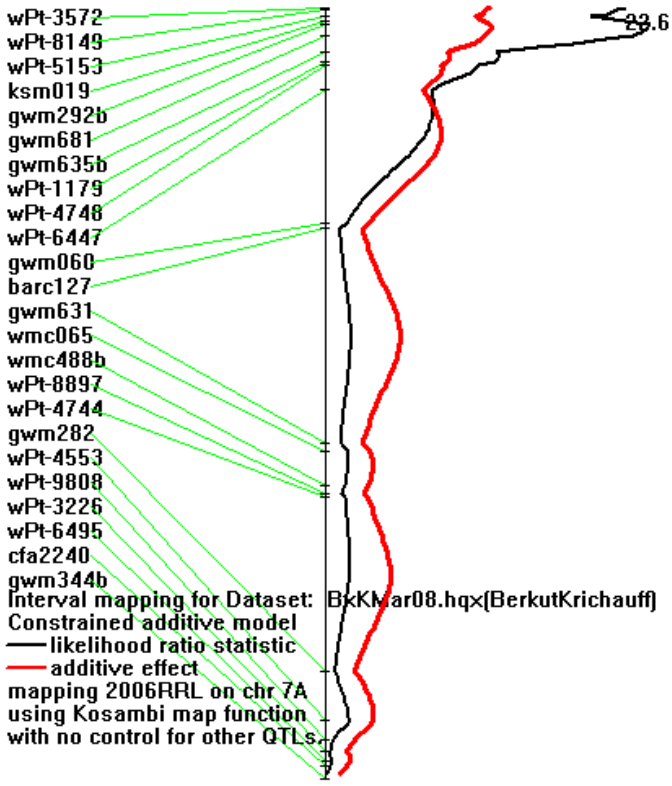


Figure 6.17: Interval map of chromosome 7A for relative root length in bicarbonate for the Berkut/Krichauff bread wheat population.

Analysis of the data for the mean root length in bicarbonate treatment (RL) identified markers on four chromosomes associated with the trait. Markers on chromosomes 3D, 6A and 7A, relating to Krichauff, were significantly linked to RL with an LRS of 13.7 (LOD=2.9), 13.6 (LOD=2.9), and 14.3 (LOD=3.1), respectively, and each accounting for 10% of trait variation. The 7A markers were identical to those identified for the RRL data. Markers on chromosome 7B, relating to Berkut, were also significantly linked to RL with an LRS of 17.4 (LOD=3.7) and explained 12% of the trait variation.

In the control treatment (RO), chromosome 4D from Krichuaff and 6D from Berkut were found to be highly associated with the data for the mean root length. Markers on chromosome 4D were linked highly significantly with RO with an LRS of 20.5

(LOD=4.4), explaining 14% of the trait variation. Similarly, markers on chromosome 6D had a highly significant linkage with the RO with an LRS of 23.0, accounting for 16% of the trait variation.

Population	Trait	Marker	Chromosome	LRS	% variation	Origin
RAC875/Cascades I	RRL	ACTCAT42	7A	16.4	17	Cascades
		BCD873ADra	7A	18.9	20	Cascades
		PSR115ADra	4A	11.7	13	Cascades
		ABG704BBam	7D	11.5	13	Cascades
	RL	ACTCAT42	7A	12.9	14	Cascades
		BCD873ADra	7A	18.1	19	Cascades
		WMC161	4A	16.0	17	Cascades
		Rht1mut	4B	17.1	18	RAC875
	RO	Rht1mut	4B	22.3	23	RAC875
		GDMO98	6D	16.3	17	RAC875
		ACGCAC353	1A	12.2	14	Cascades
		ACACCA205	3A	10.0	11	Cascades
RAC875/Cascades II	RRL	ACTCAT42	7A	11.4	12	Cascades
		BCD873ADra	7A	11.8	12	Cascades
	RL	BCD873ADra	7A	16.9	17	Cascades
Berkut/Krichauff	RRL	ksm019	7A	22.3	15	Cascades
		Gwm292b	7A	18.7	14	Cascades
		Gwm148	2B	11.5	8	Krichauff
	RL	wpt-9488	3D	13.7	10	Krichauff
		wpt-7063	6A	13.6	10	Krichauff
		wpt-3573	7A	14.3	10	Krichauff
		barc258	7B	17.4	12	Berkut
	RO	wpt-2378	4D	20.5	14	Krichauff
		gpw95010	6D	23.0	16	Berkut

Table 6.14: Molecular markers associated with the traits RRL, Carbonate (RL) and Control (RO) treatments, their chromosome locations, the Likelihood Ratio Statistic (LRS), and percent variation explained, in the populations RAC875/Cascades and Berkut/Krichauff.

Kukri/RAC875, Kukri/Excalibur, Meering/Yitpi, Meering/90072, and Stylet/Westonia

In all five populations the SSD or DH progeny were found to have a significantly different mean root length in bicarbonate treatment than either of their parents (Table 6.11). However, the mean root length of the DH population for Kukri/Excalibur was greater than

the mean root length of both parents. Similarly, in the control treatment the mean root length of DH population for Kukri/Excalibur was less than the mean root length of both parents. In the four other populations, the DH/SSD root length means for the bicarbonate and control treatments were between the parents.

In bicarbonate treatment the parental variances were lower than the observed variation of the DH/SSD populations with a moderate separation of parents (d=12.6 to 18.6), except for Kukri/Excalibur (d=4.3). Only the populations Kukri/RAC875 and Meering/Yitpi had an observed variance that was found to fit the expected variance of the one gene model (Table 6.12). In the control treatment the separation of mean root lengths for the parents varied from 3.6 to 46.1mm (d value) between the five populations, although only Stylet/Westonia and Meering/Yitpi had an observed variance that was found to fit the expected variance of the one and two gene models, respectively (Table 6.13).

6.4.4 Discussion

Similar to the F_2 and F_3 populations, the high bicarbonate screening intensities, indicated by low RRL, reduced the separation of parental distributions and led to overlapping data spreads for all of the populations tested. The inability to divide the SSD/DH population distributions into discrete classes prevented chi-square analysis from being performed and instead a simple quantitative model was fitted to the data. The model contained only additive effects and environmental error, with no assessment of unequal gene effects, epistasis, linkage or genotype-environment interactions. However, given the spread of the SSD/DH distributions and the multiple factors likely to be involved in the reduction of root length in the bicarbonate treatment, many of these genetic effects are expected to be occurring in the population. The results represent only an initial assessment of the number genes potentially involved in the bicarbonate tolerance response.

RAC875/Cascades (I)

The root length in bicarbonate treatment (RL) of the RAC875/Cascades (I) DH population was found to fit the model for one gene, although the result was strongly influence by high environmental error (E) and low additive effects (d value). The population and parentals had been sourced from material that had previously been grown for three years in off-site

field trials, exposing the lines to some level of seed mixing from mechanical seeding and harvesting operations. The high variability of the parental root lengths and the uneven distributions were likely caused by seed contamination and/or generational shifts in the lines. In the control treatments (RO), however, the variation in root length was very low in comparison to the bicarbonate treatment. Poor germination resulted in only eleven seedling roots of each parent being measured, reducing the ability to accurately assess the parental response in relation to the population and leading to neither the one or two genes model fitting the data.

The quantitative trait analysis using mapping software, however, identified multiple loci associated with root length in both bicarbonate and control treatments. Highly significant QTLs for root length in bicarbonate were located on chromosome 7A, 4A and 4B. The loci on 4B mapped to the marker for the dwarfing gene Rht1, known to have segregated in the DH population. The Rht1 gene was also identified as being significantly associated with the root length in the control treatment, along with another locus on chromosome 6D. So the loci of particular interest are the locus on chromosome 7A and locus on chromosome 4A, which were associated with both the root length in bicarbonate and the relative root length (RL/RO*100). In the RAC875/Cascades (I) population two loci appear to be responsible for the bicarbonate tolerance response originating from the Cascades parent.

A number of other loci were also identified as having weak associations with root length in the control on chromosomes 1A and 3A, and for relative root length on chromosome 7D. However, with a small DH population of 93 lines, QTLs can falsely be identified as significant (Lande and Thompson 1990, Bernardu 2004), so further analysis would be needed to ascertain whether the weakly associated QTLs were involved in the bicarbonate tolerance response.

RAC875/Cascades (II)

A second RAC875/Cascades DH population (II) was sourced from a collection that had been grown in research facilities on-site and was expected to be a more pure form of the same RAC875/Cascades (I) population. Parental variance, however, was still relatively high and when combined with a low additive component, the observed variance of the

population did not fit the one or two genes model for root length in either bicarbonate or the control treatments.

In the QTL analysis of the RAC875/Cascades (II) population, only one locus was found to have a highly significant linkage to root length. A locus on chromosome 7A was associated with root length in the bicarbonate treatment and the relative root length of the DH lines. The locus was also located in the identical position to the QTL found for the RAC875/Cascades (I) population. No QTLs were found to be significantly associated with the root length in the control treatments.

The difference in the results between the two RAC875/Cascades populations may have occurred due to several reasons. In population II the number of replications was increased, particularly for the control treatments, which would have reduced the chances of identifying false QTLs. The Rht1 locus was again expected to be identified in population II, but for unknown reasons the Rht1 locus was not found to be associated with root length for RL or RO. The longer mean root lengths in both the bicarbonate and control treatments from more rapid and less stressed growth may have been associated with the inability to identify a locus connected with Rht1 after 10 days growth.

From the two loci that had a highly significant linkage to the bicarbonate tolerance response in population I, only one locus on chromosome 7A was identified in population II. The main difference between the two populations, which may have led to the inconsistency in results, was in the bicarbonate treatments applied. The bicarbonate and control treatments used for population I did not contain zinc sulphate or boric acid, unlike the treatment for population II. The effect of chromosome 4A on root length may be associated with a Zn deficiency response commonly related to bicarbonate toxicity (Section 2.5.4), which would not have occurred in the treatment for population II. In general the RAC875/Cascades population appears to have a major locus involved in tolerance to bicarbonate on chromosome 7A, and possibly a second significant locus on chromosome 4A.

Berkut/Krichauff

The Berkut/Krichauff population was a more recent DH population, undergoing fewer generations of seed production, which created less environmental error (E). The population also had a greater separation of parental means for root length in bicarbonate, although the distributions for root length in bicarbonate (RL) were again partly overlapped and the DH distribution was transgressive. With transgressive segregation the additive genetic models for one and two genes were not expected or found to fit the observed data. The inability to fit the observed data to the one and two genes models is consistent with the QTL analysis, which identified four significant loci linked to root length in bicarbonate. Loci identified on chromosomes 7A, 6A and 3D from Krichauff and chromosome 7B from Berkut, also accounted for the transgressive segregation observed in the population distribution.

In the control data chromosome 4D from Krichauff and 6D from Berkut appear equally responsible for the root length response. However, bicarbonate tolerance in relation to the relative root length (RRL) is entirely associated with Krichauff, which was indicated by the RRL distribution and the identification of loci on chromosomes 7A and 2B linked to the RRL. The highly significant linkage of RRL and RL with chromosome 7A, similar to the RAC875/Cascades population, suggests a major role for a locus on 7A in the bicarbonate tolerance response. The tolerance to bicarbonate identified in both Cascades and Krichauff can be traced to a common tolerant ancestor, Aroona, which may be the original source of the QTL on chromosome 7A (Section 5.3). Further molecular assessment is being conducted to identify if the QTL on chromosome 7A in the RAC875/Cascades and Berkut/Krichauff population correspond to the same locus.

Frame/Yarralinka//Pugsley and Wk/TmWLYY9//WLYY9Tm

Two other populations were screened, Frame/Yarralinka//Pugsley and Wk/TmWLYY9//WLYY9Tm, however, no molecular mapping information was available on the lines, with only one and two gene additive models fitted. The root length in bicarbonate of the Frame/Yarralinka//Pugsley population was found to fit the one gene model, although with indications of transgressive segregation, overlapping parental distributions and moderate environmental error, other minor genes are also likely to be present. Interestingly, the bicarbonate tolerant parent Frame/Yarralinka/23/1 has strong

ancestral connections to Aroona/Warigal through both Frame and Yarralinka, while the less tolerant parent Puglsey has a more distant connection via Frame (parent of Pugsley).

The genetic models could not be fitted to the Wk/TmWLYY9//WLYY9Tm durum population since the population originated through SSD from a top cross and no seed of the TmWLYY9 and WLYY9Tm lines was available for bicarbonate screening. The distribution for root length in bicarbonate of the SSD population suggested that segregation for bicarbonate tolerance was occurring in comparison to the bread wheat parent Worrakatta (Wk) and durum parents Tamaroi (Tm) and Kalka (WLYY9). Observations of the RRL distributions indicated that no lines were more tolerant than Worrakatta, but that the bicarbonate tolerance associated with the bread wheat Worrakatta could be transferred to durum lines, since a proportion of the population had a greater tolerance than both the durum parents Kalka and Tamaroi. The absence of lines more tolerant than Worrakatta also suggests that tolerance in Kalka, above that of Tamaroi, has similar genes to Worrakatta. Worrakatta, a sister line to Krichauff, would also be expected to carry the 7A locus. However, given the spread to the RRL data in relation to the parentals, at least three genes appear to be segregating in the Wk/TmWLYY9//WLYY9Tm population.

Kukri/RAC875, Kukri/Excalibur and Stylet/Westonia

A further five sample populations were bicarbonate tested for future assessment and QTL analysis, since Kukri/RAC875, Kukri/Excalibur and Stylet/Westonia had prior mapping data. The Kukri/Excalibur population had little segregation for root length in the bicarbonate or control treatments and would not be useful for further assessment. The Stylet/Westonia population did appear to segregate for root length in bicarbonate and control treatments, but only the control treatment was found to fit the genetic model for one gene. In contrast, segregation was not identified in the control for the Kukri/RAC875 population, but in the bicarbonate treatment the observed variance for root length did fit the one gene model. From the three DH populations the Kukri/RAC875 population appears to be the most valuable for further analysis. The Kukri/RAC875 population may offer a source of bicarbonate tolerance not associated with the Aroona family, but the bicarbonate tolerant line Pitic62.

Meering/Yitpi and Meering/90072

The two Meering populations were developed by SSD and have no prior mapping data. However, the Meering/Yitpi and Meering/90072 populations segregate strongly for root length in bicarbonate and control treatments. The Meering/Yitpi population was found to fit the two genes model for both bicarbonate and control root lengths. The Meering/90072 population did not fit the genetic models, however, the data suggests that more than two genes are likely segregating in the population. Meering and Yitpi both have ancestral connections to Aroona (or WW-15), and to a lesser extent Yitpi to Pitic62. The Meering populations may provide further insight into the origins of bicarbonate tolerance in current bread wheats.

General comments

In general, bicarbonate tolerance appears to be controlled by both major and minor genes. The bicarbonate tolerance response recorded involves a number of tolerance mechanisms either directly (HCO_3^- toxicity) or indirectly (Zn or Fe efficiency). However, since the response to bicarbonate toxicity was measured as a reduction in root growth, other genetic mechanisms such as root vigour, root morphology or plant height, may be confounding the results. What had been shown though, is that bicarbonate tolerance is heritable, several loci are responsible for the bicarbonate response, similar genes are likely present in both durum and bread wheats, and that the bicarbonate tolerance measured in bread wheats can be transferred to durum wheats.

Chapter 7

Field evaluation of bread and durum wheat populations segregating for tolerance to bicarbonate.

7.1 Introduction

In genetic studies for abiotic stress factors, extensive research is often conducted under glasshouse or laboratory conditions, with few field results until late in the project. In many cases the results of laboratory screens have not translated to a yield advantage in the field (e.g. Na exclusion, Rathjen *pers. com*), particularly in the last few years with decreasing rainfall (Appendix 7). In the field, bicarbonate toxicity is poorly defined and often confounded with other stresses and wet soils. Interactions between salinity, boron and high pH (bicarbonate) occur readily in the field, which can lead to one stress masking another or shifts in the critical values for toxicity. Bicarbonate toxicity has also been associated with inducing various nutrient deficiencies, such as, Zn and Fe, which may be more detrimental than the direct effects of HCO_3^- toxicity. The purpose of laboratory screens is to reproduce the field response in a more cost-effective and timely manner. The aim of this chapter was to assess the influence of both pH (bicarbonate) and EC (salinity) on grain yield using three different homozygous populations to determine if bicarbonate tolerance identified in the homozygous populations was related to increased grain yield.

7.2 Evaluation of the DH bread wheat population RAC875/Cascades.

7.2.1 Introduction

In the previous chapter RAC875/Cascades was found to segregate for bicarbonate tolerance, with a significant QTL on chromosome 7A, which was associated with longer roots in a strongly alkaline bicarbonate treatment. A second locus on chromosome 4A was also identified as a possible bicarbonate tolerance QTL in the absence of trace elements Zn and B, while the *Rht1* locus and a locus on chromosome 6D were associated with root length in the mildly alkaline control treatment.

Under neutral pH soil conditions, the bicarbonate tolerant parent Cascades is often out-yielded by the less tolerant parent RAC875, but in highly alkaline soils the difference in yield between Cascades and RAC875 is marginal (Section 4.2.3). Few correlations had been previously identified between soil pH or EC (0-10cm and 40-50cm) and yield for either Cascades or RAC875 at alkaline sites, except at Buckleboo (Exp.2 Section 4.2.3). Yield was found to decrease with increasing topsoil pH for the less bicarbonate tolerant variety RAC875, but further evidence was not obtained to support the Buckleboo data. The aim of this section was to assess the RAC875/Cascades DH population under alkaline field conditions at multiple sites to determine if the segregation for longer root lengths in bicarbonate treatment was associated with increased grain yield.

7.2.2 Materials and Methods

Seed of the RAC875/Cascades (I) double haploid (DH) lines was sourced from 2003 field trials of the Durum Breeding group, University of Adelaide. In 2004 the RAC875/Cascades population was sown in the field at eight sites, BR, WJ, RAC, CS, WC, KH, RH and AV. However, due to barley regrowth at RH, and poor germination at AV, results were only recorded from six sites. In 2005 trials were again sown at eight sites, AV, BR, CK, CS, KH, RAC, RH, and WJ, with seed sourced from the 2004 field trails.

The field experiment consisted of 81 of the RAC875/Cascades DH lines (originally 93 lines) in two replications (total 162), with 45 parental check plots and 18 standard varietal plots, giving a total experiment size of 225 plots.

The field trials were sown, managed, scored and harvested as per the methods in section 3.1.2.

Seed for bicarbonate testing was sourced from RAC field trials, either 2004 or 2005, for comparison with their corresponding field trial. The bicarbonate test for the 2004 and 2005 seed followed the procedures outlined in section 3.3 and 5.2.

Twenty-four seeds of the ninety-three DH lines were arranged into four completely randomised replications (trays), with three seeds for each DH lines per tray, and two treatments (bicarbonate and control).

In 2004 soil samples were collected from three sites, BR, WJ and RAC, as described in section 3.1.4.

Measurements of soil pH and $EC_{1:5}$ were correlated for the three 2004 sites, RAC, WJ and BR. The yield (g/plot) was correlated with the mean soil values of the plot. The lines were also separated into height and maturity classes and the yield correlations re-calculated on the basis of these grouping. Bicarbonate root length data (bicarbonate, control and RRL) was correlated with relative yield (% control) at five of the sites, BR, CK, WJ, RAC and WC.

In 2005 no soil data was recorded for the RAC875/Cascades population, but yield was measured at the eight sites, AV, BR, CK, CS, KH, RAC, RH, and WJ, and relative yield correlated with the bicarbonate data.

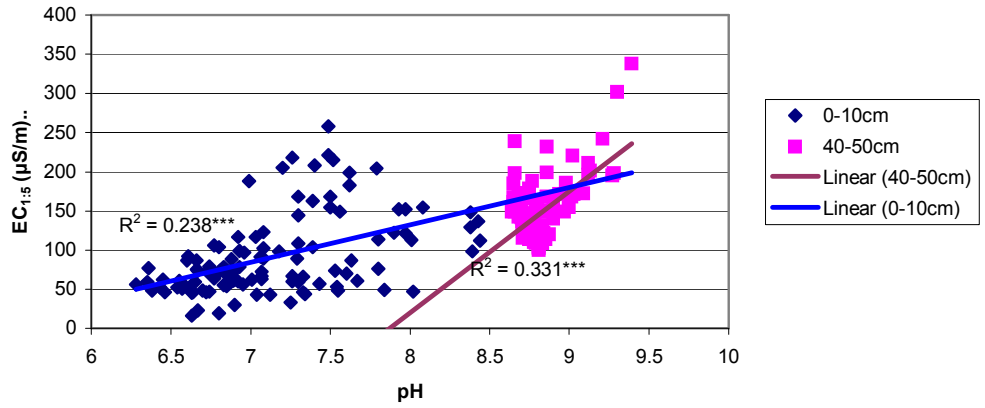
7.2.3 Results

At the three sites at which detailed soil sampling was undertaken in 2004, subsoil pH (40-50cm) reached a level (>pH8.5) that is considered to influence growth, with two sites, BR and RAC, having extreme pH values >pH 9.2 where little root growth was expected to occur (Figure 7.01). The BR site was the only site to have topsoil pH values >pH 8.5. Wanderah and Buckleboo had highly toxic subsoil $EC_{1:5}$ levels (<400µS), with only the Wanderah site having toxic topsoil $EC_{1:5}$ levels.

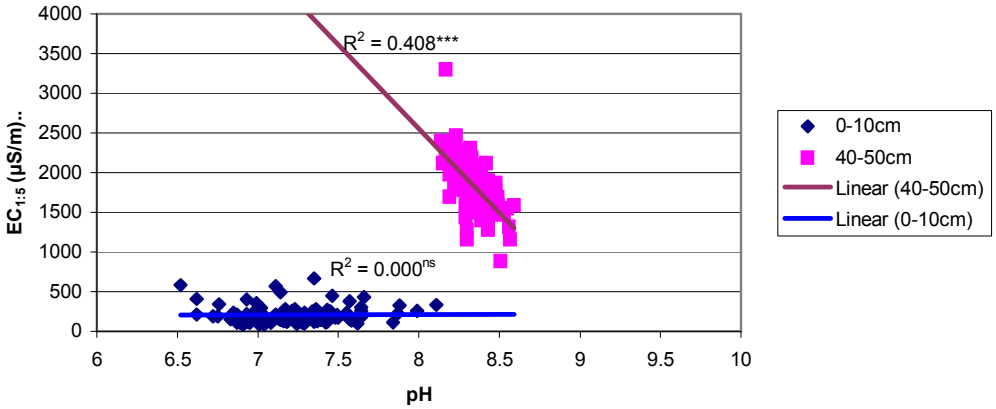
Correlation of soil pH and $EC_{1:5}$ values with grain yield in 2004 at RAC, WJ and BR.

When topsoil and subsoil pH and $EC_{1:5}$ values where correlated with grain yield (g/plot) for each of the RAC875/Cascases lines at each site, only the Wanderah site was found to have significant associations (Table 7.01). At Wanderah increasing soil pH (40-50cm) and $EC_{1:5}$ (0-10cm) was found to significantly decrease grain yield. In contradiction, increasing soil $EC_{1:5}$ (40-50cm) was found to significantly increase grain yield, although a negative association had been identified between subsoil pH and EC (Figure 7.01b).

(a) Roseworthy



(b) Wanderah



(c) Buckleboo

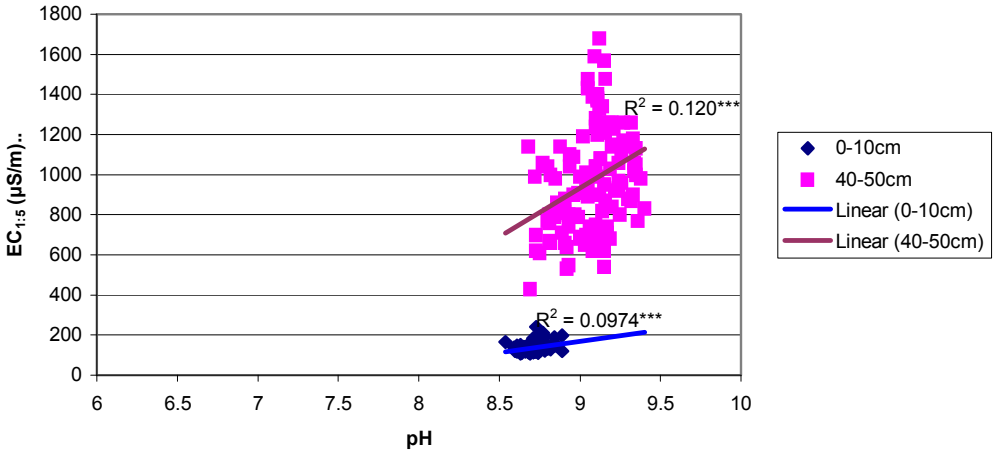


Figure 7.01: Correlation between soil pH and $EC_{1:5}$ at 0-10cm and 40-50cm depths for the sites, (a) Roseworthy, (b) Wanderah, and (c) Buckleboo.

No significant correlations were identified at Roseworthy or Buckleboo between soil pH and grain yield even though subsoil pH had reached levels regarded as highly toxic (pH >9.2). Similarly, no significant correlations were identified at Buckleboo between soil $EC_{1:5}$ and grain yield even through subsoil EC had reached toxic levels (<400µS/m).

Table 7.01: Soil pH and $EC_{1:5}$µS/m at depths 0-10cm and 40-50cm correlated with plot yield (g) for the sites, Roseworthy (RAC), Wanderah (WJ) and Buckleboo (BR) in 2004.

		Yield (g/plot)		
		RAC	WJ	BR
pH	0-10cm	-0.047^{ns}	0.060^{ns}	0.087^{ns}
	40-50cm	0.053^{ns}	-0.169**	0.047^{ns}
$EC_{1:5}$	0-10cm	-0.091^{ns}	-0.236***	-0.009^{ns}
	40-50cm	0.045^{ns}	0.204**	0.040^{ns}

ns not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

In the RAC875/Cascades population two dwarfing genes were known to be segregating, one from each of the semi-dwarf RAC875 and Cascades parents, producing dwarf, semi-dwarf and tall growth habits in the DH progeny. Plots were scored for height at two sites, Roseworthy and Wanderah, to determine if the plant height was associated with differences in plot yield or the assessment of soil pH and EC effects on yield.

The calculation of the mean grain yield for dwarf, semi-dwarf and tall types for each site found that the semi-dwarf types were higher yielding than the tall types which were both higher yielding than the dwarf types at all sites. However, while the dwarf types were lower yielding at all sites, the difference between semi-dwarf and tall types was smaller at the lower yielding sites (WJ and BR), and significantly different only at WJ (Table 7.02).

Correlations between soil pH and EC and grain yield (g/plot) for each of the height classes identified few significant relationships (Table 7.03). Wanderah was again found to have a significant negative correlation between soil pH (40-50cm) and grain yield, while having a significant positive correlation between soil $EC_{1:5}$ (40-50cm) and grain yield, although only for the tall types. Roseworthy was also found to have a weakly significant negative correlation between soil $EC_{1:5}$ (0-10cm) and grain yield, although topsoil EC at RAC was not in the range considered toxic to plants.

Table 7.02: Mean yield (g/plot) and standard deviation of the height classes, dwarf, semi-dwarf and tall, at the sites, Roseworthy (RAC), Wanderah (WJ) and Buckleboo (BR) in 2004.

		Yield (g/plot)					
		RAC		WJ		BR	
Height	No. Lines	Mean	St. Dev.	Mean	St. Dev.	Mean	St.Dev.
Dwarf	18	282.47	148.23	254.94	122.66	69.70	31.22
Semi-dwarf	36	456.51	120.10	351.21	101.98	106.96	36.41
Tall	27	335.52	97.07	312.28	101.21	104.22	21.78
Total	81	358.17	121.80	306.14	105.28	93.63	29.80
l.s.d (0.05) =		48.6		38.8		12.6	

Table 7.03: Soil pH and $EC_{1:5}$ at depths 0-10cm and 40-50cm correlated with plot yield (g) for the sites, Roseworthy (RAC), Wanderah (WJ) and Buckleboo (BR) in 2004, for the height classes, Dwarf (D), Semi-dwarf (S) and Tall (T).

		Yield (g/plot)								
		RAC			WJ			BR		
		D	S	T	D	S	T	D	S	T
pH	0-10cm	-0.103^{ns}	-0.030^{ns}	0.257^{ns}	0.185^{ns}	-0.081^{ns}	0.160^{ns}	-0.017^{ns}	0.026^{ns}	-0.172^{ns}
	40-50cm	-0.264^{ns}	0.057^{ns}	0.017^{ns}	-0.236^{ns}	0.144^{ns}	-0.388**	0.062^{ns}	0.091^{ns}	0.039^{ns}
$EC_{1:5}$	0-10cm	-0.405*	-0.131^{ns}	0.063^{ns}	-0.210^{ns}	-0.181^{ns}	-0.219^{ns}	-0.122^{ns}	-0.081^{ns}	0.014^{ns}
	40-50cm	-0.014^{ns}	-0.035^{ns}	0.182^{ns}	0.138^{ns}	-0.005^{ns}	0.429**	0.198^{ns}	0.030^{ns}	0.082^{ns}

ns not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

During September large differences were apparent in the maturity of lines within the RAC875/Cascades population. Maturity scores where therefore recorded throughout September and October to determine if the maturity of the different lines was influencing plot yield or the assessment of soil pH and EC effects on yield.

The calculation of the mean for early, mid, late and very late maturing classes found that plant maturity significantly influenced the final grain yield (Table 7.04) and was closely associated with climate.

At Roseworthy above average rainfall was recorded from June to September, however, in October the monthly rainfall was well below average with only 5.2mm recorded (Appendix 2). October also had an observed maximum temperature of 2.7°C higher than expected, and with temperatures extreme >42°C on the 12th, when the grain fill of the mid maturity types would have been affected. Rainfall in the first week of November (38.4mm)

would have benefited the late maturing types, before temperatures again reach >40°C at the end of November.

Wanderah had a similar weather pattern, although with milder temperatures and lower rainfall (Appendix 2). Wanderah recorded above average rainfall for June to August, before diminishing to below average in September and October, with only 8.6mm recorded for October. October rainfall was also accompanied by an observed maximum temperature 2.1°C higher than expected for the month. Early maturing types at Wanderah were comparable in grain yield to the mid and later maturing types, although the very late types would have been severely affected by low rainfall and temperature >40°C.

The maximum observed temperatures were again higher than expected (3.0°C) at Buckleboo (Kimba) for October, although remained below 40°C (Appendix 2). Rainfall was average from June to August, but low in September and October, with only 0.2mm recorded in October. The absence of significant rainfall from January to August resulted in minimal stored subsoil moisture so that later maturing types were severely affected by low available soil moisture.

Table 7.04: Mean yield (g/plot) and standard deviation of the maturity classes, early, mid, late and very late, at the sites, Roseworthy (RAC), Wanderah (WJ) and Buckleboo (BR) in 2004.

		Yield (g/plot)					
		RAC		WJ		BR	
Maturity	No. Lines	Mean	St. Dev.	Mean	St. Dev.	Mean	St.Dev.
Early	25	386.54	133.09	328.02	112.05	120.66	28.22
Mid	26	376.40	130.36	332.44	102.71	108.40	21.97
Late	21	404.64	142.42	322.67	102.07	76.17	25.91
Very Late	9	293.06	163.99	248.38	86.94	50.67	24.86
Total	81	365.16	142.47	307.88	100.94	88.98	25.24
	l.s.d (0.05) =	76.2		57.7		14.0	

Correlations between soil pH and EC and grain yield (g/plot) for each of the maturity classes again identified few significant relationships (Table 7.05). Within the maturity classes at Wanderah there was not a significant relationship between grain yield and soil pH (40-50cm) or $EC_{1:5}$ (40-50cm) as previously identified. However, a significant negative correlation between topsoil $EC_{1:5}$ (0-10cm) and grain yield was again found, consistent

with the adverse effects of magnesia patch (surface salinity) at the site, although only for the mid maturity types.

The grain yield of mid maturity types were also significantly correlated with soil pH and EC at the Roseworthy site. However, correlations between soil pH (40-50cm) and $EC_{1:5}$ (40-50cm) with grain yield were positive, even though subsoil pH was in the moderately to highly alkaline range and $EC_{1:5}$ was below the toxic range. Multiple linear regression analysis found that pH (40-50cm) alone accounted for 12.5% of the variation in grain yield.

Table 7.05: Soil pH and $EC_{1:5}$ at depths 0-10cm and 40-50cm correlated with plot yield (g) for the sites, Roseworthy (RAC), Wanderah (WJ) and Buckleboo (BR) in 2004, for the maturity classes, early, mid, late and very late.

		Yield (g/plot)											
		RAC				WJ				BR			
		Early	Mid	Late	Very late	Early	Mid	Late	Very late	Early	Mid	Late	Very late
pH	0-10cm	0.156^{ns}	-0.314*	-0.009^{ns}	0.097^{ns}	0.050^{ns}	0.003^{ns}	0.186^{ns}	0.094^{ns}	0.263^{ns}	-0.230^{ns}	-0.183^{ns}	-0.068^{ns}
	40-50cm	-0.062^{ns}	0.377*	-0.003^{ns}	-0.414^{ns}	-0.216^{ns}	-0.140^{ns}	0.054^{ns}	-0.138^{ns}	0.088^{ns}	0.033^{ns}	0.187^{ns}	-0.304^{ns}
$EC_{1:5}$	0-10cm	-0.132^{ns}	-0.084^{ns}	-0.124^{ns}	-0.186^{ns}	-0.188^{ns}	-0.277*	-0.224^{ns}	-0.251^{ns}	0.049^{ns}	-0.247^{ns}	-0.069^{ns}	-0.010^{ns}
	40-50cm	-0.103^{ns}	0.304*	0.214^{ns}	-0.295^{ns}	0.222^{ns}	0.272^{ns}	-0.036^{ns}	0.288^{ns}	0.188^{ns}	-0.126^{ns}	0.042^{ns}	0.121^{ns}

ns not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

Correlation of bicarbonate tolerance with relative grain yield at all six sites in 2004

The relative yield of the lines (% check variety) was compared to the root lengths in bicarbonate (RL) and control (RO) treatments, and to the relative root lengths (RRL) to identify significant correlations. The relative grain yield of five sites, Buckleboo (BR), Claypans (CK), Wanderah (WJ), Roseworthy (RAC) and Winulta (WC) was significantly correlated with RO, RL or RRL, at each site (Table 7.06).

The highly alkaline (pH 8.8-9.2) Buckleboo site had a positive correlation between relative grain yield and the root measurements RO, RL and RRL. The grain yield at Wanderah was found to have a significant positive relationship for only the RO data, although subsoil pH was lower, in the mildly alkaline range of pH 8.2-8.6. Similarly, grain yield at Claypans (pH 8.2-8.4) was found positively correlated with RO, and also RL, while grain yield at Winulta (pH 7.8-8.4) was positively correlated with only RL. The Roseworthy site had

contrary results, since a significant negative correlation was identified between relative grain yield and RRL, even though the subsoil was highly alkaline (pH 8.7-9.3).

Table 7.06: Correlation between relative grain yield (% control) at the sites Buckleboo (BR), Claypans (CK), Wanderah (WJ), Roseworthy (RAC) and Winulta (WC) in 2004, and the mean root length in a moderately alkaline calcareous control treatment (RO), a highly alkaline bicarbonate treatment (RL), and the relative root length (RRL = RO/RL*100).

	Relative yield					
Treatment	BR	CK	WJ	RAC	WC	Mean
RO	0.471***	0.436***	0.228*	0.154^{ns}	0.070^{ns}	0.466***
RL	0.520***	0.349**	0.124^{ns}	-0.119^{ns}	0.377***	0.252*
RRL	0.298**	0.119^{ns}	0.005^{ns}	-0.254*	0.070^{ns}	-0.020^{ns}

ns not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

The division on the basis of height for RO, RL and RRL found that dwarf types, while being significantly lower yielding, also had significantly shorter root lengths in both bicarbonate and control treatments (Table 7.07). The semi-dwarf types were often higher yielding than the tall types, however, no significant difference was found between semi-dwarf or tall types for RO, RL, and RRL.

Table 7.07: Relative grain yield (% control) at the sites Buckleboo (BR), Claypans (CK), Wanderah (WJ), Roseworthy (RAC) and Winulta (WC) in 2004, and the mean root length in control treatment (RO), bicarbonate treatment (RL) and the relative root length (RRL = RO/RL*100), for each height class, dwarf, semi-dwarf and tall.

	Relative yield (% control)						Treatment		
	BR	CK	WJ	RAC	WC	Mean	RO	RL	RRL
Dwarf	74.00	57.00	72.00	73.10	59.00	66.30	189.56	68.02	35.90
Semi-dwarf	103.80	89.60	82.80	95.30	87.00	89.70	209.51	74.55	35.56
Tall	102.50	85.40	74.80	71.80	70.80	75.00	205.40	75.63	36.90

l.s.d (0.05) between height classes= 11.19

Correlations between RO, RL or RRL and relative grain yield for each of the height classes identified no significant relationships for the dwarf types at any site, only the semi-dwarfs and the tall types (Table 7.08). The highly alkaline Buckleboo site was found to have a positive correlation between relative grain yield and the root measurements RO, RL and RRL for the semi-dwarfs and only RL for the tall types. Winulta had a positive correlation between both RL and RO, and relative grain yield, for both semi-dwarf and tall types.

Roseworthy also retained the significant negative correlations between relative grain yield and RRL for the tall types, but also had significant negative correlations between relative grain yield and both RO and RL for the semi-dwarf types. The less alkaline sites Wanderah and Claypans, were no longer found to have a correlation between root length and relative grain yield.

Table 7.08: Correlation between relative grain yield (% control) at the sites Buckleboo (BR), Claypans (CK), Wanderah (WJ), Roseworthy (RAC) and Winulta (WC) in 2004 and the mean root length in control treatment (RO), bicarbonate treatment (RL) and the relative root length (RRL = RO/RL*100), for each height class, dwarf, semi-dwarf and tall.

		Treatment		
Site	Height	RO	RL	RRL
Buckleboo	Dwarf	0.135^{ns}	0.286^{ns}	0.231^{ns}
	Semi-Dwarf	0.452**	0.539***	0.375*
	Tall	0.115^{ns}	0.416*	0.343^{ns}
Claypans	Dwarf	0.293^{ns}	0.353^{ns}	0.205^{ns}
	Semi-dwarf	0.282^{ns}	0.261^{ns}	0.145^{ns}
	Tall	0.014^{ns}	0.134^{ns}	0.112^{ns}
Wanderah	Dwarf	-0.164^{ns}	0.014^{ns}	0.085^{ns}
	Semi-dwarf	0.104^{ns}	0.104^{ns}	0.024^{ns}
	Tall	0.114^{ns}	0.026^{ns}	-0.052^{ns}
Roseworthy	Dwarf	0.307^{ns}	0.171^{ns}	0.005^{ns}
	Semi-dwarf	-0.396*	-0.434**	-0.289^{ns}
	Tall	0.221^{ns}	-0.338^{ns}	-0.436*
Winulta	Dwarf	0.423^{ns}	0.164^{ns}	-0.114^{ns}
	Semi-dwarf	0.403*	0.401*	0.238^{ns}
	Tall	0.397*	0.384*	0.182^{ns}
Mean	Dwarf	0.296^{ns}	0.190^{ns}	0.010^{ns}
	Semi-dwarf	0.207^{ns}	0.162^{ns}	0.071^{ns}
	Tall	0.348^{ns}	0.163^{ns}	0.020^{ns}

ns not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

The separation of lines into maturity classes found that the early types were significantly higher yielding than the late or very late types, particularly at the lower rainfall sites of BR and CK (Table 7.09). The early types in comparison to the late types also had significantly longer root lengths in the RO treatment, marginally longer root lengths in the RL, but no significant difference for RRL.

Table 7.09: Relative grain yield (% control) at the sites Buckleboo (BR), Claypans (CK), Wanderah (WJ), Roseworthy (RAC) and Winulta (WC) in 2004, and the mean root length in control treatment (RO), bicarbonate treatment (RL) and the relative root length (RRL = RO/RL*100), for each maturity class, early, mid, late and very late.

	Relative yield (% control)						Treatment		
	BR	CK	WJ	RAC	WC	Mean	RO	RL	RRL
Early	111.92	92.32	78.76	82.32	84.52	84.84	212.21	78.36	36.97
Mid	103.27	83.85	79.23	81.04	74.23	79.58	204.06	75.22	36.87
Late	71.95	67.10	75.62	87.33	69.00	75.43	197.22	68.17	34.69
Very late	51.00	46.22	57.44	68.33	53.11	57.44	194.21	67.08	34.59

l.s.d (0.05) between maturity classes = 13.55

No significant correlations were identified between RO, RL or RRL and relative grain yield for either the late or very late types at any site (Table 7.10). Buckleboo again had positive correlations between relative grain yield and the root measurements RO, RL and RRL, but only for early or mid maturity types. At Claypans a significant positive correlation between RL and relative grain yield was identified for the mid maturing types, but Wanderah again had no significant correlations. Winulta had a positive correlation between RL or RO and relative grain yield for mid maturity types. The correlations for Roseworthy, while being mostly negative, were not found to be significant, although unlike the previous results, a significant positive correlation between RL and relative grain yield for mid maturity types was identified.

Soil pH and $EC_{1:5}$ measurements and grain yield in 2005

In 2005 the field trials for RAC875/Cascades was repeated, although no specific soil sampling or assessment for height or maturity was conducted. Soil data was, however, sampled in adjacent trials, providing an indication of the soil pH and EC at the RAC875/Cascades trial site (Table 7.11). During soil sampling little soil moisture was observed past 30cm, and similar to 2004, trial sites suffered extended periods of water stress throughout the growing season. Below average rainfall was recorded in 2004 and very little rainfall fell from January to May in 2005 (Appendix 2). No subsoil moisture was present at the beginning of seeding in 2005, and although average to above average rainfall was recorded for most of the trial sites for the June to October growing period, minimal water movement into the subsoil was observed.

Table 7.10: Correlation between relative grain yield (% control) at the sites Buckleboo (BR), Claypans (CK), Wanderah (WJ), Roseworthy (RAC) and Winulta (WC) in 2004, and the mean root length in control treatment (RO), bicarbonate treatment (RL) and the relative root length (RRL = RO/RL*100), for each maturity class, early, mid, late and very late.

		Treatment		
Site	Height	RO	RL	RRL
Buckleboo	Early	0.415*	0.281^{ns}	0.090^{ns}
	Mid	0.197^{ns}	0.440*	0.393*
	Late	0.299^{ns}	0.414^{ns}	0.180^{ns}
	Very late	0.597^{ns}	0.365^{ns}	0.017^{ns}
Claypans	Early	0.130^{ns}	-0.257^{ns}	0.130^{ns}
	Mid	0.383^{ns}	0.469*	0.309^{ns}
	Late	0.235^{ns}	0.122^{ns}	-0.077^{ns}
	Very late	0.500^{ns}	0.253^{ns}	-0.040^{ns}
Wanderah	Early	0.355^{ns}	0.129^{ns}	-0.030^{ns}
	Mid	0.189^{ns}	0.114^{ns}	0.008^{ns}
	Late	-0.066^{ns}	-0.324^{ns}	-0.066^{ns}
	Very late	-0.042^{ns}	-0.108^{ns}	-0.110^{ns}
Roseworthy	Early	-0.234^{ns}	-0.411*	-0.318^{ns}
	Mid	0.474*	0.214^{ns}	-0.030^{ns}
	Late	0.065^{ns}	-0.353^{ns}	-0.405^{ns}
	Very late	0.399^{ns}	-0.168^{ns}	-0.433^{ns}
Winulta	Early	0.268^{ns}	0.167^{ns}	0.046^{ns}
	Mid	0.687***	0.393*	0.004^{ns}
	Late	0.314^{ns}	0.009^{ns}	-0.267^{ns}
	Very late	0.569^{ns}	0.373^{ns}	0.048^{ns}
Mean	Early	0.212^{ns}	-0.039^{ns}	-0.140^{ns}
	Mid	0.624***	0.397*	0.047^{ns}
	Late	0.212^{ns}	-0.211^{ns}	-0.386^{ns}
	Very late	0.469^{ns}	0.096^{ns}	-0.193^{ns}

ns not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

Table 7.11: Mean soil pH and $EC_{1:5}$(µS/m) measurements and standard deviation for eight field sites in 2005 at depths 0-10cm, 30-40cm, 50-60cm. pH>8.5, $EC_{1:5}$>400µS/m

	pH			$EC_{1:5}$ µS/m		
Site	0-10cm	30-40cm	50-60cm	0-10cm	30-40cm	50-60cm
Angas Valley	8.77 ± 0.21	9.73 ± 0.22	10.21 ± 0.06	104.5 ± 13.9	269.8 ± 69.0	513.2 ± 92.5
Buckleboo	8.70 ± 0.08	9.14 ± 0.17	9.56 ± 0.19	172.0 ± 74.5	866.8 ± 227.4	1187.0 ± 100.0
Claypans	8.61 ± 0.06	9.03 ± 0.19		169.7 ± 10.0	209.2 ± 22.6	
Coonalpyn	8.33 ± 0.04	8.90 ± 0.18	9.39 ± 0.29	208.4 ± 12.5	187.7 ± 34.0	261.8 ± 84.3
Kapunda	8.04 ± 0.28	8.80 ± 0.27	9.12 ± 0.18	164.5 ± 39.1	199.3 ± 72.5	223.7 ± 69.4
Roseworthy	7.51 ± 0.73	9.44 ± 0.29	9.69 ± 0.21	130.9 ± 48.6	744.9 ± 305.2	1189.8 ± 356.3
Redhill	6.41 ± 0.23	8.61 ± 0.19	8.34 ± 0.24	776.8 ± 611.3	2073.6 ± 425.8	3943.8 ± 976.2
Wanderah	7.24 ± 0.35	8.35 ± 0.28	8.59 ± 0.26	204.9 ± 91.1	1139.1 ± 445.2	1528.8 ± 338.9

Correlation of bicarbonate tolerance with relative grain yield in 2005

Relative grain yield was compared to mean root lengths in bicarbonate testing, which used seed from the 2005 field trials. Unlike the 2004 results, no significant correlations were identified between RO and relative grain yield (Table 7.12). The Roseworthy site again had significant negative correlations between RL or RRL and relative grain yield. However, contrary to the 2004 results, the Claypans and Wanderah trials also had significant negative correlations between RL and RRL and relative grain yield, along with Angas Valley and Coonalpyn, which had significant correlations with RRL and RL, respectively. Despite all sites having mildly to strongly alkaline subsoils, the higher the level of bicarbonate tolerance, measured as length in bicarbonate treatment, the lower the relative grain yield (g/plot).

Note: Combining the 2004 and 2005 yield data resulted in non-significant correlations due to large G x E effects and was therefore not included in this section.

Table 7.12: Correlation between relative grain yield (% control) in 2005 and the mean root length in a moderately alkaline calcareous control treatment (RO), a highly alkaline bicarbonate treatment (RL), and the relative root length (RRL = RO/RL*100).

	Relative Yield (% control)							
	Angas Valley	Buckleboo	Claypans	Coonalpyn	Kapunda	Roseworthy	Redhill	Wanderah
RO	-0.095^{ns}	0.205^{ns}	0.047^{ns}	0.146^{ns}	-0.014^{ns}	-0.041^{ns}	0.066^{ns}	0.001^{ns}
RL	-0.371***	-0.150^{ns}	-0.269*	-0.262*	-0.211^{ns}	-0.365***	-0.182^{ns}	-0.355***
RRL	-0.274*	-0.169^{ns}	-0.223*	-0.320**	-0.132^{ns}	-0.319**	-0.137^{ns}	-0.281*

ns not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

7.2.4 Discussion

Buckleboo 2004

In 2004 below average rainfall across South Australia led to grain yields of less than half the long-term average yield for all the trial sites, particularly in the marginal areas, such as, Buckleboo, where the mean grain yield of plots was only 91g/plot (0.3t/ha). At Buckleboo water stress was considered the main abiotic stress factor affecting grain yield. No correlations were therefore found between soil pH and EC, even though topsoil and subsoil pH was >8.5, and subsoil $EC_{1:5}$ was >400µS/m. Low rainfall also stunted plant growth at BR resulting in semi-dwarf and tall plant types having a similar height, and dwarf types too short to effectively mechanical harvest resulting in lower grain yields for the dwarf types and similar grain yields for the semi-dwarf and tall types. The extended dry period following initial opening rains also led to successively lower yields from the early to late maturing types.

The relationship between higher yields at Buckleboo and lines with longer root lengths in a strongly alkaline bicarbonate treatment, a moderately alkaline control treatment, and the relative root length, is likely associated with the lines ability to both extend roots further into the strongly alkaline soil and extract more water from the water deficient soil. The shorter root length of the dwarf types in the control treatment would have exacerbated the water deficit and added to the lower grain yields, preventing the detection of significant correlations between bicarbonate tolerance and grain yield. Correlations were only identified for the semi-dwarf and tall types. Similarly, the shorter root length of the later maturity types in the control treatment would have reduced the plants ability to extract water, resulting in a lower grain yield and inability to detect significant correlations between bicarbonate tolerance and grain yield. Correlations were only identified for the early and mid maturity types.

Wanderah 2004

At Wanderah grain yield of plots was also severely affected by low rainfall, but it was located in a more coastal position and had milder temperatures, reaching a mean yield of 307g/plot (1t/ha). Water deficiency at Wanderah was a major abiotic stress and combined

with areas of high topsoil EC, or 'magnesia patch', germination was also uneven across the trial site. Increasing topsoil $EC_{1:5}$ to levels >400µS/m was therefore found to decrease grain yield at Wanderah. Interestingly, increasing subsoil EC up to 2500µS/m was found to increase grain yield and increasing subsoil pH up to 8.5 was found to decrease grain yield.

The identification of both positive and negative correlations between pH and grain yield, and EC and grain yield at Wanderah was associated with a negative relationship between the moderately alkaline subsoil pH and the highly toxic subsoil EC. Generally, the presence of Na^+ leads to an increase in pH due to the higher solubility of Na_2CO_3 and $NaHCO_3$, compared to $CaCO_3$. For pH to rise above 8.3, Na^+ needs to constitute >15% of the exchangeable cations in the soil (Tan 1998). However, pH is not directly related to the soil Na^+ concentration, but only to the available faction of Na^+ in the soil and that, which is not electrically balanced by SO_4, Cl, and NO_3, the 'non-alkaline' anions (Mashhady and Rowell 1978). At Wanderah subsoil $EC_{1:5}$ reaches up to 2500µS/m while pH reaches only a modest pH 8.5. High soil Na^+ is therefore likely balanced by high concentrations of Cl^- (or SO_4 and NO_3) providing very high NaCl (EC values), and a low Na^+:Ca^{2+} resulting in the pH being <8.3. Lower concentrations of Cl^- would reduced the formation of NaCl (lower EC), increase the ratio of Na^+:Ca^{2+} and increase pH above 8.3. If increasing subsoil pH was decreasing grain yield, then a negative relationship between pH and EC may arbitrarily result in the positive correlation between subsoil EC and yield. Alternatively, increasing subsoil EC may have increased grain yield by minimising early season biomass and maximising grain production under low soil moisture, which led to the corresponding correlation with subsoil pH.

Similar to Buckleboo, the dwarf type plants at Wanderah were lower yielding, although at Wanderah the tall types were also lower yielding than the semi-dwarf types. The lower yield of the dwarf types was associated with a shorter root length, but also to lower biomass production and yield potential, and poor competitiveness with weeds. The tall types also have a lower yield potential associated with a higher biomass to grain ratio, and were prone to lodging. However, only the tall types had significant correlations between yield and the contrasting subsoil pH and EC values.

The Wanderah site was located in a region typically associated with early maturing crops. Only a few days separated the early, mid and late maturing crops at Wanderah, with the

trial site mainly ripening in the hot weather in the middle of October. The yield of the different maturity types was similar, except for the very late maturing lines. The yield of the late maturity types was likely to be affected, to a larger extent, by low rainfall and high temperatures during flowering and grain fill, than by soil pH and EC, resulting in no significant correlations for the late maturity types.

Wanderah was found to have no association between bicarbonate tolerance for the RAC875/Cascades population and yield, and only a weak correlation between the root length in the control treatment and grain yield. However, the correlation for root length could not be isolated to particular height or maturity types. Bicarbonate tolerance in the RAC875/Cascades population did not have a yield advantage at the Wanderah site, corresponding with the lack of significant correlations between soil pH and yield. At the Wanderah site soil EC (surface and subsoil) was likely a dominant abiotic factor, along with soil moisture, in influencing grain yield. Magnesia patches across the site affected germination and early yield potential, while extremely high subsoil EC may have reduced early tillering, reducing early season biomass, conserving soil moisture, and allowing a better quality end of season grain fill.

Roseworthy 2004

Roseworthy was the highest yielding site averaging a grain yield of 361g/plot (1.2t/ha), which again was well below average due to low rainfall. At Roseworthy an increase in both topsoil and subsoil pH was associated with an increase in soil EC, although at levels below 400µS/m, EC was not expected to influence plant growth. No correlations were found between soil EC and grain yield. Subsoil pH, however, was between pH 8.8 and 9.3, yet while considered toxic within the strongly alkaline range, soil pH was not found to affect grain yield.

When divided into height classes, dwarfs were lower yielding than the semi-dwarf and tall types, and tall types were lower yielding than the semi-dwarfs, similar to Wanderah. The progressively higher yields at the sites, Buckleboo, Wanderah and Roseworthy, were associated with a greater difference between the height classes, highlighting the superiority of the semi-dwarf types in relation to biomass, yield potential, lodging and effectiveness of mechanical harvesting.

Similar to the sites Buckleboo and Wanderah, maturity was found to have a significant effect of grain yield. At Roseworthy, hot dry weather in October, with one day exceeding 40°C, ripened the early and mid maturity types, followed by rain in early November, which benefited the later types, although the very late types, were again affected by hot dry weather. The seasonal conditions were reflected in the grain yield of the different types, with late types yielding the highest, although the very late maturity types were significantly lower yielding. The separation of lines into maturity classes showed that only the mid maturity types were affected by soil pH and EC. An increase in topsoil pH from acidic (pH 6.2) to moderately alkaline (pH 8.5) was weakly associated with a decrease in grain yield. However, an increase in subsoil pH and EC was also weakly associated with an increase in grain yield. Similar to Wanderah, subsoil pH or EC may have reduced biomass, lowering water requirements, leading to a better grain fill at the end of the season under minimal soil moisture.

The decrease in grain yield of the more bicarbonate tolerant lines grown at Roseworthy supports the notion that the ratio of biomass to grain yield (i.e. harvest index) was a significant factor. Early season rainfall at Roseworthy produced considerable biomass in both the trial plots and surrounding cereal crops. Minimal follow-up rainfall throughout the growing season resulted in plants relying on stored soil moisture, which was depleted rapidly by high plant biomass, with little to no soil moisture retained for seed fill. Plots that had fewer tillers and less tissue growth due to constraints, such as high soil pH, and lack of bicarbonate tolerance, probably had more available soil moisture at seed set, and consequently had higher grain yield.

Trial sites in 2005

In 2005 the RAC875/Casades trial was repeated at eight sites and, similar to 2004, the trials endured below average rainfall at the beginning of the growing season. In 2005 sites received below average rainfall from January to the middle of June, with plots sown under low moisture conditions, with almost no subsoil moisture available from below average rainfall in 2004. In contrast to 2004, RAC875/Cascades lines with a longer root length in the control treatment were not significantly higher yielding than those with shorter root length. Average to above average rainfall throughout the growing season (June-Oct), may

have resulted in soil moisture not being a significant limiting factor, and therefore root length and higher water acquisition from a larger root system had no added benefit to yield potential.

In contrast to the 2004 results, lines in the RAC875/Cascades population that had greater tolerance to bicarbonate found to be significantly lower yielding than bicarbonate intolerant lines at the majority of the sites. The ability of plant roots to extend into the subsoil under moderately to highly alkaline conditions appeared to be detrimental to the final grain in RAC875/Cascades population in 2005. The adverse relationship between bicarbonate tolerance and grain yield may be associated with the availability of soil moisture throughout the growing season. Virtually no subsoil moisture was present at the beginning of June, and with consistent low rainfall periods from mid-June to October, crops were able to source moisture almost entirely from the top 30cm of the soil profile (Appendix 2 and 7). Bicarbonate tolerant lines capable of growing into the alkaline subsoil may have received little added benefit where moisture in the topsoil was not limiting, but were exposed to other toxic soil conditions, such as boron and salinity, which was also present at depth at most of the sites. Bicarbonate intolerant lines may have been able to avoid the toxic nature of the subsoil by maintaining root growth mainly in the top 30cm.

7.3 Evaluation of the SSD durum wheat population Wk/TmWLYY9//WLYY9Tm.

7.3.1 Introduction

The Wk/TmWLYY9//WLYY9Tm single-seed decent population had previously been found to segregate for bicarbonate tolerance with three or more genes responsible for the tolerance response (Section 6.4). Tolerance in the population was likely due to genes in both the bread wheat parent Worrakatta (possibly on 7A), and genes in the durum parents, Kalka and Tamaroi, with genes in common between the three parents.

In medium rainfall areas (>400mm p.a.) durum wheats, Tamaroi and Kalka, often have comparable yields to the more tolerant bread wheat parent Worrakatta, however, in lower rainfall (<350mm p.a.) alkaline soils, Worrakatta is often observed as having far superior grain yield. No previous soil pH or EC (0-10cm, 30-40cm, 50-60cm) and grain yield correlations had been calculated for Worrakatta and few correlations were previously

identified between soil pH or EC and yield for either Kalka or Tamaroi at alkaline sites (Section 4.2.3). The aim of this section was to assess the Wk/TmWLYY9//WLYY9Tm SSD population under alkaline field conditions at multiple sites to determine whether the segregation for longer root lengths in bicarbonate treatment was associated with an increase in yield.

7.3.2 Materials and Methods

Seed of the Wk/TmWLYY9//WLYY9Tm single-seed descent (SSD) lines was sourced from 2003 field trials of the Durum Breeding group, University of Adelaide. In 2005 the Wk/TmWLYY9//WLYY9Tm population was sown in the field at eight sites, Buckleboo, Two Wells, Redhill, Claypans, Jamestown, Angas Valley, Roseworthy and Wanderah. Field data on the grain yield of each line was also obtained from 2003 trials at the sites Angas Valley, Buckleboo, Claypans, Coonalpyn, Kapunda and Two Wells.

The field experiment consisted of 350 of the Wk/TmWLYY9//WLYY9Tm SSD lines with no replication, 90 durum check plots and 10 standard varietal plots, giving a total experiment size of 450 plots. The field trials were sown, managed, scored and harvested as per the methods in section 3.1.2.

The bicarbonate testing followed the procedures outlined in section 3.3 and 5.2. Twenty-four durum wheat seeds of 167 randomly selected SSD lines were arranged into four completely randomised replications (2 trays per replication), with three seeds for each SSD lines per tray, and two treatments (bicarbonate and control).

In 2006 soil samples were collected from the sites Angas Valley, Roseworthy and Wanderah, and measured for soil pH and EC as described in section 3.1.4. Measurements of soil pH and $EC_{1:5}$ were correlated for the three 2005 sites, Angas Valley, Roseworthy and Wanderah. Bicarbonate data was correlated with relative yield (% control) at five of the sites, Buckleboo, Claypans, Wanderah, Roseworthy and Winulta.

Seed for boron testing was sourced from RAC field trials in 2005 for comparison with 2003 and 2005 field trial data. Seedlings were screened to determine the level of tolerance to boron by methods adapted from Chantachume *et. al.* (1995) and Campbell *et. al.* (1994).

Seed was germinated as for bicarbonate screening described in section 3.3. When seedling roots were 20mm long, the trays were placed into 3L rectangular plastic containers containing 2L of boron treatment solution, which consisted of 1000ppm boron, added as H_3BO_3, and 500µM $Ca(NO_3)_2.4H_2O$ and 2.5µM $ZnSO_4.7H_2O$. Air was supplied to the containers via 10" x ½" airstones, run by a aqua pump, through 4mm air hose. The root length of each seedling was measured after 10 days and the average root length of the well (3 seeds) recorded.

Chi-square analysis was conducted on the data for the boron solution screen to determine the number of genes segregating. The boron data was also correlated with relative yield (% control) at five of the sites, Buckleboo, Claypans, Wanderah, Roseworthy and Winulta.

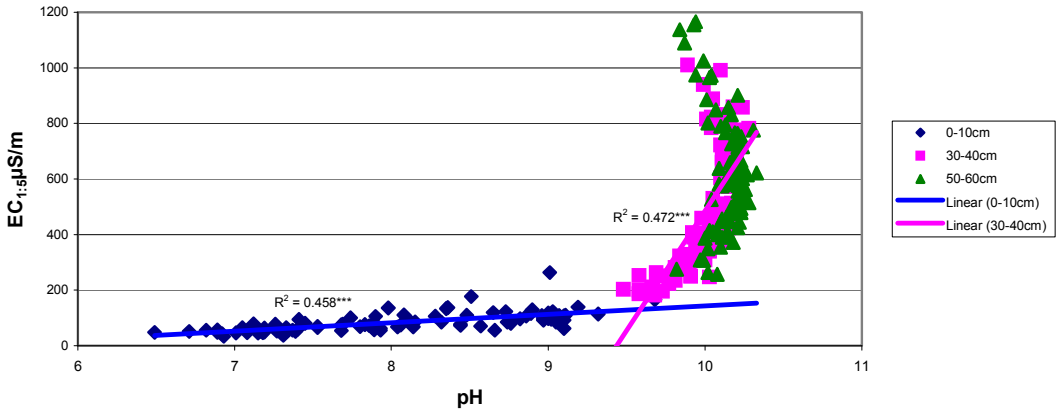
Seed from six Tamaroi check plots were collected from 2003 field trials at six sites, Angas Valley, Buckleboo, Claypans, Coonalpyn, Kapunda, and Two Wells, and sent to the Waite Analytical Services unit for ICP (inductively coupled plasma) spectrometry analysis to determine the grain boron concentration.

No soil data was recorded in 2003 for the Wk/TmWLYY9//WLYY9Tm population, but yield data was obtained for the six sites, Angas Valley, Buckleboo, Claypans, Coonalpyn, Kapunda and Two Wells, and relative grain yield correlated with the bicarbonate and boron data.

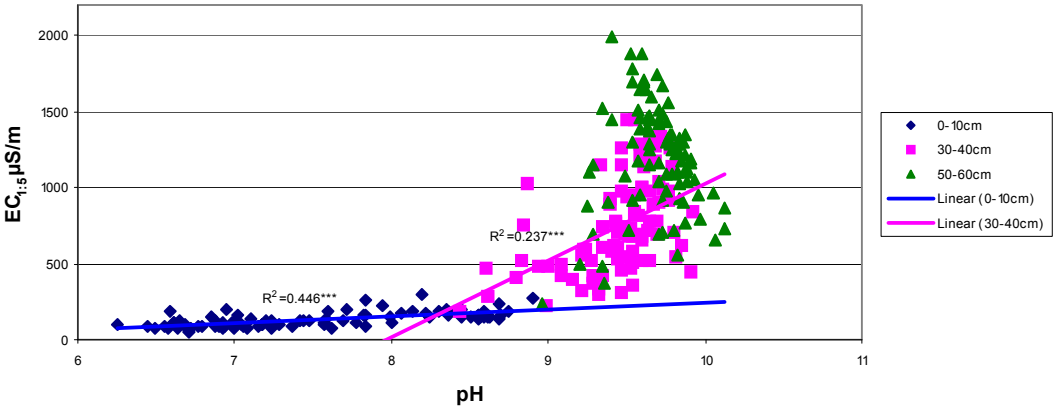
7.3.3 Results

Ninety soil samples were collected at three depths, 0-10cm, 30-40cm and 50-60cm, for three sites, AV, RAC, WJ and measured for pH and $EC_{1:5}$. At all three sites subsoil pH reached a level (>pH8.5) that is considered to influence growth, with two sites, AV and RAC, having extreme pH values >pH 9.2 where little root growth is expected to occur (Figure 7.02). The AV and RAC sites also had topsoil pH values >pH 8.5, although remained <pH 9.2. All three sites had highly toxic subsoil $EC_{1:5}$ levels (<400µS/m), although only the Wanderah site had toxic topsoil $EC_{1:5}$ levels.

(a) Angas Valley



(b) Roseworthy



(c) Wanderah

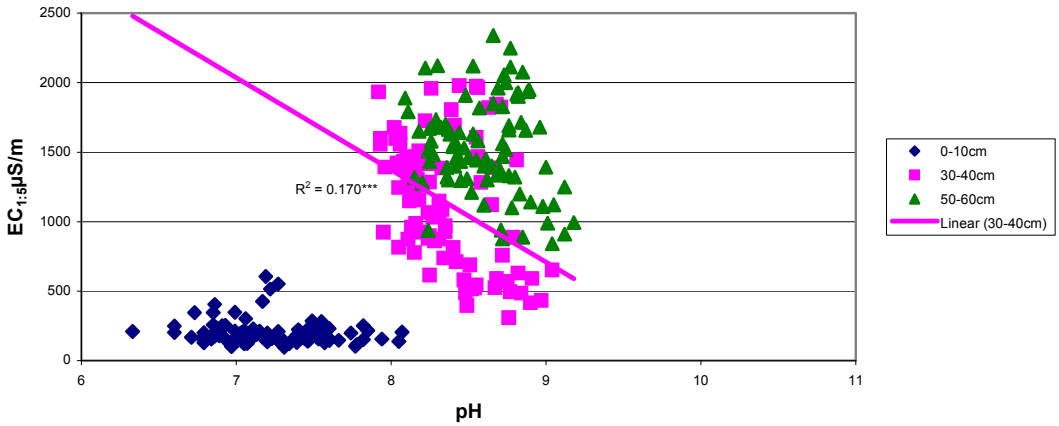


Figure 7.02: Correlation between soil pH and $EC_{1:5}$ at 0-10cm, 30-40cm and 50-60cm depths for the sites, (a) Angas Valley, (b) Roseworthy, and (c) Wanderah, in 2005.

At the other fives field sites, BR, TW, RH, CK, and JC, only 10 random soil measurements were taken. All the sites recorded subsoil pH levels in the moderately or highly alkaline level, although RH and JC did not reach pH values (>pH 8.5) that were expected to significantly influence plant growth (Table 7.13). Soil cores could not be taken at the depth of 50-60cm for trials at Claypans and Buckleboo due to sheet limestone and high soil strength, respectively.

Correlation of soil pH and $EC_{1:5}$ measurements with grain yield in 2005

When topsoil and subsoil pH and $EC_{1:5}$ values where correlated with plot yield (g/plot), only the Rosewothy and Wanderah sites were found to have significant associations (Table 7.14). At Roseworthy increasing subsoil $EC_{1:5}$ (30-40cm and 50-60cm) was found to significantly decrease grain yield, with $EC_{1:5}$ (50-60cm) accounting for 28 percent of the variation in grain yield. Similarly, at Wanderah increasing subsoil $EC_{1:5}$ (30-40cm and 50-60cm) was found to significantly decrease grain yield. However, at Wanderah increasing topsoil pH (0-10cm) in the range of pH 6.3 to 8.1 was also found to increase grain yield, with pH (0-10cm) and $EC_{1:5}$(50-60cm) accounting for 33 percent of the variation in grain yield.

No significant correlations were identified at Angas Valley between soil pH and grain yield, although subsoil pH had reached levels considered extremely toxic (pH 9.2) and little root growth was observed past 20-30cm in depth.

Table 7.13: Mean soil pH and $EC_{1:5}$(µS) measurements and standard deviation for eight field sites at depths 0-10cm, 30-40cm, 50-60cm, in 2005. pH>8.5, $EC_{1:5}$>400µS/m

	pH			$EC_{1:5}$ µS/m		
Site	0-10cm	30-40cm	50-60cm	0-10cm	30-40cm	50-60cm
Buckleboo	8.65 ± 0.05	9.23 ± 0.16		151.1 ± 8.1	619.0 ± 294.3	
Two Wells	7.48 ± 0.33	9.39 ± 0.18	9.34 ± 0.20	301.3 ± 56.5	791.0 ± 205.0	1204.2 ± 181.7
Redhill	6.41 ± 0.23	8.61 ± 0.19	8.34 ± 0.24	776.8 ± 611.3	2073.6 ± 425.8	3943.8 ± 976.2
Claypans	8.61 ± 0.06	9.03 ± 0.19		169.7 ± 10.0	209.2 ± 22.6	
Jamestown	6.15 ± 0.38	7.69 ± 0.71	8.63 ± 0.13	231.0 ± 96.5	188.8 ± 53.8	479.7 ± 73.9
Angas Valley	8.05 ± 0.79	10.0 ± 0.17	10.14 ± 0.10	83.6 ± 35.2	486.3 ± 218.8	615.5 ± 204.9
Roseworthy	7.51 ± 0.73	9.44 ± 0.29	9.69 ± 0.21	130.9 ± 48.6	744.9 ± 305.2	1189.8 ± 356.3
Wanderah	7.24 ± 0.35	8.35 ± 0.28	8.59 ± 0.26	204.9 ± 91.1	1139.1 ± 445.2	1528.8 ± 338.9

Table 7.14: Soil pH and $EC_{1:5}$ at depths 0-10cm, 30-40cm and 50-60cm correlated with plot yield (g) for the sites, Angas Valley (AV), Roseworthy (RAC) and Wanderah (WJ), in 2005.

		Yield (g/plot)		
		AV	RAC	WJ
pH	0-10cm	0.111^{ns}	0.082^{ns}	0.481***
	30-40cm	0.144^{ns}	-0.069^{ns}	-0.046^{ns}
	50-60cm	0.044^{ns}	-0.017^{ns}	-0.111^{ns}
$EC_{1:5}$	0-10cm	0.181^{ns}	-0.095^{ns}	-0.202^{ns}
	30-40cm	0.174^{ns}	-0.288*	-0.349**
	50-60cm	0.214^{ns}	-0.288*	-0.466***

ns not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

Correlation of bicarbonate tolerance with relative grain yield in 2005

The relative yield of lines (% control) from the eight sites was compared to the root lengths in bicarbonate (RL) and control (RO) treatments, and to the relative root lengths (RRL) to identify significant correlations. The relative grain yield at five of the sites, Angas Valley, Buckleboo, Claypans, Jamestown, and Roseworthy was found significantly correlated with RO or RRL (Table 7.15). The extreme pH and transient saline site of Angas Valley had a positive correlation between relative grain yield and the root length in the control treatment (RO), but not with the root length in the bicarbonate treatment (RL) or with relative root length (RRL). Similarly, Buckleboo, Claypans, Jamestown and Rosewothy, had a positive correlation between relative grain yield and RO, although sites varied considerably for pH levels. No significant correlations were identified between relative grain yield and RL for any of the eight sites. However, for seven of the sites, a negative correlation was identified between relative grain yield and RRL, only at the lowest rainfall sites, Buckleboo and Claypans. At Buckleboo and Claypans increasing RRL was associated with a decrease in the relative grain yield, even though the sites were calcareous and highly alkaline. Sites such as Redhill, Two Wells and Wanderah, with the highest salt concentrations had the lowest correlations for RO, RL or RRL and relative grain yield.

Table 7.15: Correlation between relative grain yield (% control) in 2005 at the sites Angas Valley (AV), Buckleboo (BR), Claypans (CK), Jamestown (JC), Roseworthy (RAC), Redhill (RH), Two Wells (TW) and Wanderah (WJ), and the mean root length in a moderately alkaline calcareous control treatment (RO), a highly alkaline bicarbonate treatment (RL), and the relative root length (RRL = RO/RL*100).

	Relative yield								
Treatment	AV	BR	CK	JC	RAC	RH	TW	WJ	Mean
RO	0.194*	0.217**	0.269***	0.240**	0.190*	0.005^{ns}	0.077^{ns}	0.042^{ns}	0.304***
RL	-0.026^{ns}	-0.129^{ns}	-0.129^{ns}	0.040^{ns}	0.058^{ns}	-0.062^{ns}	-0.020^{ns}	0.085^{ns}	0.014^{ns}
RRL	-0.108^{ns}	-0.214**	-0.251***	-0.096^{ns}	-0.042^{ns}	-0.042^{ns}	-0.057^{ns}	0.057^{ns}	-0.145^{ns}

ns not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

Correlation of bicarbonate tolerance with relative grain yield in 2003

Yield data from 2003 was also compared to the root lengths in bicarbonate (RL) and control (RO) treatments, and to the relative root lengths (RRL) to identify significant correlations. The relative grain yield at five of the sites, Buckleboo, Angas Valley, Coonalpyn, Kapunda and Two Wells was found significantly correlated with RO, RL or RRL (Table 7.16). No soil tests were conducted at the sites in 2003, although soil tests from 2004 and 2006 (Table 7.11 and 7.13) indicate that the subsoils are likely to range from moderately alkaline to strongly alkaline. Similar to the 2005 results, BR had a significant positive correlation between relative grain yield and RO, but all correlations between relative grain yield and RL or RRL were negative. At Buckleboo, Angas Valley, Coonalpyn, Kapunda and Two Wells, an increase in the relative grain yield of lines was associated with a significant decrease in the lines RL and RRL.

Table 7.16: Correlation between relative grain yield (% control) in 2003 at Angas Valley (AV), Claypans (CK), Coonalpyn (CS), Kapunda (KH), and Two Wells (TW), and the mean root length in control treatment (RO), bicarbonate treatment (RO) and the relative root length (RRL).

	Relative yield						
Treatment	AV	BR	CK	CS	KH	TW	Mean
RO	0.026^{ns}	0.175*	0.102^{ns}	-0.062^{ns}	0.111^{ns}	0.017^{ns}	0.066^{ns}
RL	-0.193*	-0.319***	-0.097^{ns}	-0.265***	-0.193*	-0.218**	-0.320***
RRL	-0.177*	-0.365***	-0.137^{ns}	-0.197*	-0.221**	-0.194*	-0.307***

ns not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

Boron tolerance

The Wk/TmWLYY9//WLYY9Tm population was expected to segregate for boron tolerance, since Worrakatta (Wk) contained the boron tolerance locus *Bo1* on chromosome 7BL and Kalka (WLYY9) contained the boron tolerance loci *Bot2* (*Bo1* equivalent) on chromosome 7B and *Bot3*. The frequency distribution indicated that one or two genes were segregating in the population (Figure 7.03). One gene segregating (*Bo1* from Wk) in the topcross population would be expected to have a ratio of 1:3, equating to 219:73 for the 292 lines tested. Chi-square analysis for a ratio of 1:3 had a value of 1.83 (separated between 85 and 86mm) or 3.58 (separated between 90 and 91mm), which were both lower than the 3.84 required for significance to reject the hypothesis at the 5% level of confidence. In the absence of boron data for the parents TmWLYY9 and WLYY9Tm, the parental origins of boron tolerance loci can only be assumed. Since progeny lines were measured with a level of tolerance at the Kalka level (*Bot2*, *Bot3*), above that of Worrakatta (*Bo1*), and with much of the population at the Tamaroi level (intolerant), it is likely that both *Bo1*(or *Bot2*) and *Bot3* are segregating in the population.

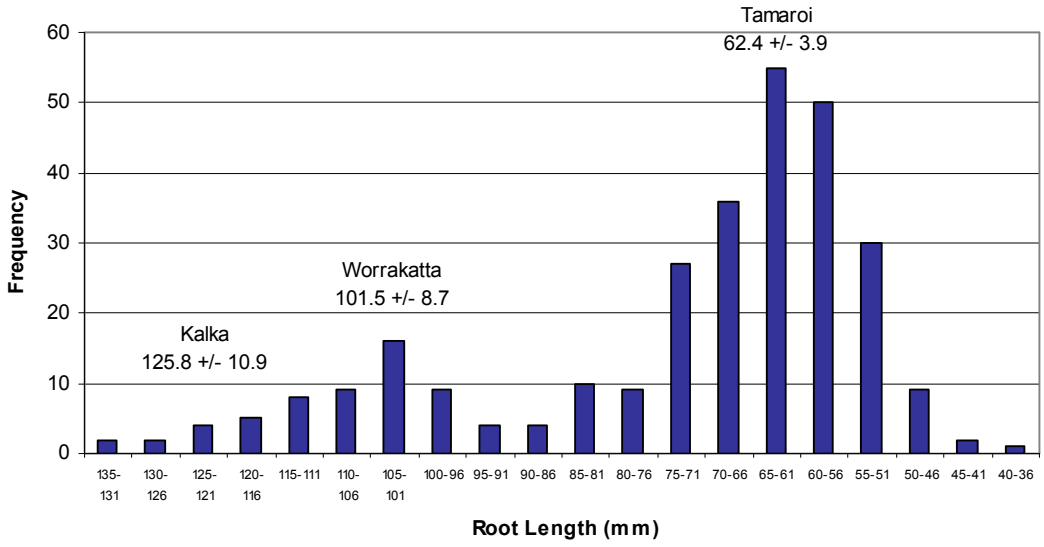


Figure 7.03: Frequency distribution of the Wk/TmWLYY9//WLYY9Tm population for root length in boron solution.

Correlation of boron tolerance with relative grain yield in 2005 and 2003

The root length in boron solution was correlated with the 2005 spatially adjusted grain yields for 292 boron test lines (350 lines in field trials) (Table 7.17). Two of the eight sites were found to have a significant negative correlation between grain yield and root length in boron. At Claypans and Jamestown a greater tolerance to boron was associated with a reduction in grain yield. Claypans and Jamestown are low boron sites in comparison to Redhill, Two Wells and Wanderah.

Table 7.17: Correlation between the mean root length in boron treatment and the adjusted grain yield in 2005 for eight field sites, Angas Valley (AV), Buckleboo (BR), Claypans (CK), Jamestown (JC), Roseworthy (RAC), Redhill (RH), Two Wells (TW) and Wanderah (WJ).

	Adjusted grain yield								
Treatment	AV	BR	CK	JC	RAC	RH	TW	WJ	Mean
Boron	0.045^{ns}	-0.080^{ns}	-0.168***	-0.170***	-0.059^{ns}	-0.079^{ns}	-0.022^{ns}	-0.028^{ns}	-0.093^{ns}

ns not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

The root length in boron solution was also correlated with 2003 spatially adjusted grain yields (Table 7.18). Two of the six sites were again found to have a significant negative correlation between grain yield and root length in boron.

Grain from the boron sensitive durum variety Tamaroi was sampled from each of the 2003 field trials and analysed for grain boron concentration as an indication of the soil boron status (Cartwright *et. al.* 1986). Grain from Two Well was found to contain almost twice the boron concentration of any other site and had the lowest association between root length in boron and grain yield (Table 7.19). The sites, Buckleboo, Claypans, Coonalpyn and Kapunda, had similar grain B concentrations and all had negative correlations despite having grain boron concentrations substantially above 2ppm, but only Buckleboo and Kapunda had significant negative correlations.

Table 7.18: Correlation between the mean root length in boron treatment and the adjusted grain yield in 2003 for six field sites, Angas Valley (AV), Buckleboo (BR), Claypans (CK), Coonalpyn (CS), Kapunda (KH) and Two Wells (TW).

	Relative yield						
Treatment	AV	BR	CK	CS	KH	TW	Mean
Boron	-0.093^{ns}	-0.220***	-0.112^{ns}	-0.059^{ns}	-0.165**	-0.003^{ns}	-0.134*

ns not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

Table 7.19: Mean grain boron concentration (B mg/kg) of the durum variety Tamaroi from six field sites, Angas Valley (AV), Buckleboo (BR), Claypans (CK), Coonalpyn (CS), Kapunda (KH) and Two Wells (TW) in 2003.

	Site					
B (mg/kg)	AV	BR	CK	CS	KH	TW
Mean	3.33	4.28	4.43	4.27	4.62	8.73
St. Dev.	0.92	0.46	1.10	0.73	0.94	1.01

7.3.4 Discussion

Angas Valley 2005

The 2005 season again had below average rainfall across South Australia, although was notably better than 2004. In the Murray Mallee, at the site Angas Valley, yields were near average with the mean grain yield of plots at 405g/plot (1.3t/ha) for the durum wheat population Wk/TmWLYY9//WLYY9Tm. The trial site was located in an area with extremely alkaline (>pH 10) and saline ($EC_{1:5}$ >400µS) subsoil, prone to hard-setting, resulting in no root growth observed past 30cm in depth. No correlations were therefore found between subsoil pH and EC, and grain yield. Topsoil pH (0-10cm) ranged from slightly acid to strongly alkaline, within a range expected to influence root growth, but again no correlation could be identified between topsoil pH and grain yield.

Similarly, greater tolerance to bicarbonate in Wk/TmWLYY9//WLYY9Tm lines had no influence on grain yield. However, longer root length in a mildly alkaline control was significantly correlated with an increase in grain yield. At Angas Valley in 2005, alkaline soil was not a dominant factor in influencing grain yield and bicarbonate tolerance had no effect on increasing grain yields. The association between longer root lengths (RO) and

grain yield may have related to better water or nutrient acquisition (not tested) in a below average rainfall season.

Roseworthy 2005

The Roseworthy site in the lower mid north also had slightly below average rainfall, but still produced a grain yield of 722g/plot (2.4t/ha) for the Wk/TmWLYY9//WLYY9Tm population. Similar to Angas Valley, the site was located in an area with extremely alkaline (pH>9.2) and saline ($EC_{1:5}$ 400 to 2000µS/m) subsoil, with minimal root growth observed past 40-50cm. The subsoil pH was within a range expected to influence grain yield, however, with extremely toxic EC levels, only EC (30-40cm) and EC (50-60cm) were found to significantly decease grain yield. Since soil EC was a dominant factor over soil pH, no correlations were identified between higher bicarbonate tolerance and improved grain yield. A correlation was found between longer root length in the control and increased grain yield, which again may be associated with better water acquisition in a highly saline soil.

Wanderah 2005

The Wanderah site had slightly below average rainfall in 2005, with the mean grain yield of plots 227g/plot (0.75t/ha) for the Wk/TmWLYY9//WLYY9Tm population. The site had a lower subsoil pH than both Angas Valley and Roseworthy, although was still within a range (pH 8.0 to 9.2) expected to influence plant growth. Similar to the Wanderah site in 2004, the 2005 site again had a negative correlation between subsoil pH and EC. A decrease in the alkaline subsoil pH was associated with an increase in subsoil EC, at extremely toxic levels (400 to 2400µS/m). As previously described in Section 7.2.4, the negative relationship is likely associated with the balance between Na^+, Ca^{2+} and the 'non-alkaline' anions Cl, SO_4 and NO_3. Although, the site also contained a calcareous ridge that runs through the centre of the trial, where pH was between 8.0 and 8.5 and $EC_{1:5}$ was <800µS/m, weakening the negative correlation. Despite an alkaline subsoil pH, a highly toxic EC at Wanderah was again found to significantly decrease grain yield, masking any effect high subsoil pH may have on yield. Consequently, no correlations were identified between higher bicarbonate tolerance and improved grain yield.

Buckleboo, Claypans, Jamestown, Two Wells and Redhill 2005

Five other trial sites where used to determine if bicarbonate tolerance led to improved grain yield. Soil sampling had shown that the sites varied in both pH and EC, and also mean grain yield of plots. The lowest yielding site, Buckleboo had a mean grain yield of 212g/plot (0.7t/ha), with strongly alkaline and moderately toxic EC levels in the subsoil (30-40cm). An average subsoil pH of 9.2 would be expected to negatively effect grain yield, however, contrary to the expected, a higher level of bicarbonate tolerance in Wk/TmWLYY9//WLYY9Tm lines was associated with a reduction in grain yield. Similarly, at the low yielding site Claypans (282g/plot, 0.9t/ha), with strongly alkaline and non-toxic subsoil EC, bicarbonate tolerant lines were lower yielding than bicarbonate intolerant lines.

A negative relationship between bicarbonate tolerance and grain yield had previously been identified for the RAC875/Cascades population in 2005 and associated with avoidance of subsoil constraints by intolerant lines under adequate soil moisture (Section 7.2). A similar effect may be occurring for the Wk/TmWLYY9//WLYY9Tm population in 2005, since a general negative relationship was observed for most of the sites. Alternatively, higher biomass production for bicarbonate tolerant lines due to a greater root growth in the alkaline soils may have exhausted soil nutrients, such as nitrogen and sulphur, and soil moisture (limited in subsoil) prior to anthesis, compared to plants with fewer tillers and tissue mass, reducing the final grain yield of the bicarbonate tolerant durum lines. In the absence of subsoil moisture, the intermittent drying of the topsoil at seed set may have led to the reduced yields in lines with greater biomass, which is evident for the lowest rainfall sites, Buckleboo and Claypans.

The Jamestown site located in the northern Mt Lofty Ranges had a much higher mean grain yield of 1076g/plot (3.6t/ha), although pH was only mildly alkaline and the EC marginally toxic in the subsoil. Similar to Buckleboo and Claypans a positive relationship was identified between root length in the control treatment and grain yield, which was likely associated with improved water and nutrient acquisition. No correlations were identified between bicarbonate tolerance and grain yield.

The Two Wells and Redhill sites were also found to have no correlation between bicarbonate tolerance and grain yield, even though subsoil pH averaged 9.4 and 8.6, respectively. However, unlike Buckleboo, Claypans and Jamestown, the Two Wells and Redhill sites had extremely toxic EC levels of 1204µS and 3944µS, respectively, which undoubtably had a significant effect on grain yield and likely masked any pH effect.

Bicarbonate tolerance and grain yield in 2003

Yield data from 2003 was collected to determine if the reduction in grain yield for lines with higher bicarbonate tolerance was occurring in seasons with more average rainfall. In 2003 rainfall varied from below average to above average across South Australia, following a state-wide drought in 2002 (Appendix 7).

Five of the six trial sites were found to have a significant negative correlation between the level of bicarbonate tolerance and grain yield. From 2005 soil testing at the sites Angas Valley, Buckleboo, Claypans, Coonalpyn, Kapunda, and Two Wells, all had subsoil pH values ranging from moderately alkaline to extremely alkaline and EC levels ranging from non-toxic to toxic. However, given the high pH and variable levels of both EC and yield at each site, higher bicarbonate tolerance was associated with reduced grain yield in 2003. Consistent negative correlations between bicarbonate tolerance and grain yield in the Wk/TmWLYY9//WLYY9Tm population may have resulted from either increased moisture stress from high biomass, or avoidance of other subsoil constraints by bicarbonate intolerant types.

The bicarbonate tolerance in the durum wheat population Wk/TmWLYY9// WLYY9Tm originates from both the bread wheat parent Worrakatta (Wk) and the durum parent Kalka (WLYY9). In the cross between bread wheat (ABD) and durum wheat (AB), chromosomal rearrangement in the genomes when reverting to a durum type (AB), may be associated with a deleterious loci linked to the bicarbonate tolerance from the bread wheat Worrakatta. Alternatively, the mechanism leading to improved bicarbonate tolerance in the Worrakatta parent, may be deleterious in the durum wheat genetic background.

Boron tolerance and grain yield in 2003 and 2005

Similar to the bicarbonate response for 2005 data, sites recorded a negative correlation between boron tolerance and grain yield, although only Jamestown and Claypans were significant. At Jamestown and Claypans, a greater level of boron tolerance was strongly associated with a reduction in grain yield.

In 2003 sites again had negative correlations for boron tolerance and grain yield, although only Buckleboo and Kapunda were significant. Grain boron measurements for 2003 seed indicated that sites had a moderate to high levels of boron, well above the likely critical level of about 2ppm, with Two Wells having double the grain boron concentration, and the least significant correlation, so at all sites a positive relationship between boron tolerance and grain yield would have been expected. At moderately high boron sites (4mg/kg), lines with a greater level of boron tolerance were associated with reduced grain yield. The tolerant boron lines were only comparable to the intolerant lines at the extremely high boron site of Two Wells. The poor grain yield associated with both bicarbonate and boron tolerance may also be associated with the chromosomal rearrangements occurring between the bread and durum wheat parents, and this potentially has serious implications for the use bread wheats in improving tolerance to abiotic stresses in durum wheats. More likely, the unexpected negative correlations with boron tolerance and yield reflect the seasonal conditions and especially the lack of plant available moisture in the subsoil.

7.4 Evaluation of the bread wheat SSD population Frame/Yarralinka//Pugsley.

7.4.1 Introduction

The Frame/Yarralinka//Pugsley single-seed descent population had previously been found to segregate for bicarbonate tolerance with at least one gene responsible for the tolerance response (Section 6.4). Tolerance in the population was mainly from the Frame/Yarralinka/23/1 parent, which has ancestral connections to Aroona and potentially contains the 7A locus. Minor genes for bicarbonate tolerance are also likely present in the population.

Under neutral soil conditions the bicarbonate intolerant parent Pugsley out-yields the more bicarbonate tolerant parent Frame/Yarralinka/23/1, but in highly alkaline soils the difference in yield between Pugsley and Frame/Yarralina/23/1 is reduced (Section 4.2.3).

Correlations were previously identified between soil pH or EC (0-10cm, 30-40 and 50-60cm) and yield for both Frame/Yarralinka/23/1 and Puglsey at alkaline sites (Section 4.2). Grain yield was found to decrease with increasing subsoil pH for the bicarbonate intolerant variety Pugsley at the highly alkaline site Angas Valley - North. The aim of this section was to assess the Frame/Yarralinka//Puglsey SSD population under alkaline field conditions at multiple sites to assess the influence of soil pH and EC on yield and to determine if the segregation for longer root lengths in bicarbonate treatment is associated with an increase in grain yield.

7.4.2 Materials and Methods

Seed of the Frame/Yarralinka//Puglsey single-seed descent (SSD) lines was sourced from 2004 field trials of the Durum Breeding group, University of Adelaide. In 2005 the Frame/Yarralinka//Puglsey population was sown in the field at eight sites, Angas Valley-North, Angas Valley-South, Buckleboo, Kapunda, Roseworthy-West, Roseworthy-East, Redhill and Wanderah. Field data on the grain yield of each line was also obtained from 2006 trials at the site Angas Valley, Buckleboo, Claypans, Coonalpyn, Roseworthy, Redhill, Two Wells and Winulta.

The field experiment consisted of 144 Frame/Yarralinka//Puglsey SSD lines with no replication, and 45 parental check plots and 36 standard varietal plots, giving a total experiment size of 225 plots.

The field trials were sown, managed, scored and harvested as per the methods in section 3.1.2.

Seed for bicarbonate testing was sourced from Roseworthy-East field trials in 2005. The bicarbonate testing of seedlings followed the procedures outlined in section 3.3 and 5.2. Twenty-four bread wheat seeds of the 144 SSD lines were arranged into four completely

randomised replications (2 trays per replication), with three seeds for each SSD line per tray, and two treatments (bicarbonate and control).

In 2006 soil samples were collected from the sites Buckleboo, Kapunda, and Redhill, and measured for soil pH and EC as described in section 3.1.4. Measurements of soil pH and $EC_{1:5}$ were correlated for the five 2005 sites, Angas Valley-South, Angas Valley-North, Roseworthy-West, Roseworthy-East and Wanderah. The soil pH and EC values at the three depths, 0-10cm, 30-40cm and 50-60cm were compared to the spatially adjusted grain yield for each plot.

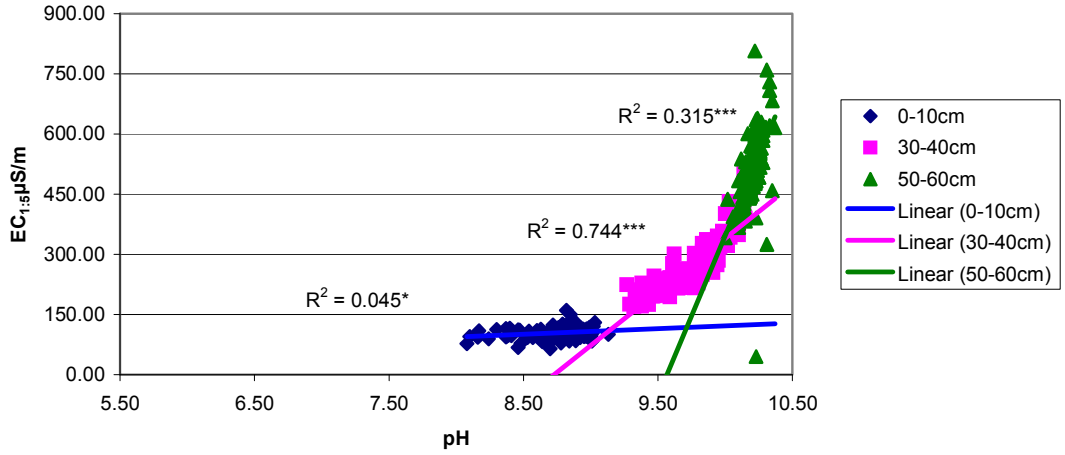
Bicarbonate data (RO, RL and RRL) was correlated with spatially adjusted grain yield for the eight sites, Angas Valley-North, Angas Valley-South, Buckleboo, Kapunda, Roseworthy-West, Roseworthy-East, Redhill and Wanderah in 2005.

No soil data was recorded in 2006 for the Frame/Yarralinka//Puglsey population, but yield data was obtained for the eight sites, Angas Valley, Buckleboo, Claypans, Coonalpyn, Roseworthy, Redhill, Two Wells and Winulta, and relative grain yield correlated with the bicarbonate data.

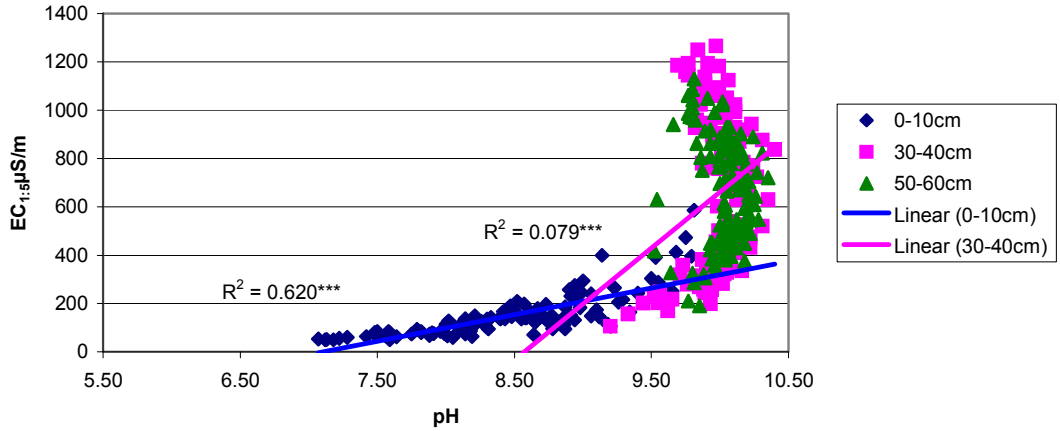
7.4.3 Results

One hundred and thirteen soil samples were collected at three depths, 0-10cm, 30-40cm and 50-60cm, for five sites, Angas Valley-South, Angas Valley-North, Roseworthy-East, Roseworthy-West and Wanderah, and measured for pH and $EC_{1:5}$. All five sites reached the subsoil pH levels (>pH 8.5) expected to influence plant growth, with all except WJ, having extreme pH values >pH 9.2. All sites also had highly toxic subsoil $EC_{1:5}$ levels (<400µS/m), but only the Wanderah site had toxic topsoil $EC_{1:5}$ levels (Figure 7.04). The pH and EC values of the paired Angas Valley and Roseworthy sites, found that the Roseworthy-East and Angas Valley-South, both on calcareous rises (Woorinen formation), had higher topsoil pH, lower subsoil pH and a substantially lower subsoil EC, than the neighbouring sites, Roseworthy-West and Angas Valley-North (Blanchtown clay), situated further down the slope.

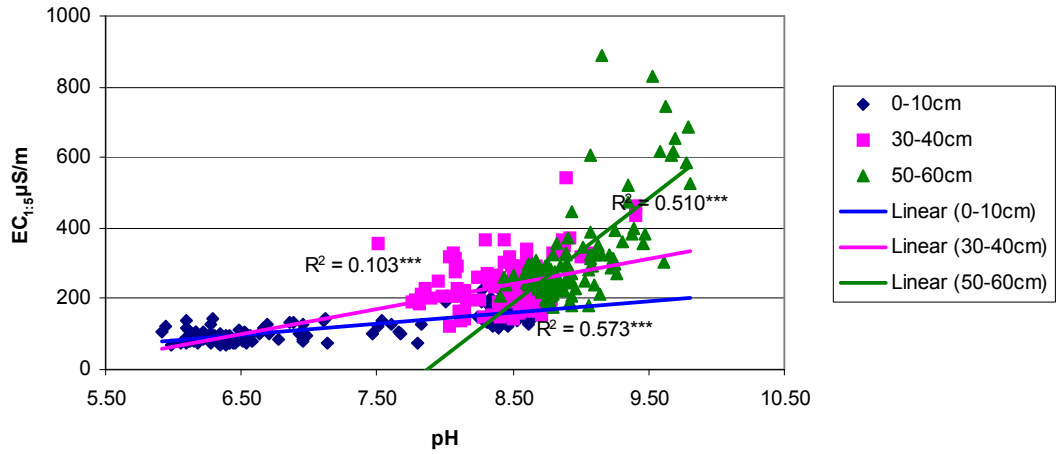
(a) Angas Valley - South



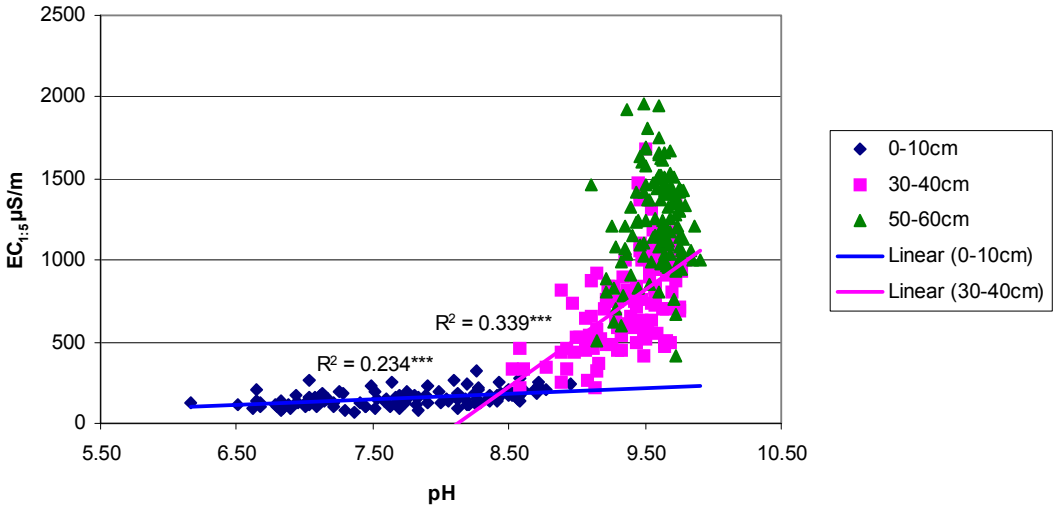
(b) Angas Valley - North



(c) Roseworthy - East



(d) Roseworthy - West



(e) Wanderah

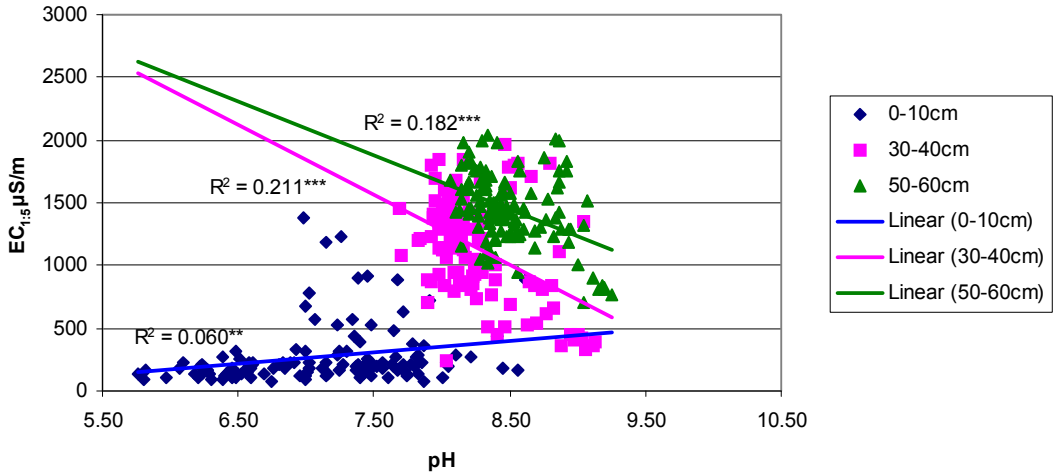


Figure 7.04: Correlation between soil pH and $EC_{1:5}$ at 0-10cm, 30-40cm and 50-60cm depths for the sites, (a) Angas Valley - South, (b) Angas Valley – North, (c) Roseworthy – East (d) Roseworthy - West, and (e) Wanderah.

A further three field sites, Buckleboo, Kapunda and Redhhill, were soil sampled (10 random plots) and recorded for grain yield (Table 7.20). Buckleboo and Kapunda recorded strongly alkaline subsoils (>pH 8.8), with Buckleboo also having toxic EC levels ($EC_{1:5}$>400µS/m). Redhill had moderately alkaline subsoil pH values (pH 8.5), but extremely toxic EC levels ($EC_{1:5}$>2000µS/m).

Table 7.20: Mean soil pH and $EC_{1:5}$(µS) measurements and standard deviation for eight field sites at depths 0-10cm, 30-40cm, 50-60cm, in 2005. pH>8.5, $EC_{1:5}$>400µS/m

	pH			EC		
Site	0-10cm	30-40cm	50-60cm	0-10cm	30-40cm	50-60cm
Buckleboo	8.70 ± 0.08	9.14 ± 0.17	9.56 ± 0.19	172.0 ± 74.5	866.8 ± 227.4	1187.0 ± 100.0
Redhill	6.41 ± 0.23	8.61 ± 0.19	8.34 ± 0.24	776.8 ± 611.3	2073.6 ± 425.8	3943.8 ± 976.2
Kapunda	8.04 ± 0.28	8.80 ± 0.27	9.12 ± 0.18	164.5 ± 39.1	199.3 ± 72.5	223.7 ± 69.4
Angas Valley-Nth	8.77 ± 0.21	9.73 ± 0.22	10.21 ± 0.06	104.5 ± 13.9	269.8 ± 69.0	513.2 ± 92.5
Angas Valley-Sth	8.45 ± 0.65	9.98 ± 0.20	10.01 ± 0.22	148.8 ± 91.6	649.8 ± 324.1	676.2 ± 224.1
Roseworthy-Est	7.26 ± 0.99	8.46 ± 0.31	8.93 ± 0.32	124.5 ± 43.1	239.2 ± 69.3	311.5 ± 132.0
Roseworthy-Wst	7.75 ± 0.65	9.38 ± 0.28	9.59 ± 0.16	159.2 ± 57.5	748.1 ± 287.5	1242.4 ± 302.6
Wanderah	7.12 ± 0.64	8.25 ± 0.32	8.51 ± 0.28	273.5 ± 241.1	1145.8 ± 395.4	1447.3 ± 283.5

Correlation of soil pH and $EC_{1:5}$ measurements with grain yield in 2005

Correlations between topsoil and subsoil pH and $EC_{1:5}$ values and plot yield (g/plot) were found significant for the sites, Angas Valley-South, Angas Valley-North and Wanderah (Table 7.21). At Angas Valley-South increasing topsoil pH and $EC_{1:5}$ (0-10cm) was found to significantly decrease grain yield, although $EC_{1:5}$ was at a level regarded as non-toxic (<150 µS/m). No root growth was observed or expected past 30cm in depth due to extreme pH (>9.2) and low soil moisture. At Angas Valley-North increasing subsoil pH (50-60cm) was found to significantly decrease grain yield, although subsoil EC (30-40cm and 50-60cm) was found to significantly increase grain yield, even through subsoil EC was >400µS. Similar to Angas Valley-South, no root growth was expected past 30cm in depth due to extreme pH (>9.2) and a lack of soil moisture throughout the growing season.

No significant correlations were identified at either Roseworthy site between soil pH and grain yield, even though both subsoil pH and EC had reached levels regarded as extremely toxic (pH 9.2, $EC_{1:5}$>400µS/m) to plant growth.

At Wanderah increasing both topsoil (0-10cm) and subsoil $EC_{1:5}$ (30-40cm and 50-60cm) at toxic levels, was found to significantly decrease grain yield. However, subsoil pH was also highly alkaline, but contrary to expected, increasing pH was significantly correlated with an increase in grain yield. The decrease in grain yield associated with both increasing subsoil EC and decreasing subsoil pH, was likely due to the negative relationship between subsoil pH and EC.

Table 7.21: Soil pH and $EC_{1:5}$ at depths 0-10cm, 30-40cm and 50-60cm correlated with plot yield (g) for the sites, Wanderah (WJ), Angas Valley-South (AV-S), Angas Valley-North (AV-N), Roseworthy-West (RAC-W) and Roseworthy-East (RAC-E), in 2005.

		Yield (g/plot)				
		AV-S	AV-N	RAC-W	RAC-E	WJ
pH	0-10cm	-0.160*	0.079^{ns}	0.003^{ns}	0.079^{ns}	0.050^{ns}
	30-40cm	-0.060^{ns}	-0.120^{ns}	-0.108^{ns}	0.103^{ns}	0.401***
	50-60cm	0.017^{ns}	-0.160*	0.010^{ns}	0.101^{ns}	0.430***
$EC_{1:5}$	0-10cm	-0.275**	0.081^{ns}	0.062^{ns}	0.086^{ns}	-0.371***
	30-40cm	-0.010^{ns}	0.214*	-0.118^{ns}	0.078^{ns}	-0.365***
	50-60cm	-0.001^{ns}	0.239**	-0.156^{ns}	0.082^{ns}	-0.330***

ns not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

Correlation of bicarbonate tolerance with relative grain yield in 2005

The spatially adjusted grain yield of plots from the eight sites was compared to the root lengths in bicarbonate (RL) and control (RO) treatments, and to the relative root lengths (RRL) to identify significant correlations. The relative grain yield at four of the sites, Angas Valley-South, Angas Valley-North, Kapunda, and Roseworthy-East was found significantly correlated with RO or RRL (Table 7.22). Both Angas Valley sites had a positive correlation between grain yield and the root length in the control treatment (RO), but not with the root length in the bicarbonate treatment (RL) or with relative root length (RRL), even though subsoil pH was extremely alkaline. Similarly, Kapunda and Roseworthy-East had a positive correlation between grain yield and the RO, although Kapunda also had a significant negative correlation between grain yield and RRL. At Kapunda a higher RRL, or tolerance to bicarbonate, resulted in a reduction in grain yield, even though subsoil pH was highly alkaline. Interestingly, Kapunda was the only site to have non-toxic subsoil EC levels ($EC_{1:5}$<200µS/m). No significant correlations were identified between relative grain yield and RL for any of the eight sites.

Correlation of bicarbonate tolerance with relative grain yield in 2006

Yield data from 2006 was also compared to the root lengths in bicarbonate (RL) and control (RO) treatments, and to the relative root lengths (RRL). No soil tests were conducted at the sites in 2006, although soil tests from 2004 and 2006 (Table 7.11, 7.14 and 7.21) indicate that the subsoils are likely to range from moderately alkaline to strongly

alkaline. Only the grain yield at Angas Valley was found significantly correlated with RL and RRL (Table 7.23). At Angas Valley increasing RL and RRL, or bicarbonate tolerance, led to an increase in grain yield.

Table 7.22: Correlation between spatially adjusted grain yield in 2005 at the sites Angas Valley-South (AV-S), Angas Valley-North (AV-N), Buckleboo (BR), Kapunda (KH), Roseworthy-West (RAC-W), Roseworthy-East (RAC-E), Redhill (RH) and Wanderah (WJ), and the mean root length in a highly alkaline bicarbonate treatment (RL), a mildly alkaline calcareous control treatment (RO) and the relative root length (RRL = RO/RL*100).

	Relative yield								
Treatment	AV-S	AV-N	BR	KH	RAC-W	RAC-E	RH	WJ	Mean
RO	0.232*	0.174*	-0.036^{ns}	0.375**	-0.014^{ns}	0.168*	0.052^{ns}	-0.062^{ns}	0.261**
RL	0.014^{ns}	0.069^{ns}	0.096^{ns}	-0.087^{ns}	-0.014^{ns}	0.033^{ns}	0.126^{ns}	0.110^{ns}	0.057^{ns}
RRL	-0.114^{ns}	-0.028^{ns}	0.108^{ns}	-0.267***	-0.009^{ns}	-0.059^{ns}	0.087^{ns}	0.134^{ns}	-0.088^{ns}

ns not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

Table 7.23: Correlation at eight sites in 2006 between spatially adjusted grain yield at Angas Valley (AV), Buckleboo (BR), Claypans (CK), Coonalpyn (CS), Roseworthy (RAC), Redhill (RH), Two Wells (TW) and Winulta (WC), and the mean root length in bicarbonate treatment (RL), control treatment (RO) and the relative root length.

	Relative yield								
Treatment	AV	BR	CK	CS	RAC	RH	TW	WC	Mean
RO	-0.076^{ns}	0.020^{ns}	-0.098^{ns}	0.010^{ns}	0.008^{ns}	0.002^{ns}	0.002^{ns}	0.004^{ns}	-0.050^{ns}
RL	0.203*	-0.037^{ns}	-0.143^{ns}	-0.073^{ns}	0.000^{ns}	-0.016^{ns}	-0.019^{ns}	-0.126^{ns}	-0.063^{ns}
RRL	0.227**	0.020^{ns}	-0.075^{ns}	-0.077^{ns}	0.003^{ns}	-0.013^{ns}	-0.017^{ns}	-0.120^{ns}	-0.026^{ns}

ns not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

The Frame/Yarralinka//Pugsley population had previously been screened for boron tolerance, with no segregation for root length found, consistent with both Frame/Yarralinka/23/1 and Pugsley containing the *Bo1* locus.

7.4.4 Discussion

Angas Valley 2005

The Frame/Yarralinka//Pugsley population was sown adjacent the Wk/TmWLYY9// WLYY9Tm population in 2005, with similarly low rainfall and below average grain yields. The Angas Valley-South site was sown on a calcareous rise, with a mean grain yield of

419g/plot (1.4t/ha). The Angas Valley-South trial site had extremely alkaline and moderately saline subsoil, with minimal root growth observed past 40cm in depth. Correlations were therefore only found between topsoil pH and EC, and grain yield, with increasing pH and EC associated with a decrease in grain yield. However, while topsoil pH was >8.5 and expected to influence plant growth, topsoil EC remained below $EC_{1:5}$ 160µS and was unlikely to be directly reducing plant growth. The significant positive relationship between topsoil pH and EC may be responsible for the apparent association between topsoil EC and grain yield.

Greater tolerance to bicarbonate did not increase grain yield in the Frame/Yarralinka//Pugsley population even though a reduction in yield was associated with increasing topsoil pH in the moderately alkaline range. However, longer root length in a moderately alkaline control was significantly correlated with an increase in grain yield. The moderately alkaline control treatment (pH 8.4) was a better indicator of grain yield than the highly alkaline bicarbonate treatment (pH 9.2) at Angas Valley-South. At Angas Valley-South in 2005, minimal soil moisture and toxic subsoils (high pH and EC) resulted in reliance on the topsoil moisture for growth, with root growth and grain yield partly reduced by moderately alkaline soil conditions.

The Angas Valley-North site was slightly lower yielding than the Angas Valley-South site with a mean grain yield of 366g/plot (1.2t/ha). The trial site was located on soil that was less calcareous, with a higher clay content, which resulted in a pH >9.2 and an $EC_{1:5}$ >400µS/m at 30-40cm in depth. The subsoil was also prone to hard-setting from high sodicity, with no root growth observed past 30cm in depth. However, increasing subsoil pH was associated with decreased grain yield, and increasing subsoil EC was found to significantly increase grain yield. The contradictory results for subsoil pH and EC (50-60cm) relate to the negative correlation between pH and EC at 50-60cm in depth. The extreme pH levels may be adversely affecting grain yield, which leads to a superficial correlation between subsoil EC and grain yield. Alternatively, high subsoil EC may have reduced biomass production in the early grown stages, conserving soil moisture, resulting in improved grain production. The association between subsoil constraints and grain yield rather than topsoil factors is unclear, since minimal root growth was observed in the subsoil, but movement of soil moisture by capillary from depth may have occurred.

Similar to Angas Valley-South, greater tolerance to bicarbonate at the highly alkaline Angas Valley-North site did not lead to increased grain yield. However, longer root length in a moderately alkaline control was significantly correlated with an increase in grain yield. Longer root length in the moderately alkaline topsoil would have increased the plants ability to acquire water and nutrients in a below average rainfall season. At Angas Valley-North in 2005, the Frame/Yarralinka//Pugsley population was being significantly affected by toxic soil conditions, possibly from the highly variable alkaline topsoil (pH 7.0-9.6) or the toxic subsoil (pH 9.2-10.5, $EC_{1:5}$ >400µS/m), either directly from bicarbonate or sodium toxicity, or indirectly through water availability.

Roseworthy 2005

At the Roseworthy site, similar to Angas Valley, Roseworthy-East was sown on a calcareous rise, although had a higher mean grain yield of 832g/plot (2.8t/ha) for the Frame/Yarralinka//Pugsley population. The Roseworthy-East site had moderately to highly alkaline subsoil, with few plots reaching toxic EC levels at the 50-60cm depth. Soil EC was not expected to influence grain yield, resulting in no significant correlations between soil EC and grain yield. Subsoil pH (50-60cm) was within a range expected to influence grain yield, but no correlations were identified between subsoil pH and grain yield. Root growth past 50cm in depth was likely minimal in the absence of subsoil moisture. Subsoil pH (30-40cm) was lower, ranging from pH 7.5-8.8, but had no relationship with grain yield, even though root growth was observed at 30-40cm in depth.

In the absence of a strong pH affect at RAC-E, greater bicarbonate tolerance in the Frame/Yarralinka//Pugsley lines did not lead to increased grain yield. However, similar to Angas Valley-South and Angas Valley-North, longer root length in a moderately alkaline control was significantly correlated with an increase in grain yield. Longer root length in the moderately alkaline subsoil would have increased the plants growth rate and final grain yield. At Roseworthy-East in 2005, the Frame/Yarralinka//Pugsley population was minimally affected by moderately alkaline soils, and with no detrimental affects on grain yield due to salinity, other more significant constraints were likely affecting the grain yield of the population. In 2005, stripe rust was prevalent at Roseworthy and may have affected grain yield in the Frame/Yarralinka//Pugsley population, which varied for a moderate level

of resistance, arising from the bicarbonate intolerant, moderately resistant parent Pugsley (VPM gene).

Similarly, at the Roseworthy-West site, stripe rust was prevalent in the plots and may have influenced the outcome of soil pH and EC affects on grain yield. Roseworthy-West had a slightly higher mean yield of 856g/plot (2.9t/ha) for the Frame/Yarralinka//Pugsley population. The Roseworthy-West site was located on alluvial soil (Blanchtown clay), with moderately to highly alkaline subsoil and highly toxic EC levels. Minimal root growth was observed past 40cm in depth due toxic soil conditions, pH >9.2 and $EC_{1:5}$ 1000µS/m, and low subsoil moisture. High pH and EC at 30-40cm in depth was expected to influence plant growth, but no significant correlations were identified between subsoil pH and EC, and grain yield. Furthermore, greater tolerance to bicarbonate in Frame/Yarralinka//Puglsey lines had no influence on grain yield. At Roseworthy-West in 2005, alkaline pH at high levels was not a dominant factor in determining grain yield and consequently, greater bicarbonate tolerance in Frame/Yarralinka//Puglsey lines did not lead to a yield improvement.

Wanderah 2005

The Wanderah trial site in 2005 for Frame/Yarralinka//Puglsey was adjacent to the Wk/TmWLYY9//WLYY9Tm population, and had similar site characteristics. Below average rainfall for 2005 resulted in the lowest mean grain yield of 305g/plot (1t/ha) of the five sites. The topsoil had numerous magnesia patches (EC>400µS) resulting in a significant negative correlation between topsoil EC and grain yield. Subsoil $EC_{1:5}$ was also extremely high and was again negatively correlated with grain yield. However, as previously found for other trials at Wanderah (Section 7.2 and 7.3), contrary to expected, increasing subsoil pH in the moderately alkaline range was associated with an increase in grain yield. The extremely high subsoil EC was expected to be the dominant soil factor at the site, more so than the moderately alkaline pH, with the apparent positive correlation between pH and grain yield produced from the negative association between subsoil pH and EC.

Buckleboo, Redhill and Kapunda 2005

Three other trial sites where used to determine if bicarbonate tolerance led to improved grain yield. The lowest yielding site, Buckleboo had a mean grain yield of 248g/plot (0.8t/ha), with extremely alkaline and highly toxic EC levels in the subsoil. No correlations were identified between grain yield and either bicarbonate tolerance or longer root lengths in the control. At Buckleboo, alkaline pH did not appear to be significantly affecting grain yield, but EC was a possible dominant factor. Similarly, at Redhill, the mean yield was below average at 388g/plot (1.3t/ha), with the site having moderately alkaline and extremely toxic EC levels. Extreme EC (to 4000μS) was likely the dominant factor affecting grain yield, resulting in no significant correlations between grain yield and either bicarbonate tolerance or longer root lengths in the control.

At Kapunda, the subsoil EC was below 400μS/m and the pH was moderately alkaline. The subsoil pH was within a range expected to influence plant growth. A positive relationship was identified between root length in the control treatment and grain yield, however, a negative relationship was identified between bicarbonate tolerance and grain yield. The Kapunda site was in a high rainfall, high yielding area, but the trial yielded well below the district average at 786g/plot (2.6t/ha) in 2005, with below average rainfall. Similar to previous experiments, bicarbonate tolerant lines may have had greater early season biomass, leading to premature water stress and poor grain fill.

Bicarbonate tolerance and grain yield in 2006

The trials were repeated in 2006 to determine if the absence of correlations between grain yield and bicarbonate tolerance re-occurred in other more average rainfall seasons. For seven of the sites no significant correlations were identified between grain yield and either bicarbonate tolerance or longer root lengths in the control, even though sites were likely to vary in both pH and EC values. Only Angas Valley was found to have a significant correlation, with increased bicarbonate tolerance related to an increase in grain yield.

General Comments

The Frame/Yarralinka//Pugsley population, along with RAC875/Cascades and Worrakatta/TmWLYY9//WLYY9Tm, appear to maintain a general positive correlation for root length in the mildly alkaline control treatment and grain yield, but a negative relationship between relative root length (bicarbonate tolerance) and grain yield, in 2004, 2005 and 2006. The negative relationship also appears to coincide with a negative association between boron tolerance and grain yield in 2003 and 2005 for the Wk/TmWLYY9//WLYY9Tm population, contrary to the results expected. The negative association may be associated with deleterious effects of loci for boron or bicarbonate tolerance within these populations, although the negative effect is more likely associated with the weather patterns and soil moisture regimes across the years 2003-2006, with virtually no subsoil moisture present throughout the growing season. With plant growth relying on topsoil moisture, improved growth may be associated with avoidance of subsoil constraints rather than tolerance, or greater water use efficiency. The move towards high biomass production early in the season, from high nitrogen use or greater tolerance to environmental constraints, increases water use, causing more frequent periods of water stress, particularly at anthesis when temperatures increase and rainfall declines, resulting in a lower final grain yield.

Chapter 8

General Discussion

Soil pH is one of the most important parameters in assessing plant growth responses, as an indicator of soil chemical processes and the availability of many essential nutrients or solubility of toxic ions. In South Australia extensive soil mapping of agricultural regions by Maschmedt and colleagues (2002) of PIRSA Land Information identified over 80% of the cropping area as having alkaline soils, with mostly moderately to highly alkaline subsoils. Not surprisingly, in the work for this thesis, soil samples extracted in 2004 and 2006, up to a depth of 50 or 60cm, from several areas around South Australia, were slightly acid to alkaline in the topsoil, but as pH increased with depth, subsoils were entirely alkaline, ranging from moderately to strongly alkaline (Chapter 4 and 7).

Strongly alkaline soils (pH_w >9.2) often occur when sodium is present, forming highly soluble sodium carbonate. These sodic-alkaline soils tend to be impermeable with low porosity, poor water-soil relations and are dispersive in nature (Rengasamy 2002). Salinity is also common to these semi-arid areas where rainfall is insufficient to leach from the soil the base forming cations, such as sodium, calcium, and magnesium, that are slowly released from the weathering of rocks and minerals or deposited from rainfall. A further consequence of impermeable subsoils with minimal leaching is the accumulation of toxic concentrations of boron in evaporative deposits at the upper surface layer of the B horizon (Biggar and Fireman 1960). The occurrence of salinity (transient) and boron toxicity at sites identified as having high pH subsoils was almost universal (Chapter 4 and 7).

Bread and durum wheat varieties were grown at several sites in 2004 or 2005, where pH values reached >pH 8.5 in the subsoil, yet increasing pH in the alkaline range was not found to decrease grain yield, except for the bread wheat variety Puglsey at Angas Valley-South in 2005 (Chapter 4). However, Pugsley at Angas Valley-South was also found to have reduced yield from increasingly toxic EC levels. Soil EC was measured at toxic levels ($EC_{1:5}$ >400µS/m) for all, except one, field site and appeared to be a significant factor at five of the field sites in 2004 and 2005, where increasing soil EC significantly reduced grain yield in both the durum and bread wheats. However, correlations calculated for most sites failed to identify significant relationships for either soil pH or EC at varying depths

and grain yield. The inability to separate a distinct high pH effect on grain yield was not entirely unexpected, since all sites suffered from low available soil moisture, particularly in 2004, and a range of abiotic stresses, such as salinity and boron, and biotic stresses, such as crown rot in the durums and stripe rust in the bread wheats.

Similarly, bread and durum wheat populations were grown at several sites in 2004 or 2005, yet only the RAC875/Cascades and Frame/Yarralinka//Pugsley populations at Wanderah and Angas Valley-North, respectively, had a reduction in yield corresponding to an increase in subsoil pH (Chapter 7). At all eleven field sites, subsoil pH was >8.5 and $EC_{1:5}$ >400µS/m, except for the Roseworthy site in 2004. Subsoil EC was found to decrease grain yield at two of the sites (Roseworthy and Wanderah 2005), yet interestingly, subsoil EC was also found to be correlated with an increased grain yield at two sites (Angas Valley-North 2005, Wanderah 2004), which were the same two sites where subsoil pH was correlated with decreased grain yield. The contradictory results for subsoil pH and EC relate to the negative correlation between pH and EC. For Angas Valley – North, extreme pH levels may have been adversely affecting grain yield, which led to a superficial correlation between subsoil EC and grain yield. The opposite is likely the case for Wanderah with extreme EC and moderate pH in the subsoil. Soil pH and EC, however, again failed to identify significant correlations with grain yield at most of the sites.

Most of the significant correlations in 2004 and 2005 between soil pH and EC and grain yield occurred with the bread wheats, rather than the durum wheats (Chapter 4). This is inconsistent with previous field trials by Cooper (2002) where a significant yield reduction in the durum variety Tamaroi was found at three of six sites in 2001, but bread wheats were unaffected by increasing alkaline pH. Generally, durum wheats are less tolerant to high pH (HCO_3^- toxicity) (Lui and Rathjen 1998) and salinity (Na^+ toxicity) (Munns *et. al.* 2000, Cooper 2002), and more susceptible to Zn, Mn and Fe deficiency (Seberi *et. al.* 1999, Genc *et. al.* 2006), yet under non-yield limiting constraints have a greater yield potential than bread wheats (Rathjen *pers. comm.*). In 2004/2005 the yield of the durum wheats was 20-90% less that bread wheat trials sown in adjacent plots (Table 4.02, 4.07, and 4.11). Multiple yield-limiting factors, including soil physical and chemical constraints and biotic stresses, were influencing grain yield, which in any year, were likely to inhibit the identification of a single soil constraint at the field sites, particularly a dynamic character, such as soil pH.

In many cases the poor performance of durum wheats in comparison to bread wheats on highly calcareous or sodic-alkaline soils has been associated with the deficiency of plant available nutrients, aggravated by poor nutrient uptake efficiency by the durum wheats (Zubaidi *et. al.* 1999). A comparison of two groups of durums selected on the bases of different responses to HCO_3^- was undertaken at eight sites. The subsoil pHs over the sites ranged from pH 7.1 to 9.6, the plant tissue concentration of Fe^{3+}, Mn^{2+}, Zn^{2+}, K^+ and Ca^{2+} were found to decrease with increasing pH, while Na^+, B, P and Mg^{2+} were found to increase with increasing pH (Table 4.20). Furthermore, the tissue concentration of Mn^{2+}, Cu^{2+}, Zn^{2+}, K^+, Ca^{2+}, Mg^{2+} and P were below the critical level at several sites, particularly Coonalpyn and Angas Valley that have undergone relatively recent reworking of soils (Molineaux type soils). Selection for genotypes with enhanced ability to acquire adequate nutrition on these soils would confer a significant adaptational advantage for improved production. Durum landrace lines selected for tolerance to bicarbonate solution were generally found to take up less Ca^{2+}, Mg^{2+}, Na^+, and possibly Mn^{2+}, but more Zn^{2+}, K^+, and possibly Fe^{3+} and Cu^{2+} in the field than those lines selected with lower tolerance to bicarbonate solution (Table 4.21). Tolerance to bicarbonate, or high pH soils, in durum wheats appears to be strongly related to the ability to extract adequate nutrition from alkaline soils, either by tolerance to HCO_3^- toxicity which otherwise decreases root elongation and reduces the surface area for nutrient absorption (Alhendawi *et. al.* 1997), or mitigating HCO_3^- prevention of the absorption of nutrients and depression of their translocation (Forno *et. al.* 1975, Yang *et. al.* 1993, Marschner 1995, Romheld 2000, Zuo *et. al.* 2007).

The inability to detect a significant yield penalty from increasing pH, in the presence of increasing HCO_3^-/CO_3^{2-} concentrations and reduction in plant available nutrients, may be associated with the capricious nature of soil pH and the methods of its measurement. Soil pH can be highly variable across a small area and change with depth, but can also fluctuate throughout a season, from year to year, or over decades. Soil pH values are also highly dependent on methods of pH measurement, with time in storage, mixing intensity, soil to water ratio, measurement of supernatant versus soil sediment, and type of pH instrument, affecting final pH readings (Slattery *et. al.* 1999). A single soil core sample from every 2nd or 5th plot may have been inadequate to accurately represent the average pH over the area of each plot. Furthermore, alkaline pH can limit plant growth via several paths, which may

vary within or between sites. Plants growing on high pH soils can be affected directly by high OH^- concentrations (Kopittke and Menzies 2004), or indirectly by soil physical constraints, nutritional disorders and toxicity of HCO_3^-/CO_3^{2-}.

The measurement of soil pH at field sites was used extensively as an indicator of the HCO_3^- and CO_3^{2-} concentration of the soil solution, yet the latter only represents a part of the overall pH effect on plant growth. However, in most soil solutions, the pH of alkaline soils is directly related to the concentration of HCO_3^- and CO_3^{2-} (Orlov 1992), and becomes toxic at a concentration of approximately 3-5mM HCO_3^- or pH 8.5 (Alhendawi *et. al.* 1997, Lui and Rathjen 1998), when soluble Na_2CO_3 and $NaHCO_3$ form in sodic-alkaline soils. Soil samples collected and tested at three sites found that both HCO_3^- and CO_3^{2-} increased with increasing pH, although CO_3^{2-} only became soluble after pH 8.5 (Figure 4.24 and 4.25). A pH of 8.5 has generally been regarded as the critical level for toxicity, however, the solution pH at the soil-root interface can be up to 2 units different from the bulk soil, and the root apoplasts are strongly buffered in the slightly acidic range, which can influence the form of carbonate species in contact with the plant roots (Grignon and Sentenac 1991, Guern *et. al.* 1991). An increase in both HCO_3^- and CO_3^{2-} with increasing soil pH is likely to increase the concentration of HCO_3^- at the root surface. A higher concentration of HCO_3^- was also identified in the topsoil as compared to the subsoil, with considerable variation around the trend line. At a fixed pH, the HCO_3^- concentration may vary depending on interactions with other cations, organic matter, or waterlogging, and can only be used as a general indicator for the concentration of HCO_3^- in the soil solution or in contact with the root surface.

One of the major limitations associated with measuring the level of bicarbonate toxicity occurring in the field is the inability to gauge the degree of toxicity, unlike boron or sodium toxicity, where the concentration of the ions can be detected in tissue samples. Most of the research conducted on bicarbonate toxicity in various species has been conducted in controlled solution culture experiments. This allows the isolation of bicarbonate toxicity responses from other multiple physical and chemical properties occurring in the field. A simple screening method was developed using 5mM $NaHCO_3$, 1mM Na_2CO_3, 5mM $CaCO_3$, 15µM H_3BO_3, 2.5µM $ZnSO_4.7H_2O$, adjusted with Na_2CO_3 to pH 9.1-9.2 daily, with root length measured after 9 or 10 days, and a control treatment

containing 5mM $CaCO_3$, 15µM H_3BO_3, 2.5µM $ZnSO_4.7H_2O$, which was not adjusted for pH, yet retained a pH of approximately 8.4 (Chapter 5).

Previous bicarbonate screens by Lui and Rathjen (1998) using similar screening methods found significant genetic variation between bread and durum varieties, landraces and other *Triticum* species, with the bread wheat Krichauff identified as the most bicarbonate tolerant line. To further evaluate the tolerance to bicarbonate toxicity in commercial wheat lines, a large number of durum and bread wheat varieties were tested originating from different geographical areas, with commercial release dates spanning several decades. A difference of up to 30% in relative root length between bread wheat varieties was identified, yet no varieties were found to be more tolerant that Krichuaff (Table 5.08 and 5.10). Krichauff was, at least in part, the result of breeding work at Rudall on the Eyre Peninsula on high $CaCO_3$ soil, which would have led to its high level of bicarbonate tolerance. However, many of the varieties, which have dominated the southern Australian wheat belt throughout the last 50 years, only had a low to moderate level of bicarbonate tolerance. Bicarbonate toxicity appears not to have had the level of selection pressure observed for problems such as CCN or boron, which led to the dominance of more tolerant varieties.

In the durum wheat varieties, Kalka was found to be the most tolerant of the Australian commercial varieties, although it had a significantly shorter root length in HCO_3^- solution than Krichauff. These durum varieties have mainly originated from and been bred to suit deep, heavy soils, such as northern New South Wales (analygous to the Blanchtown land types of SA), with little to no alkaline selection pressure, which likely led to the low level of bicarbonate tolerance. The lack of alkaline selection pressure is also reflected in durum wheats higher incidence of trace element deficiency, such as Zn, Mn and Fe, and high Na uptake, also commonly associated with alkaline soils. The lower tolerance of durum wheats to bicarbonate is expected to be one of the factors responsible for their poor adaptation to calcareous (Woorinen) or silicious sand (Molineaux) type soils in lower rainfall areas (<400mm p.a.), and their commercial restriction to the higher rainfall areas of the northern Mt Lofty Ranges.

Advanced durum breeding lines and landrace lines were assessed to determine if the level of bicarbonate tolerance in durum wheats could be improved to a level equal or greater

than the most tolerant bread wheat, Krichauff. Several advanced durum lines were found to exceed Krichauff, some of which contained bread wheat parentage. The improved tolerance of durum wheats (AB) from the inclusion of genetic material from bread wheat lines (ABD) therefore offers the potential for tolerant bread wheats, such as Krichauff, to be used for the improvement of bicarbonate tolerance in durum wheats.

The other advanced durum lines found to exceed Krichauff had ancestry of durum landrace lines, including Palestine 7, Karpaz and Menshia 54, that had previously been selected by Das and Cooper (unpublished) for tolerance to bicarbonate. Based on the success of these landrace lines in improving bicarbonate tolerance in durum breeding lines, further potential sources of bicarbonate tolerance were sourced from similar geographical origins of west and south Asia, and also from some of the more traditional durum growing areas of southern and eastern Europe, and north Africa. Eight percent of lines were found to exceed Kalka, with fewer equal to the bread wheat Krichauff (Table 5.14). These tolerant lines mostly originated from the central Asia region of Iran, Afganistan, Iraq and India, and to a lesser extent countries of the Former Soviet Union, such as, Kazakhstan and Turkmenistan. Previous landrace selection for both boron and salinity tolerance had identified the Asia regions of India and Iraq as sources of tolerant germplasm (Moody et. al. 1988, Paull et. al. 1992), making the region a potential target for sourcing multiple adaptational characters that may be useful in South Australian durum breeding programs.

Large variation exists between genotypes in response to alkaline soils for bread and durum wheats, yet no genetic studies had previously been conducted to determine the mode of inheritance or number of genes involved in the bicarbonate response. In an attempt to evaluate the genetic composition of bicarbonate tolerance in durum, landrace lines identified as the most tolerant were crossed with Kalka, Tamaroi, or the fixed breeding line Na49/Kalka#4, for the screening of F_2 and F_3 populations (Chapter 6). Transgressive segregation was identified in the populations AUS4897 x Tamaroi, AUS4897 x Na49/Kalka, AUS7890 x Na49/Kalka#4 and R622Sh/Tm//WLYY9///Tm x Na49/Kalka#4, and two loci identified in the Na49/Kalka#4 x Tamaroi population, which provided the potential for the populations to be used in further genetic analysis or field studies, although the parentage of some of the populations may need some clarification due to inconsistent parental root length distributions.

Further genetic analysis of bread and durum wheat mapping populations developed by single-seed descent or doubling haploids identified several putative genes involved in the bicarbonate tolerance response. A highly significant QTL for root length in bicarbonate solution was located on chromosome 7A for the RAC875/Cascades and Berkut/Krichauff populations, suggesting a major role for a locus on 7A in the bicarbonate tolerance response. The tolerance to bicarbonate identified in both Cascades and Krichauff could be traced to a common tolerant ancestor, Aroona, which may represent the original source of the QTL on chromosome 7A. Other possible loci identified in marker analysis, included regions on chromosome 4A (Cascades), 6D, 3D and 2B (Krichauff) and 7B (Berkut). Quantitative analysis of a further two populations, Frame/Yarralinka//Pugsley and Wk/TmWLYY9//WLYY9Tm, suggest that multiple genes (at least three) are segregating for root length in bicarbonate solution. While bicarbonate tolerance appears simply inherited, the total response may involve a number of other tolerance mechanisms, other than just tolerance to HCO_3^- toxicity, such as Zn or Fe efficiency, root vigour or morphology. Here the response to bicarbonate toxicity was measured as a reduction in root growth in a low concentration nutrient, sodium bicarbonate/carbonate solution.

Three populations, RAC875/Cascades, Frame/Yarralinka//Pugsley and Wk/TmWLYY9//WLYY9Tm were assessed in the field to determine whether the difference in root length when exposed to bicarbonate in high pH solution corresponded to grain yield in the field, given the presence of many abiotic and biotic stresses. A general positive correlation for root length in the (mildly alkaline) control and grain yield was found, but unexpectedly, a general negative relationship was identified between the root length in highly alkaline conditions (bicarbonate tolerance) and grain yield, in 2004, 2005 and 2006 (Table 7.06, 7.12, 7.15, 7.16, 7.22 and 7.23). Also contrary to the expected, a negative association was identified between boron tolerance and grain yield in 2003 and 2005 for the Wk/TmWLYY9//WLYY9Tm population (Table 7.17 and 7.18). The negative association may be associated with deleterious effects of loci linked to the boron or bicarbonate tolerance within these populations, although unlikely, since a number of different loci appear to be responsible for both the boron and bicarbonate responses in the populations. Alternatively, the negative response of grain yield to bicarbonate and boron tolerance may be associated with the absence or very low levels of available subsoil moisture throughout consecutive growing seasons, from 2003-2006.

The general rainfall pattern for the South Australian cropping districts has typically been to begin seeding on the late autumn rain with stored subsoil moisture from summer rainfall and carry-over from the previous season. Throughout the growing season, June to October, rainfall events are intermittent with dry periods where the topsoil dries and crops rely on the subsoil for maintaining plant growth. Wheat varieties were bred and selected to suit these conditions, with high root vigour, mid maturity and longer roots with improved tolerance to subsoil constraints for better exploration and acquisition of subsoil moisture during the dry phases. Selection for boron tolerance by selecting plants with longer root lengths in boron toxic soil and solution cultures has previously been found to increase yield by up to 19% when grown in soils with toxic concentrations of boron (Cooper 2002, Brooks 2004). However, in 2003 and 2005, boron tolerance was found detrimental to yield, along with bicarbonate tolerance in 2004, 2005 and 2006. Similar to boron tolerance, bicarbonate tolerance was selected by selecting plants with longer root lengths under toxic concentrations in solution. This selection for longer root lengths to tolerate and grow into the subsoil and lack of available soil moisture appears to be the key factors in the observed responses.

From 2003 to 2006, virtually no subsoil moisture had been present at the beginning or throughout the growing season to support growth during the intermittent dry periods. Crops had been relying on moisture almost entirely from rainfall events and dew formation from May to October, with moisture rarely moving below 40-50cm in depth. The positive correlation between longer root length in the (mildly alkaline) control treatment and grain yield indicates an advantage for improved exploration and increased root surface area for greater acquisition of nutrients and water in the topsoil, particularly in a water limited soil. In seasons where water or nutrition was not limiting, greater root growth in the topsoil would not be expected to significantly increase grain yield. Similarly, in a non-water limiting season, bicarbonate tolerance would not be expected to increase grain yield, with plants capable of sustaining growth from moisture in the topsoil (0-30cm), which is characteristically slightly acidic to moderately alkaline with non-toxic EC levels. However, during South Australia's relatively long growing season (≈6 months), sporadic drying of the topsoil is common, hence the expectation that enhanced ability to grow into high pH subsoil would be advantageous.

In a typical growing season tolerance to subsoil constraints, such as bicarbonate or boron, would be expected to be advantageous by allowing plants to draw available soil water from the subsoil to sustain growth when topsoil moisture was limiting. The negative relationship between bicarbonate tolerance (measured as longer root length in bicarbonate solution or relative root length) implies a more complex reaction. From 2003 to 2006 average to below average rainfall was recorded (Appendix 3), with the season often beginning with negative water potential in the subsoil. Tolerance to bicarbonate and root growth into the subsoil may have been of initial benefit by supplying some moisture that had percolated into the subsoil during higher rainfall events, providing a higher number of tillers and greater biomass. However, in a drier finish to the season, particularly 2004, the exhaustion of soil moisture in comparison to lower water use plants with less biomass, may have reduced the final seed set and grain fill. A similar response has been identified with increased nitrogen use, where 'haying-off' has occurred from rapid vegetative growth in response to adequate soil water and high nitrogen, followed by terminal drought, leading to grain yield disproportionately lower in relation to total dry matter production (van Herwaarden *et. al.* 1998, McDonald 1991). Premature ripening of cereal crops has occurred frequently in the last decade, which has corresponded with an increase in cereal crops (originally intended for grain production) being cut for hay, and the release of early maturing wheat varieties, such as Axe (AGT), in 2007.

Continuing drought throughout the season is not commonly associated with 'haying-off', but only when water deficiency occurs near anthesis (van Herwaarden *et. al.* 1998). In 2004, average to above average rainfall was recorded between May and September, followed by a very hot, dry October. In 2005, above average rainfall fell in June and October, but was generally below average from July to September, with low subsoil moisture due to below average rainfall in 2004. From 2002 to 2008 average September and October rainfall has been well below average (Appendix 7), which has corresponded to below average grain yields in comparison to the previous decade. In this thesis, crop performance in 2003-2006 was recorded as grain yield, with no measurements of tiller number, dry matter, kernel number or kernel weight, which would be necessary to determine if bicarbonate tolerance led to an increase in biomass. Measurements of soil water availability and use would also be valuable in understanding the bicarbonate tolerance response under varying rainfall patterns. Alternatively, characterisation of morphological or physiological differences between the root systems of bicarbonate

tolerant and intolerant lines may demonstrate that intolerant plants have a more shallow, more fibrous root system, avoiding exposure to other toxic subsoil element (under limited rainfall), or that bicarbonate tolerance in energetically expensive, from greater root elongation, active exclusion of ions, or shifts in ionic balance to compensate for exposure to toxic concentrations of HCO_3^-.

Bicarbonate tolerance clearly has an influence on plant growth in the field, but further research is necessary to unravel the complex environmental interactions affecting final grain yield. The consistent identification of negative yield effects associated with bicarbonate tolerance, boron tolerance, and sodium exclusion (Rathjen *pers. comm*.) for bread and durum wheats in the last five years in South Australia, generally associated with decreased and erratic rainfall patterns, and the absence of subsoil moisture, may represent a shift in cropping systems that requires new breeding objectives to be set for grain yield improvement.

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Appendix 1: South Australia map, including agricultural regions and trial locations (adapted from SA map in UBD 44th Edition 2006).

NOTE:
This map is included on page 341 of the print copy of the thesis held in the University of Adelaide Library.

Appendix 2: Surface charts for soil measurements of pH and EC at field sites in 2004 and 2005.

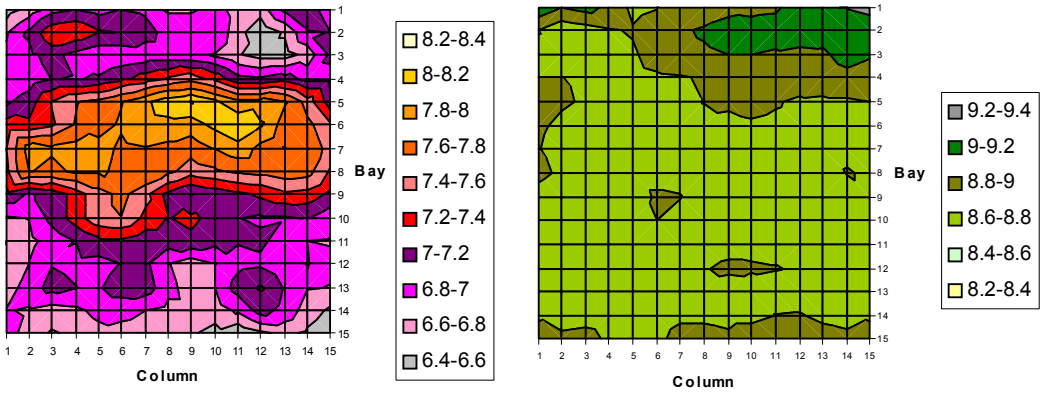


Figure 2A: Surface chart of soil pH 0-10cm and 40-50cm at Roseworthy for the RAC875/Cascades population in 2004.

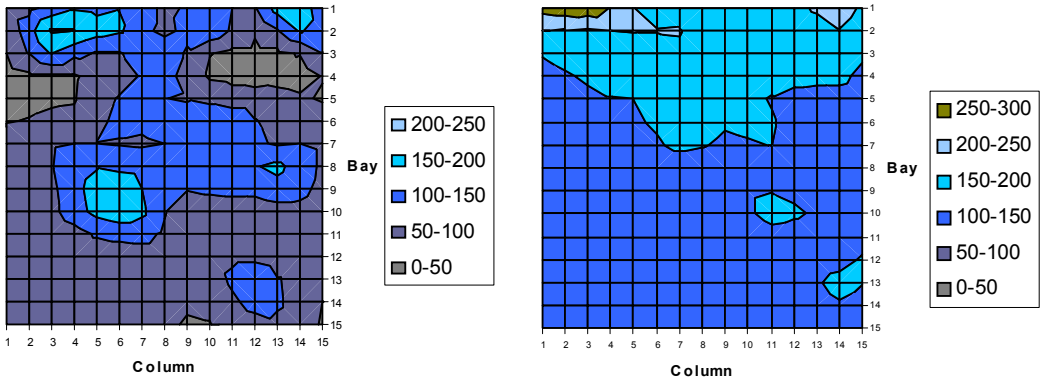


Figure 2B: Surface chart of soil $EC_{1:5}$µS/m 0-10cm and 40-50cm at Roseworthy for the RAC875/Cascades population in 2004.

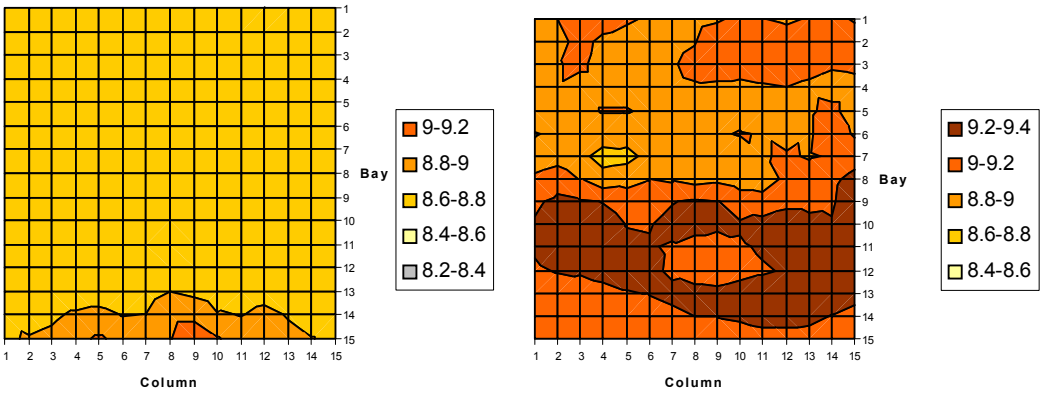


Figure 2C: Surface chart of soil pH 0-10cm and 40-50cm at Buckleboo for the RAC875/Cascades population in 2004.

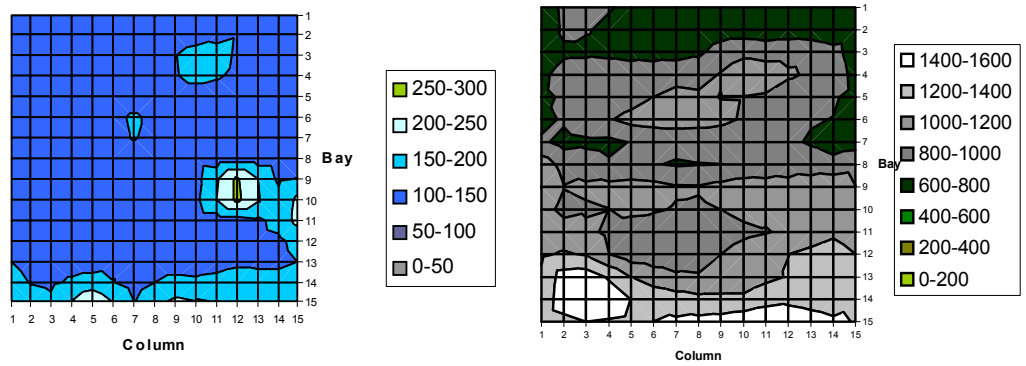


Figure 2D: Surface chart of soil $EC_{1:5}$µS/m 0-10cm and 40-50cm at Buckleboo for the RAC875/Cascades population in 2004.

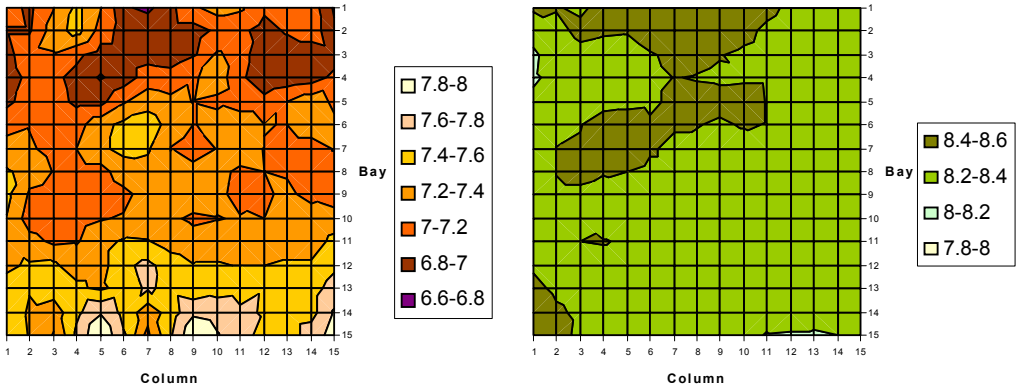


Figure 2E: Surface chart of soil pH 0-10cm and 40-50cm at Wanderah for the RAC875/Cascades population in 2004.

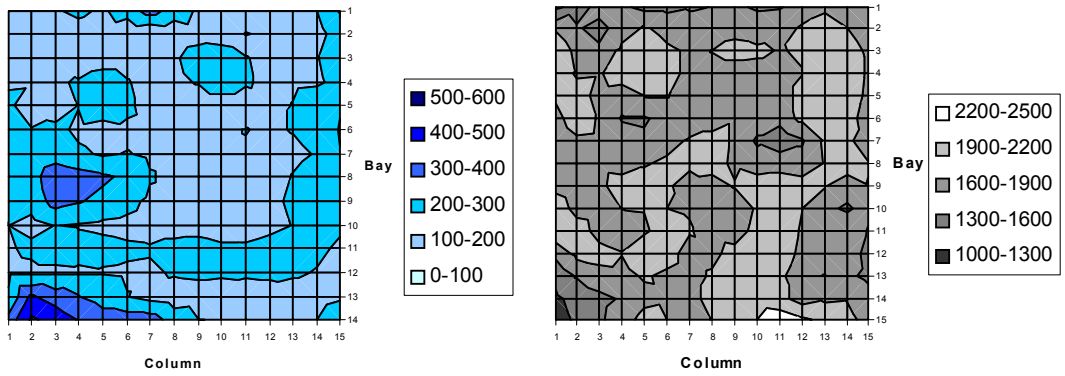
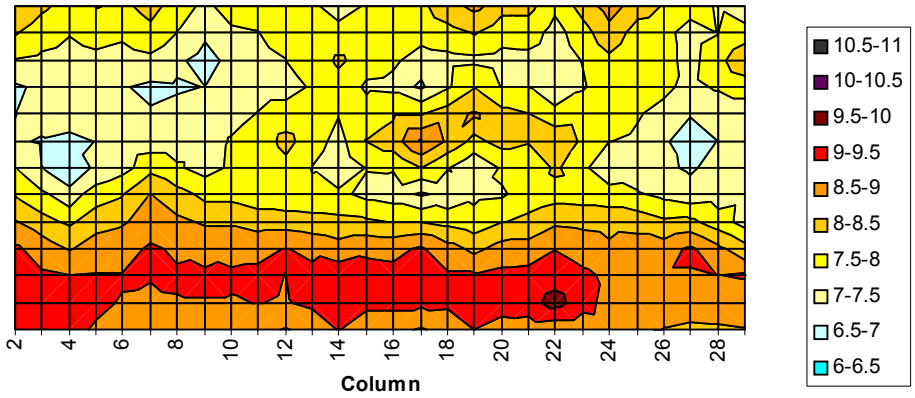


Figure 2F: Surface chart of soil $EC_{1:5}$µS/m 0-10cm and 40-50cm at Wanderah for the RAC875/Cascades population in 2004.

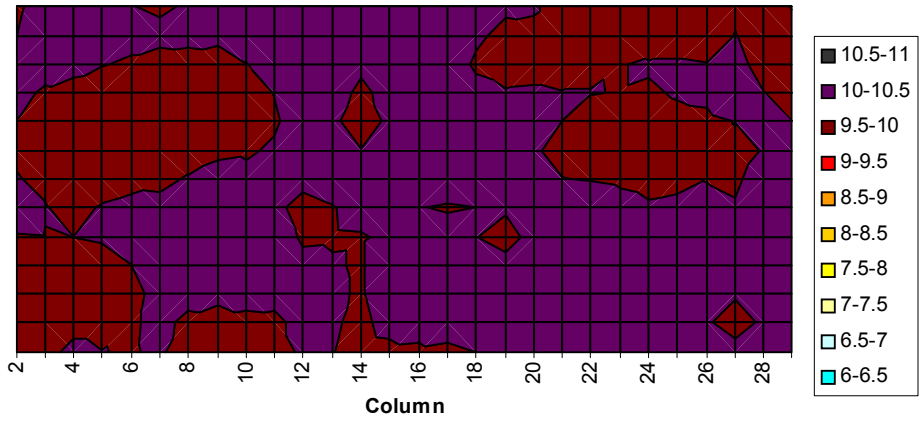
Correlations (r) Yield (g/plot)			PH		EC	
			0-10cm	40-50cm	0-10cm	40-50cm
RAC	pH	0-10cm	-	-0.198*	0.488***	-0.173^{ns}
		40-50cm	-	-	0.125^{ns}	0.575***
	EC	0-10cm	-	-	-	0.142^{ns}
		40-50cm	-	-	-	-
WJ	pH	0-10cm	-	-0.051^{ns}	0.014ns	-0.045^{ns}
		40-50cm	-	-	0.007ns	-0.638***
	EC	0-10cm	-	-	-	0.001^{ns}
		40-50cm	-	-	-	-
BR	pH	0-10cm	-	0.032^{ns}	0.312***	0.375***
		40-50cm	-	-	0.155^{ns}	0.331***
	EC	0-10cm	-	-	-	0.444***
		40-50cm	-	-	-	-

Figure 2G: Correlation between pH and EC values at 0-10cm and 40-50cm depths for Roseworthy (RAC), Wanderah (WJ) and Buckleboo (BR).

(a)



(b)



(c)

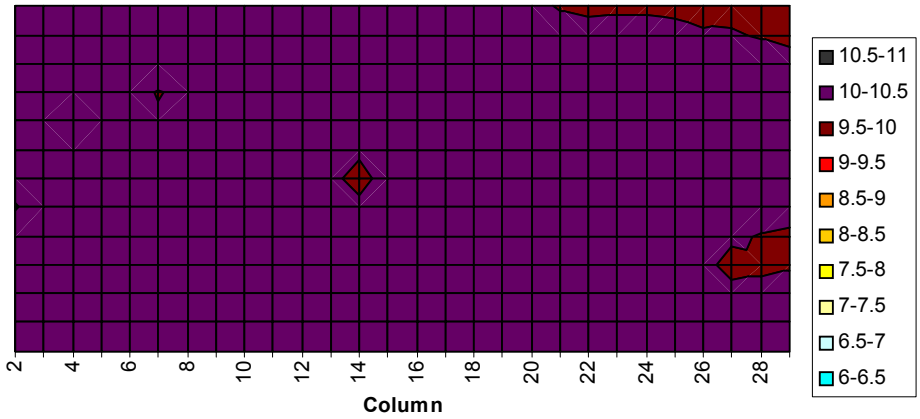
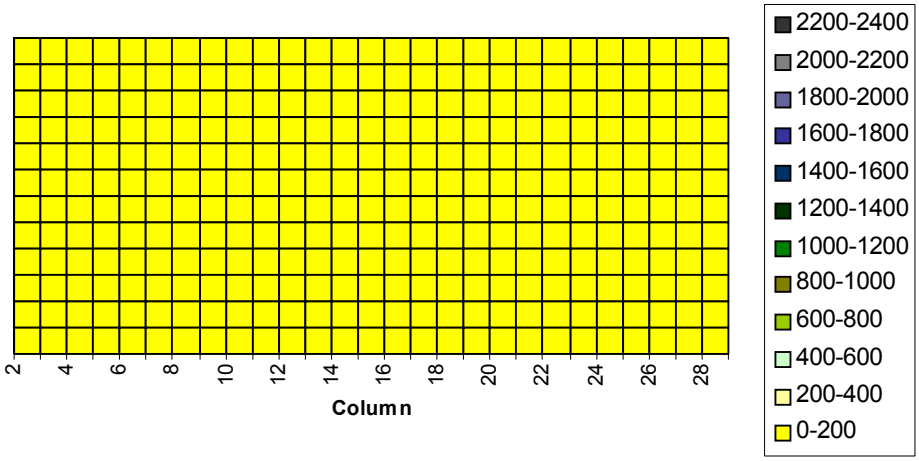
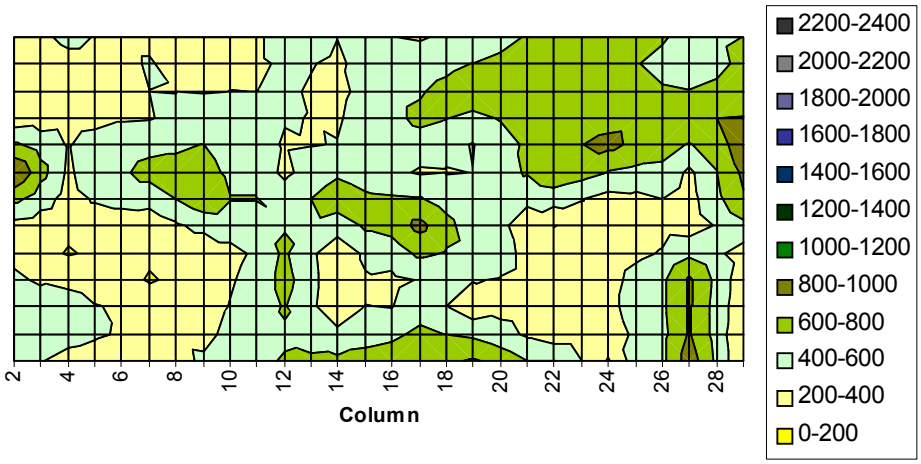


Figure 2H: Surface chart of soil pH (a) 0-10cm, (b) 30-40cm, and (c) 50-60cm at Angas Valley for the Worrakatta/TmWLYY9//WLYY9Tm population in 2005.

(a)



(b)



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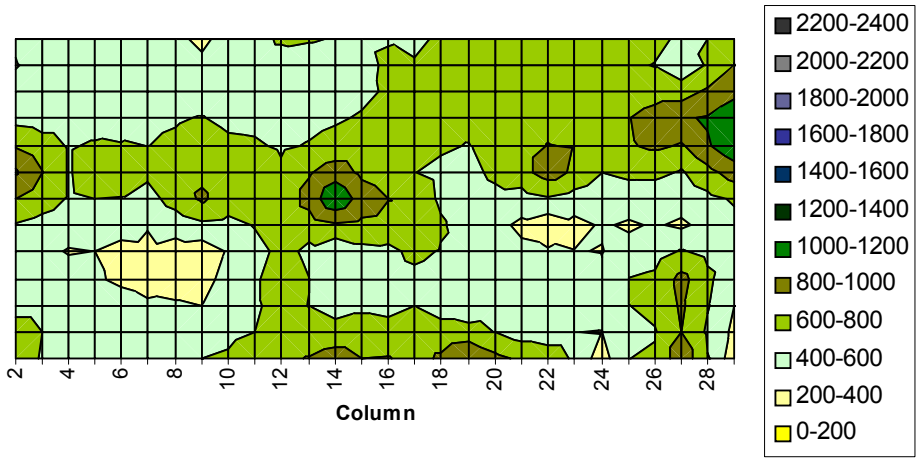
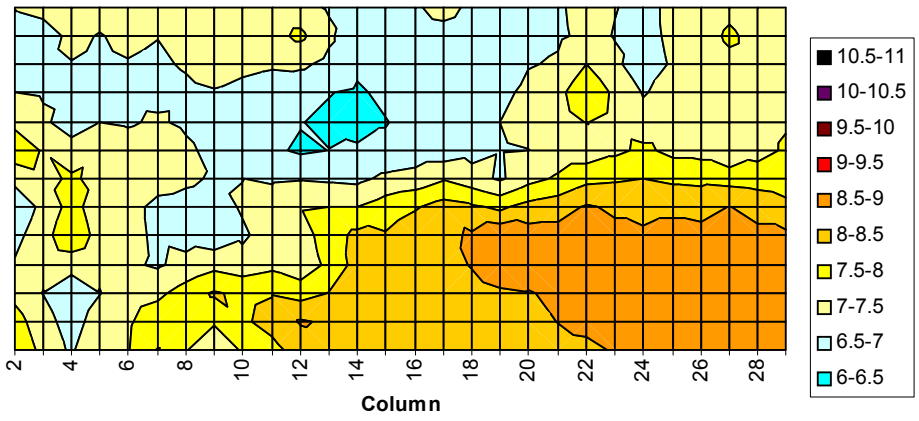


Figure 2I: Surface chart of soil $EC_{1:5}$µS/m (a) 0-10cm, (b) 30-40cm, and (c) 50-60cm at Angas Valley for the Worrakatta/TmWLYY9//WLYY9Tm population in 2005.

(a)



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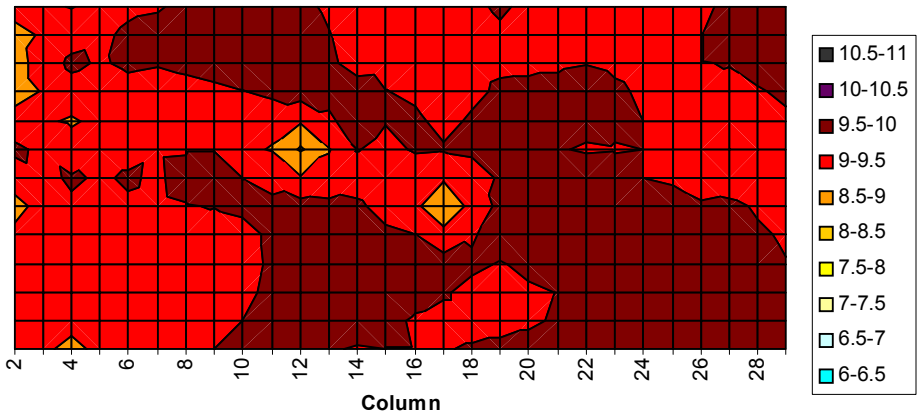

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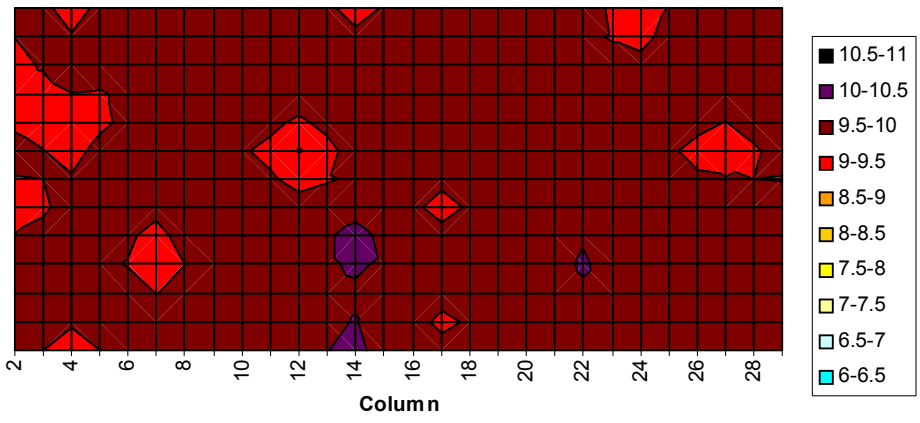


Figure 2J: Surface chart of soil pH (a) 0-10cm, (b) 30-40cm, and (c) 50-60cm at Roseworthy for the Worrakatta/TmWLYY9//WLYY9Tm population in 2005.

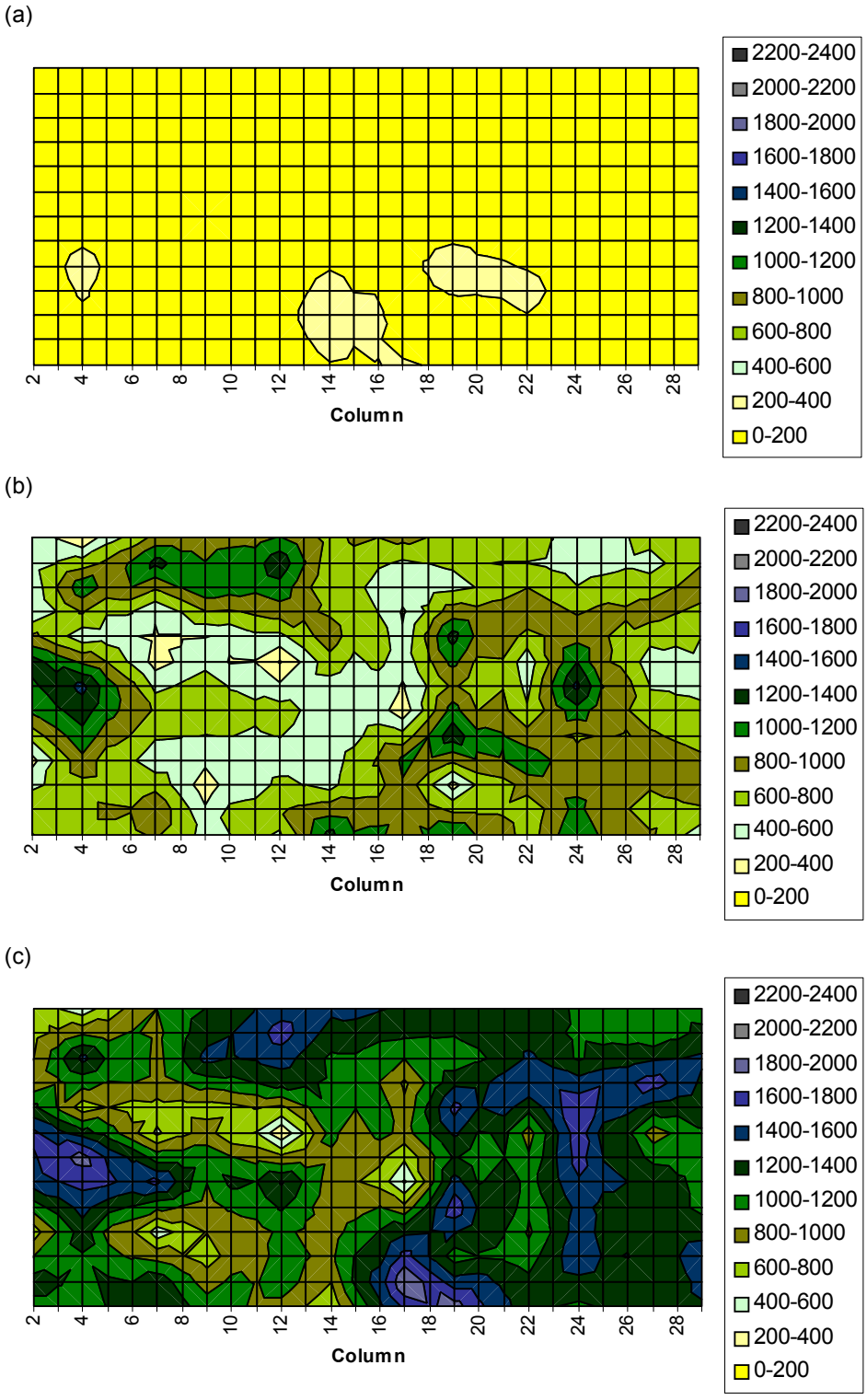
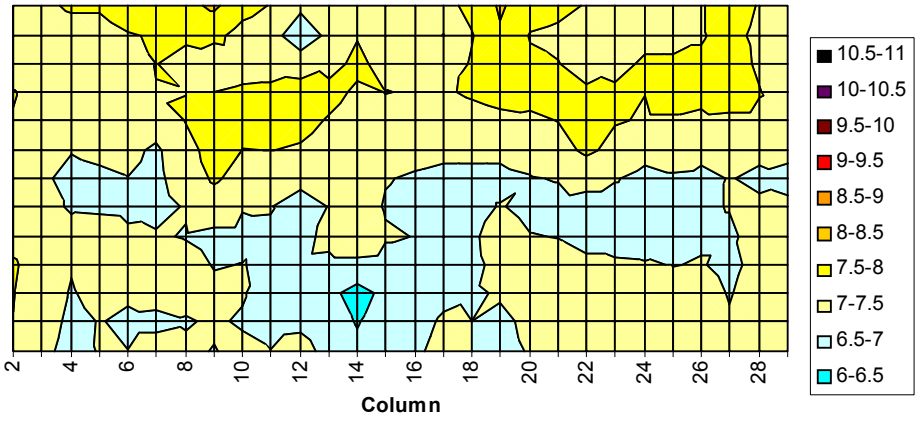
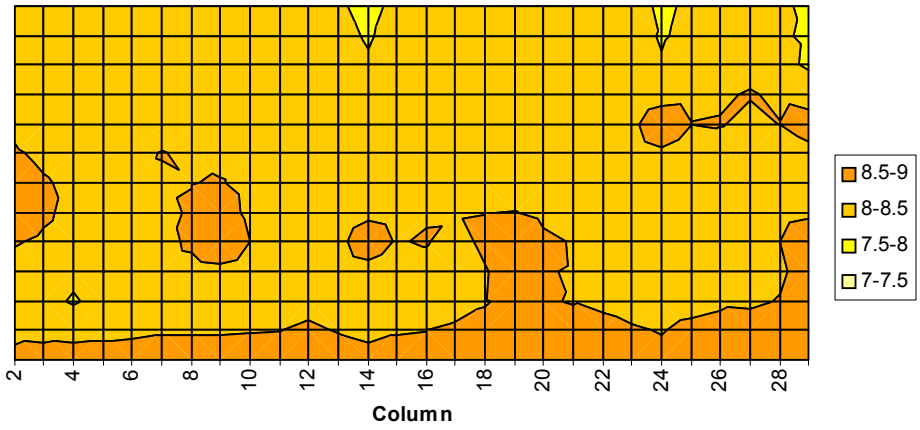


Figure 2K: Surface chart of soil $EC_{1:5}$µS/m (a) 0-10cm, (b) 30-40cm, and (c) 50-60cm at Roseworthy for the Worrakatta/TmWLYY9//WLYY9Tm population in 2005.

(a)



(b)



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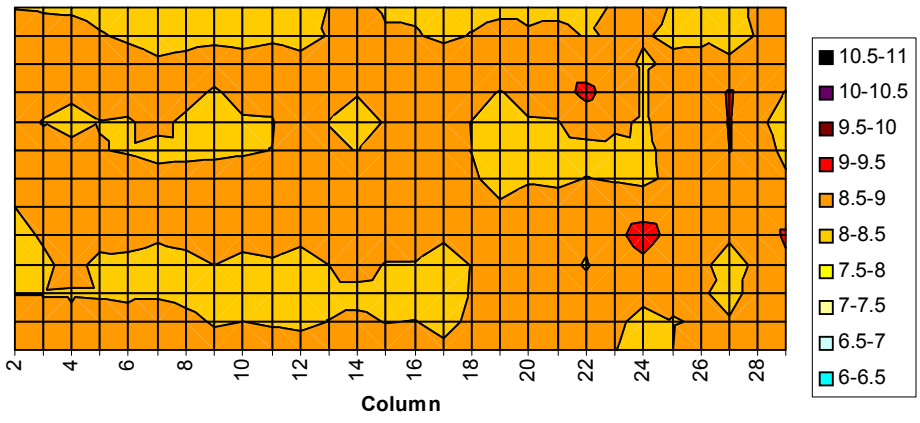
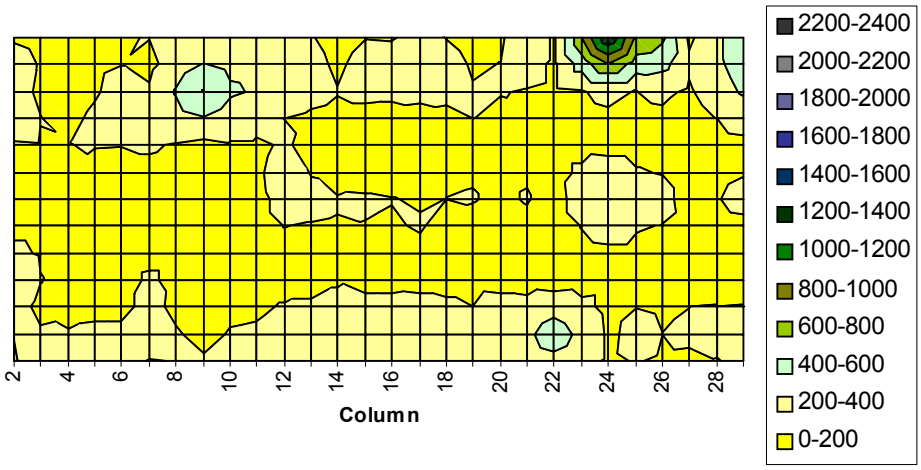
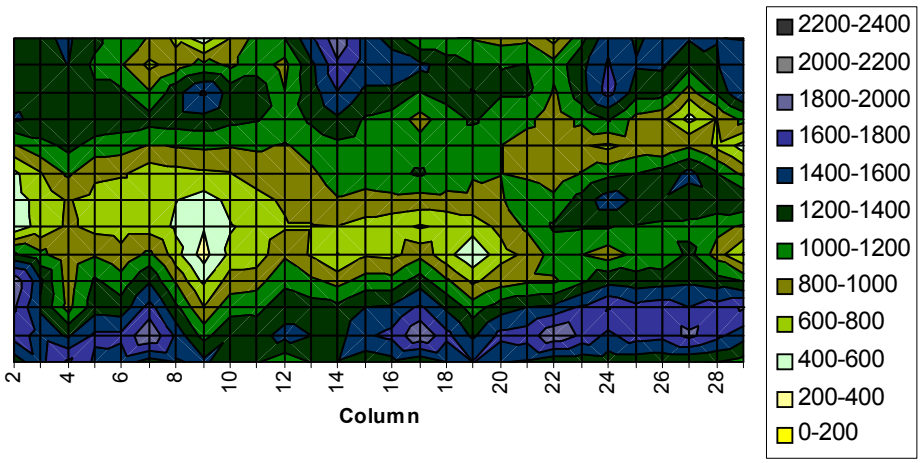


Figure 2L: Surface chart of soil pH (a) 0-10cm, (b) 30-40cm, and (c) 50-60cm at Wanderah for the Worrakatta/TmWLYY9//WLYY9Tm population in 2005.

(a)



(b)



(c)

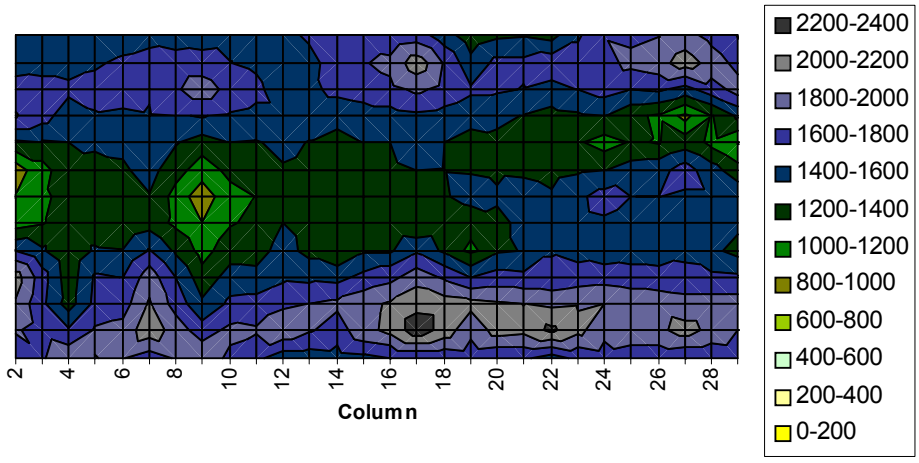


Figure 2M: Surface chart of soil $EC_{1:5}$µS/m (a) 0-10cm, (b) 30-40cm, and (c) 50-60cm at Wanderah for the Worrakatta/TmWLYY9//WLYY9Tm population in 2005.

Correlations (r)			pH			EC		
Yield (g/plot)			0-10cm	30-40cm	50-60cm	0-10cm	30-40cm	50-60cm
AV	pH	0-10cm	-	0.131^{ns}	0.202^{ns}	0.677***	0.160^{ns}	0.087^{ns}
		30-40cm	-	-	0.549***	0.062^{ns}	0.687***	0.603***
		50-60cm	-	-	-	0.135^{ns}	-0.030^{ns}	-0.204^{ns}
	EC	0-10cm	-	-	-	-	0.192^{ns}	0.076^{ns}
		30-40cm	-	-	-	-	-	0.895***
		50-60cm	-	-	-	-	-	-
RAC	pH	0-10cm	-	0.382***	0.305**	0.668***	0.259*	0.218*
		30-40cm	-	-	0.726***	0.452***	0.487***	0.503***
		50-60cm	-	-	-	0.370***	0.136^{ns}	0.007^{ns}
	EC	0-10cm	-	-	-	-	0.325**	0.227*
		30-40cm	-	-	-	-	-	0.757***
		50-60cm	-	-	-	-	-	-
WJ	pH	0-10cm	-	-0.022^{ns}	-0.122^{ns}	-0.169^{ns}	-0.128^{ns}	-0.229*
		30-40cm	-	-	0.762***	-0.159^{ns}	-0.412***	-0.295**
		50-60cm	-	-	-	-0.133^{ns}	-0.194^{ns}	-0.189^{ns}
	EC	0-10cm	-	-	-	-	0.468***	0.479***
		30-40cm	-	-	-	-	-	0.861***
		50-60cm	-	-	-	-	-	-

Figure 2N: Correlation between pH and EC values at 0-10cm, 30-40cm and 50-60cm depths for Angas Valley (AV), Roseworthy (RAC) and Wanderah (WJ).

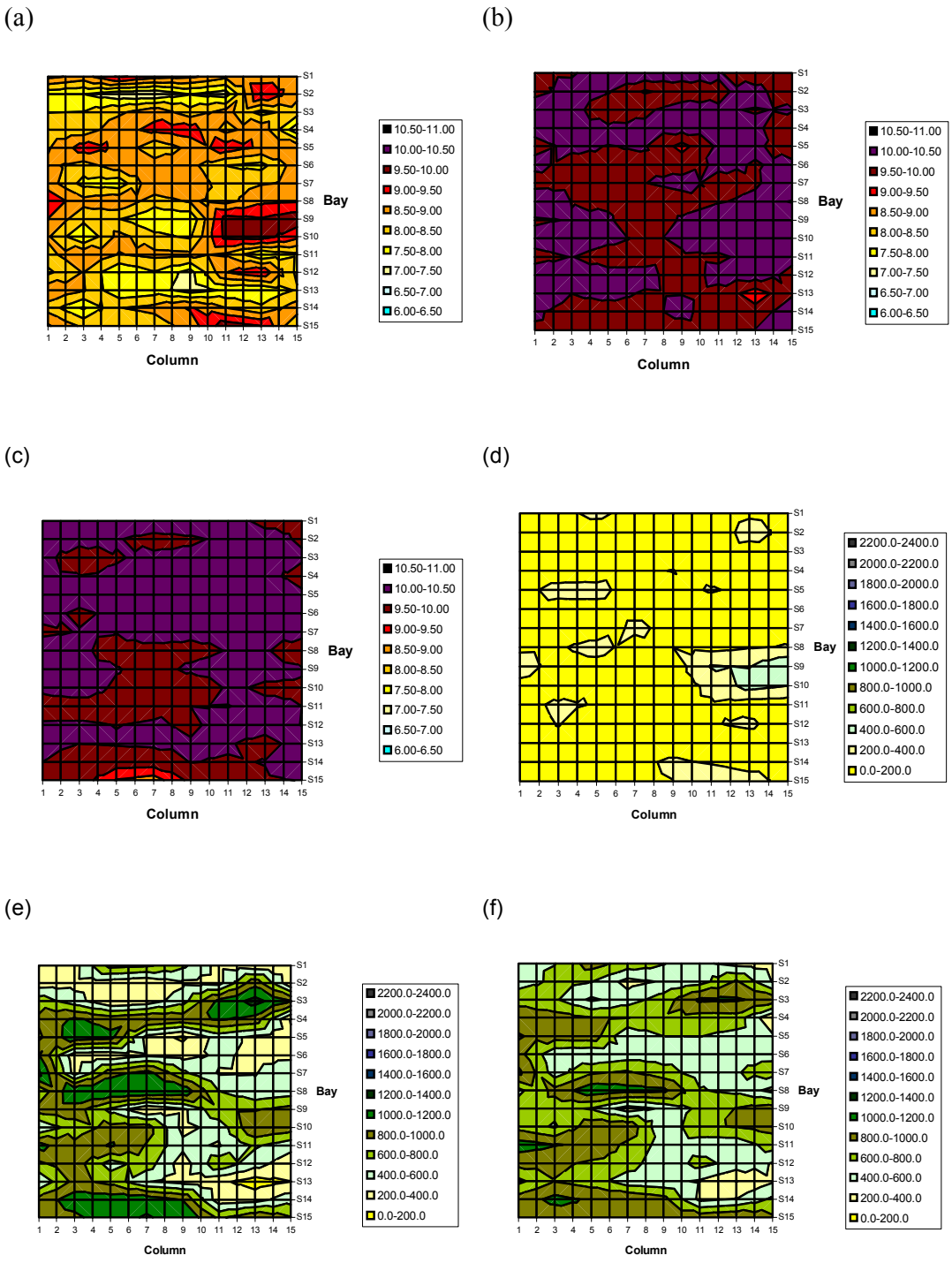


Figure 2O: Surface chart of soil pH (a) 0-10cm, (b) 30-40cm and (c) 50-60cm, and soil $EC_{1:5}$µS/m (d) 0-10cm, (e) 30-40cm and (f) 50-60cm, at Angas Valley - North for the Frame/Yarralinka//Pugsley population in 2005.

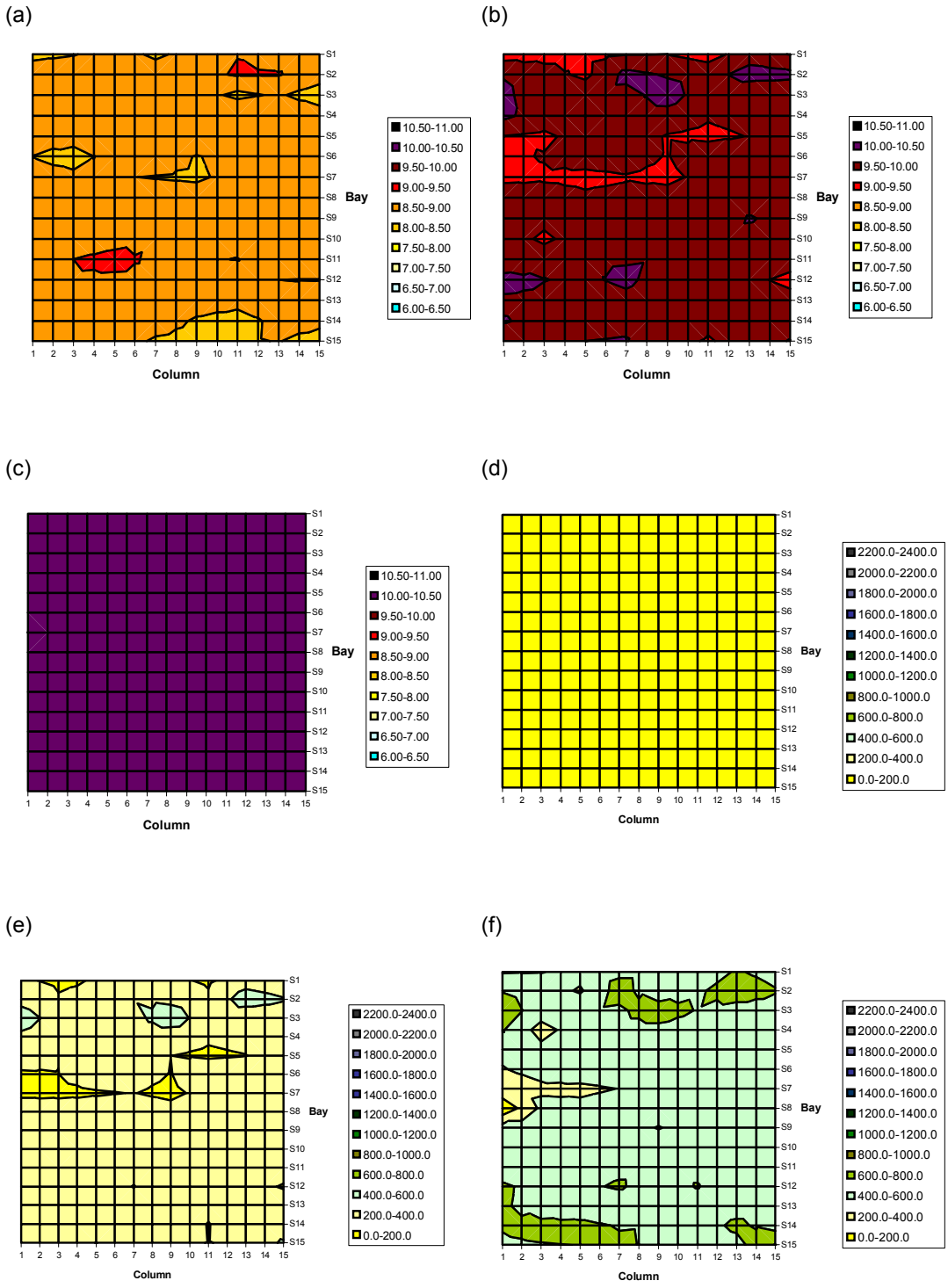


Figure 2P: Surface chart of soil pH (a) 0-10cm, (b) 30-40cm and (c) 50-60cm, and soil $EC_{1:5}$µS/m (d) 0-10cm, (e) 30-40cm and (f) 50-60cm, at Angas Valley - South for the Frame/Yarralinka//Pugsley population in 2005.

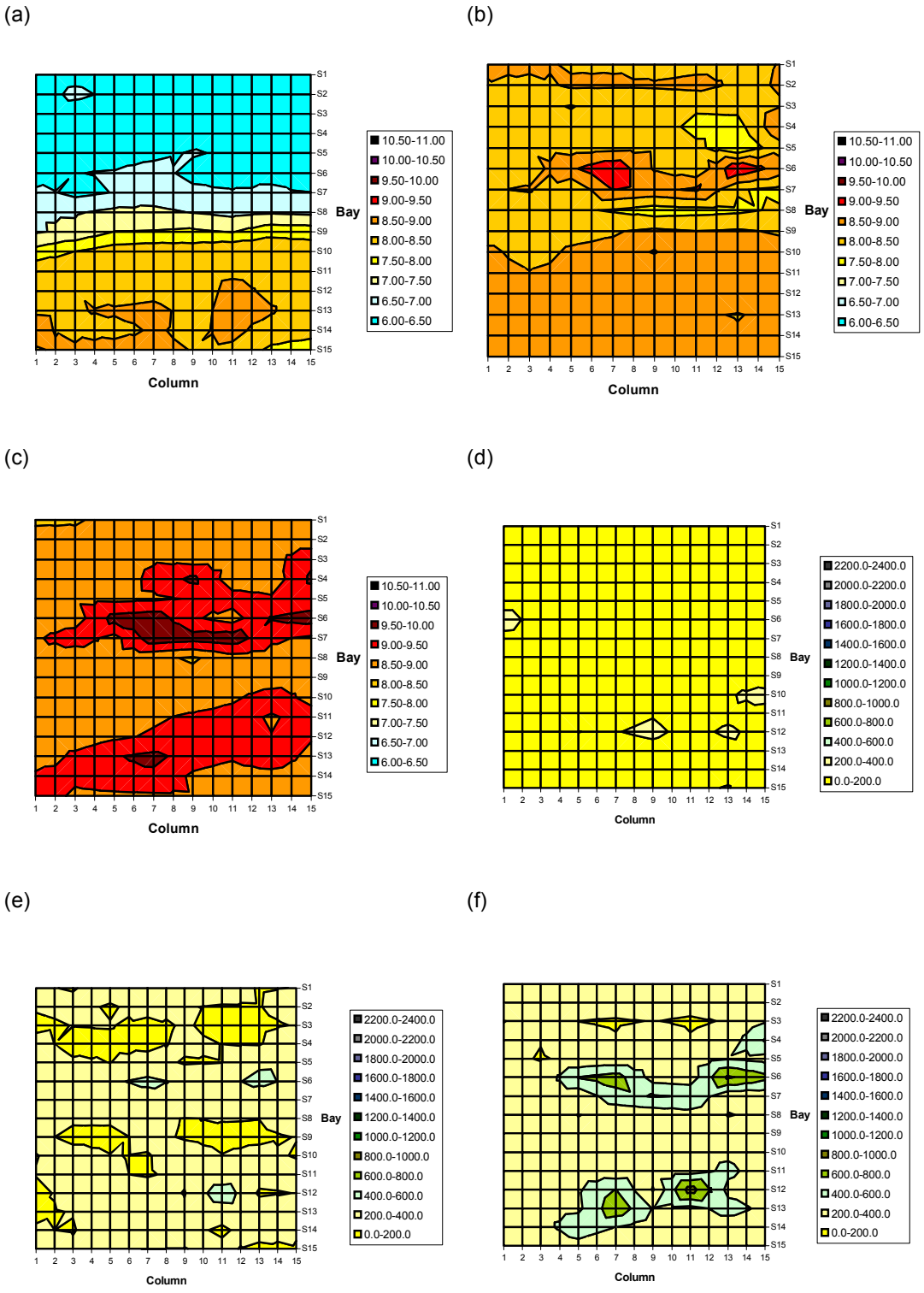


Figure 2P: Surface chart of soil pH (a) 0-10cm, (b) 30-40cm and (c) 50-60cm, and soil $EC_{1:5}$µS/m (d) 0-10cm, (e) 30-40cm and (f) 50-60cm, at Roseworthy - East for the Frame/Yarralinka//Pugsley population in 2005.

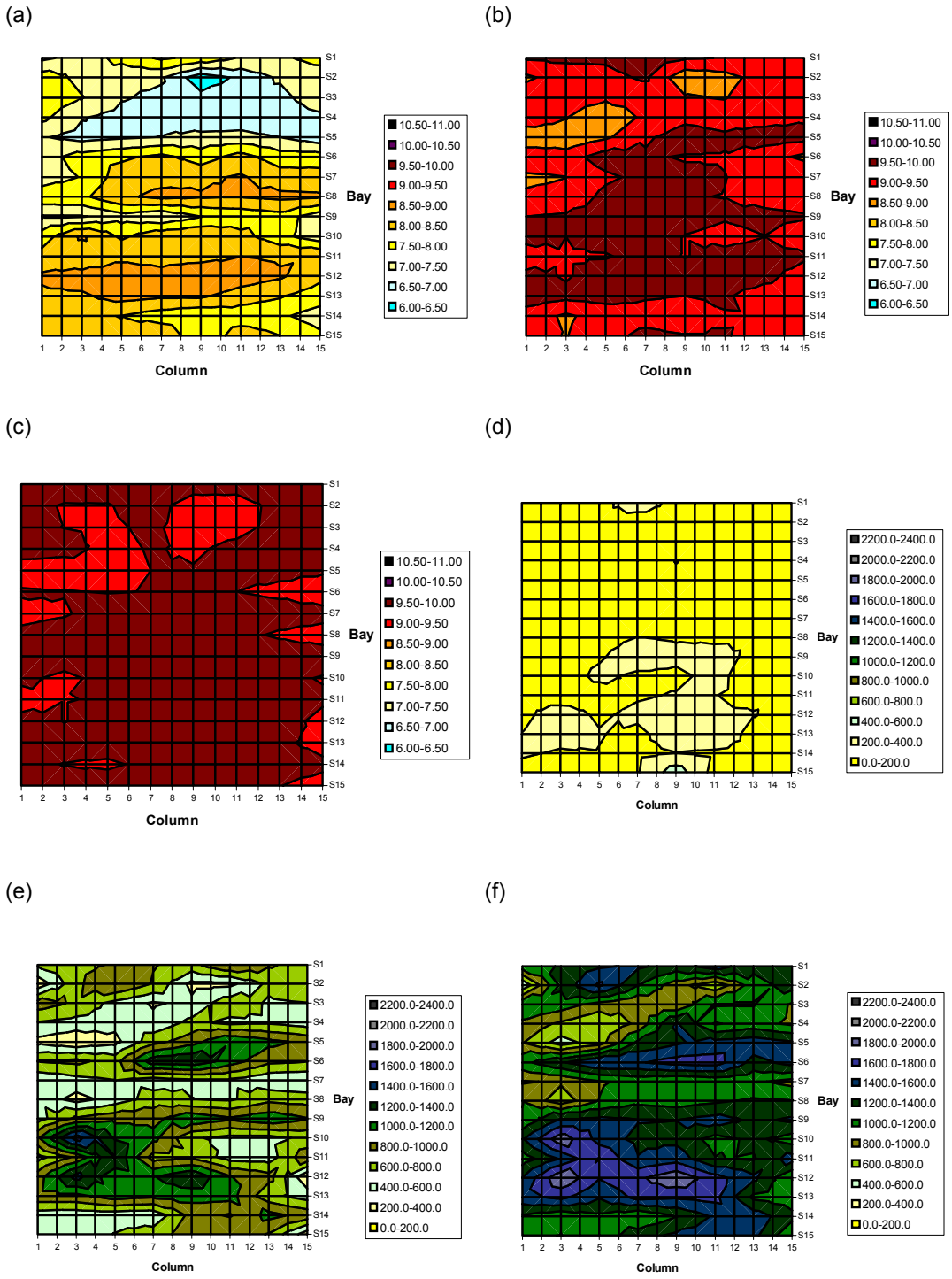


Figure 2Q: Surface chart of soil pH (a) 0-10cm, (b) 30-40cm and (c) 50-60cm, and soil $EC_{1:5}$µS/m (d) 0-10cm, (e) 30-40cm and (f) 50-60cm, at Roseworthy - West for the Frame/Yarralinka//Pugsley population in 2005.

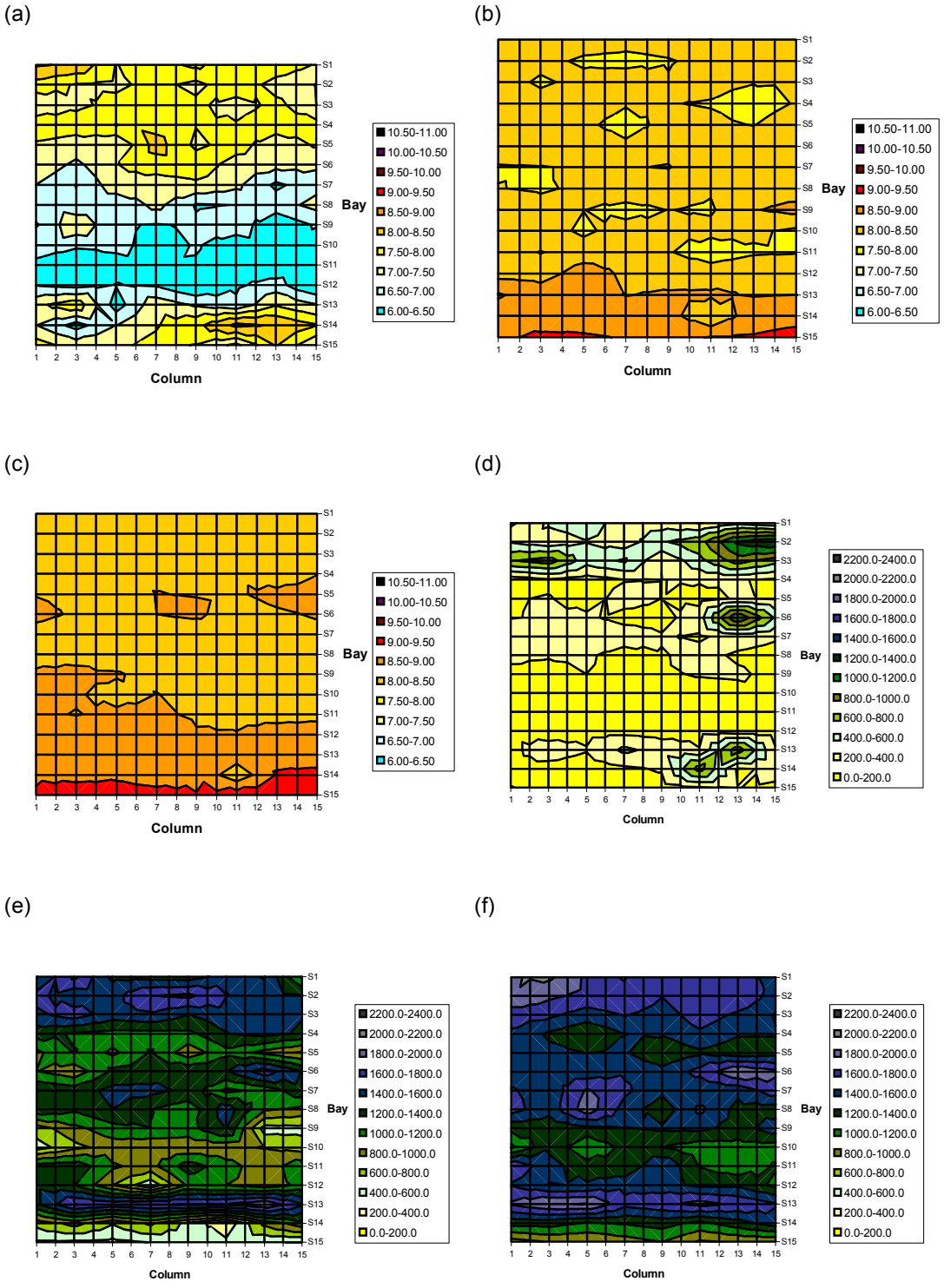


Figure 2R: Surface chart of soil pH (a) 0-10cm, (b) 30-40cm and (c) 50-60cm, and soil $EC_{1:5}$µS/m (d) 0-10cm, (e) 30-40cm and (f) 50-60cm, at Wanderah for the Frame/Yarralinka//Pugsley population in 2005.

Correlations (r)			PH			EC		
Yield (g/plot)			0-10cm	30-40cm	50-60cm	0-10cm	30-40cm	50-60cm
AV-S	pH	0-10cm	-	0.280**	0.210*	0.212*	0.181*	0.024^{ns}
		30-40cm	-	-	0.664***	0.332***	0.862***	0.644***
		50-60cm	-	-	-	0.683***	0.620***	0.561***
	EC	0-10cm	-	-	-	-	0.320***	0.324***
		30-40cm	-	-	-	-	-	0.703***
		50-60cm	-	-	-	-	-	-
AV-N	pH	0-10cm	-	0.349***	0.212*	0.787***	0.325***	0.324***
		30-40cm	-	-	0.475***	0.215*	0.282***	0.313***
		50-60cm	-	-	-	0.032^{ns}	0.188*	0.114^{ns}
	EC	0-10cm	-	-	-	-	0.381***	0.376***
		30-40cm	-	-	-	-	-	0.913***
		50-60cm	-	-	-	-	-	-
RAC-E	pH	0-10cm	-	0.432***	0.057^{ns}	0.757***	0.030^{ns}	0.145^{ns}
		30-40cm	-	-	0.601***	0.341***	0.321***	0.601***
		50-60cm	-	-	-	0.036^{ns}	0.548***	0.714***
	EC	0-10cm	-	-	-	-	0.017^{ns}	0.117^{ns}
		30-40cm	-	-	-	-	-	0.749***
		50-60cm	-	-	-	-	-	-
RAC-W	pH	0-10cm	-	0.431***	0.270**	0.382***	0.299***	0.414***
		30-40cm	-	-	0.683***	0.391***	0.582***	0.681***
		50-60cm	-	-	-	0.239**	0.157^{ns}	0.158^{ns}
	EC	0-10cm	-	-	-	-	0.335***	0.429***
		30-40cm	-	-	-	-	-	0.889***
		50-60cm	-	-	-	-	-	-
WJ	pH	0-10cm	-	0.063^{ns}	0.010^{ns}	0.245**	0.165^{ns}	0.184*
		30-40cm	-	-	0.863***	0.492***	0.459***	0.327***
		50-60cm	-	-	-	0.235**	0.407***	0.427***
	EC	0-10cm	-	-	-	-	0.424***	0.378***
		30-40cm	-	-	-	-	-	0.834***
		50-60cm	-	-	-	-	-	-

Figure 2S: Correlation between pH and EC values at 0-10cm, 30-40cm and 50-60cm depths for Angas Valley - South (AV-S), Angas Valley – North (AV-N), Roseworthy - East (RAC-E), Roseworthy – West (RAC-W) and Wanderah (WJ).

Appendix 3: Temperature and Rainfall data for the 2004 and 2005 field trial sites.

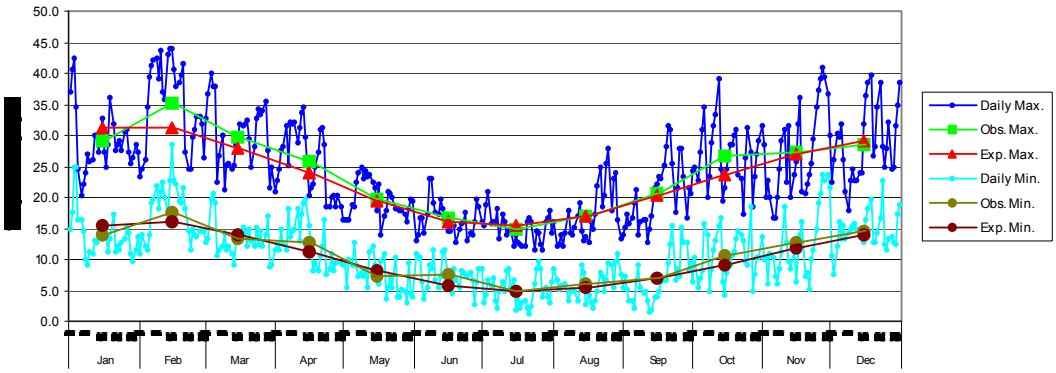


Figure 3A: The daily, and monthly observed and long-term (expected) maximum and minimum temperatures for Kimba in 2004 (Buckleboo).

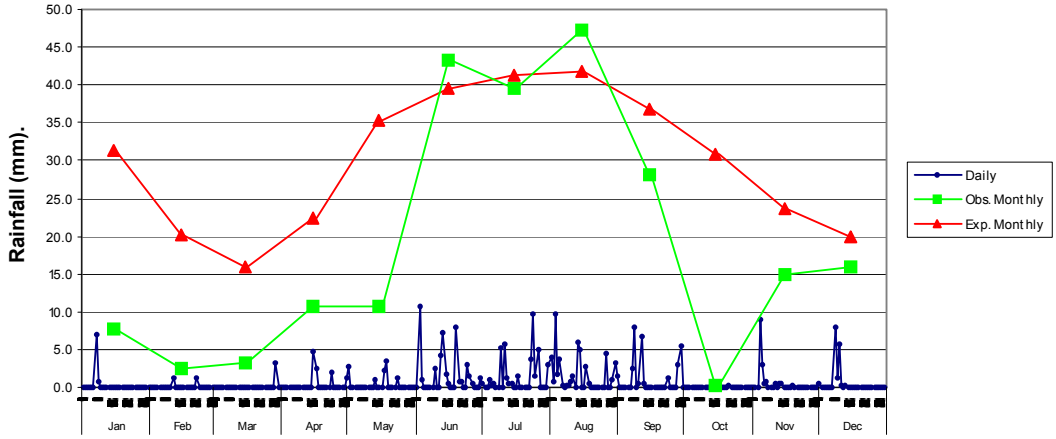


Figure 3B: The daily, and monthly observed and expected rainfall for Kimba in 2004.

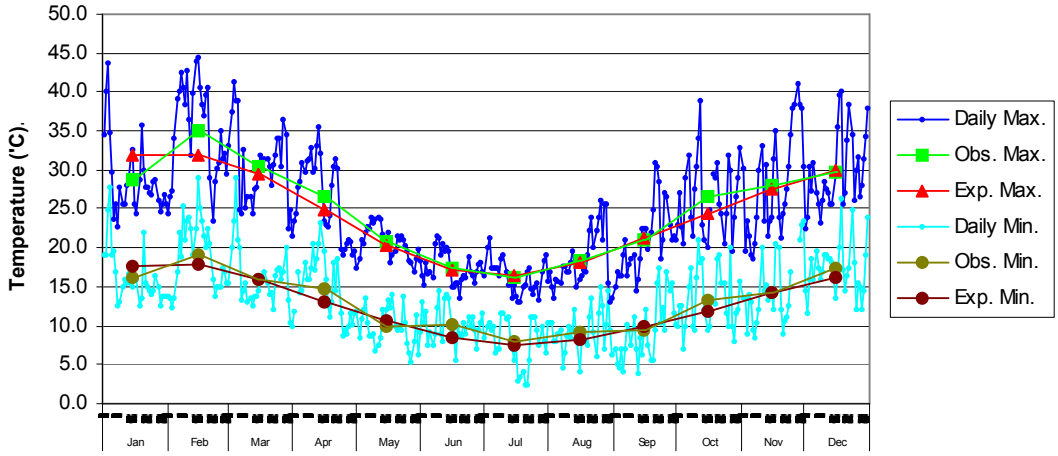


Figure 3C: The daily, and monthly observed and expected maximum and minimum temperatures for Port Pirie in 2004 (Wanderah).

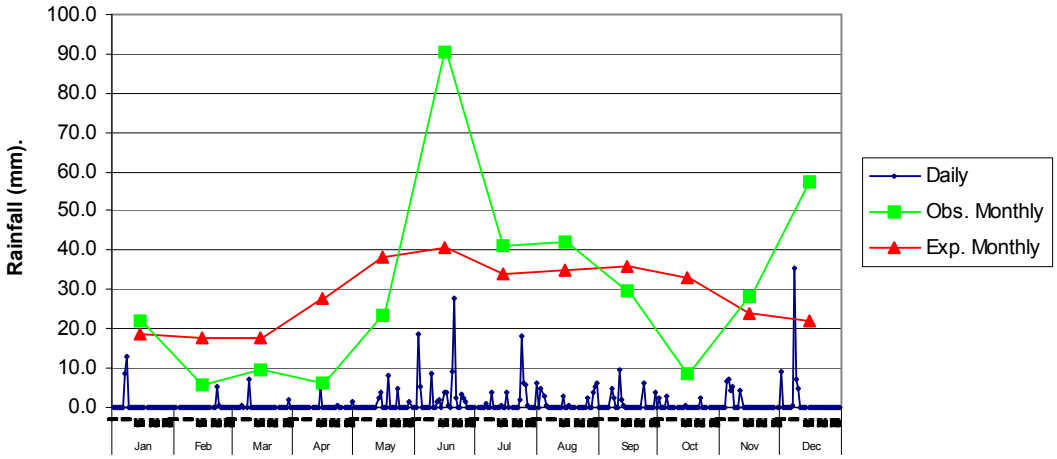


Figure 3D: The daily, and monthly observed and expected rainfall for Port Pirie in 2004.

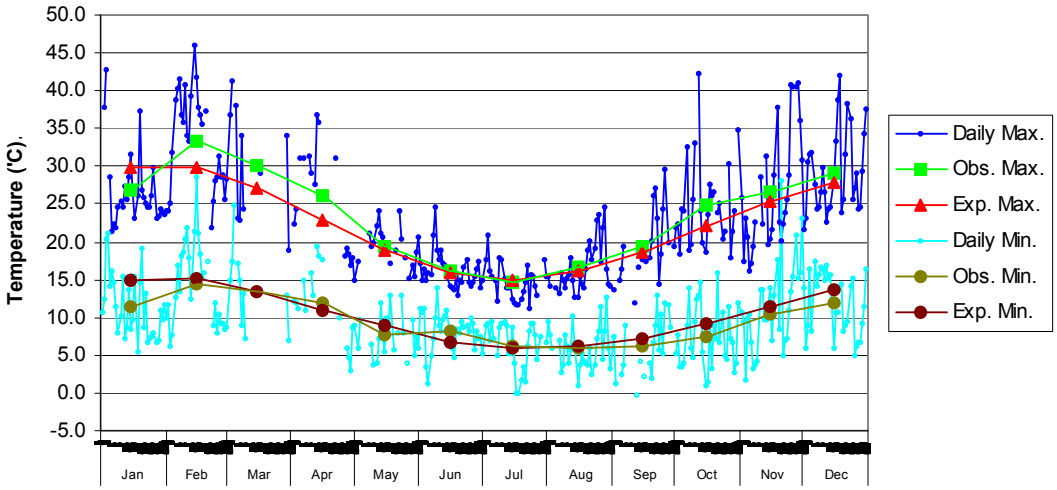


Figure 3E: The daily, and monthly observed and expected maximum and minimum temperatures for Roseworthy in 2004.

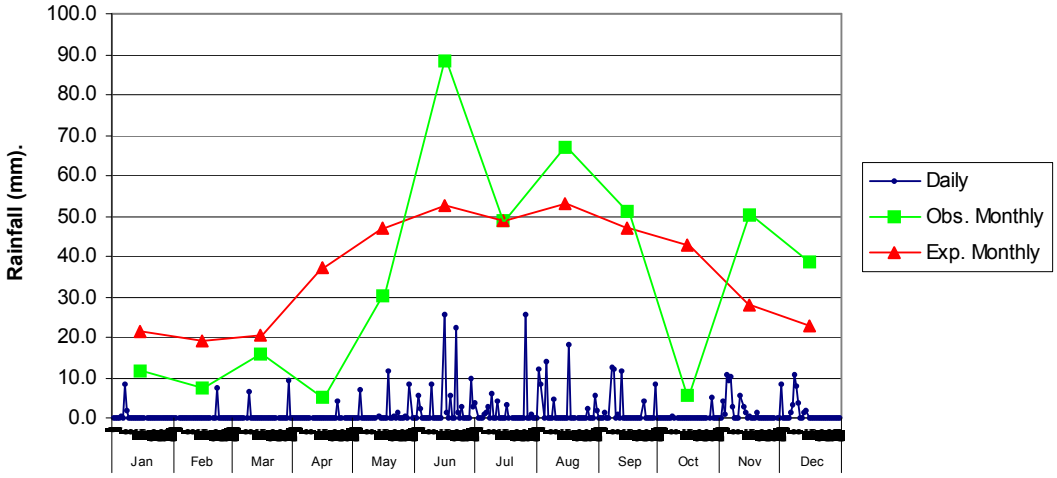


Figure 3F: The daily, and monthly observed and expected rainfall for Roseworthy in 2004.

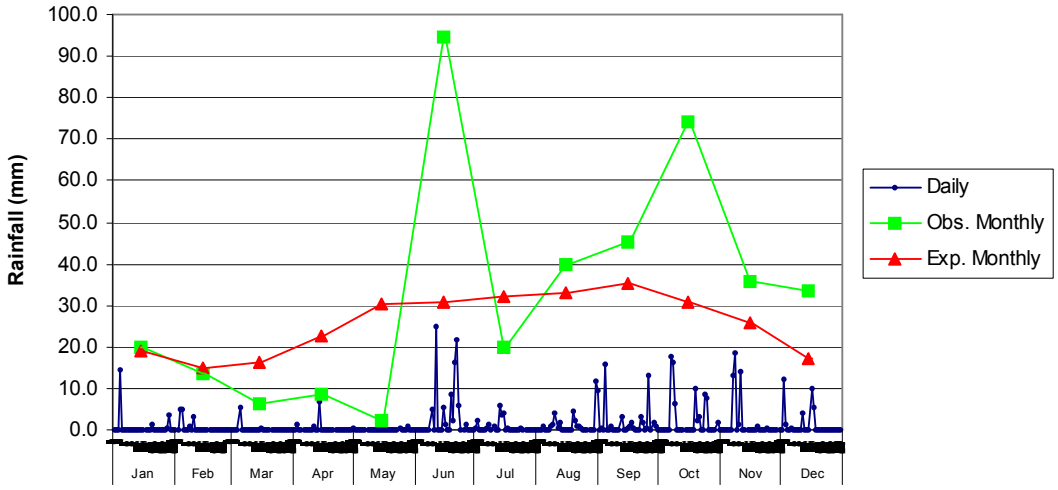


Figure 3G: The daily, and monthly observed and expected rainfall for Bowhill in 2005 (Claypans).

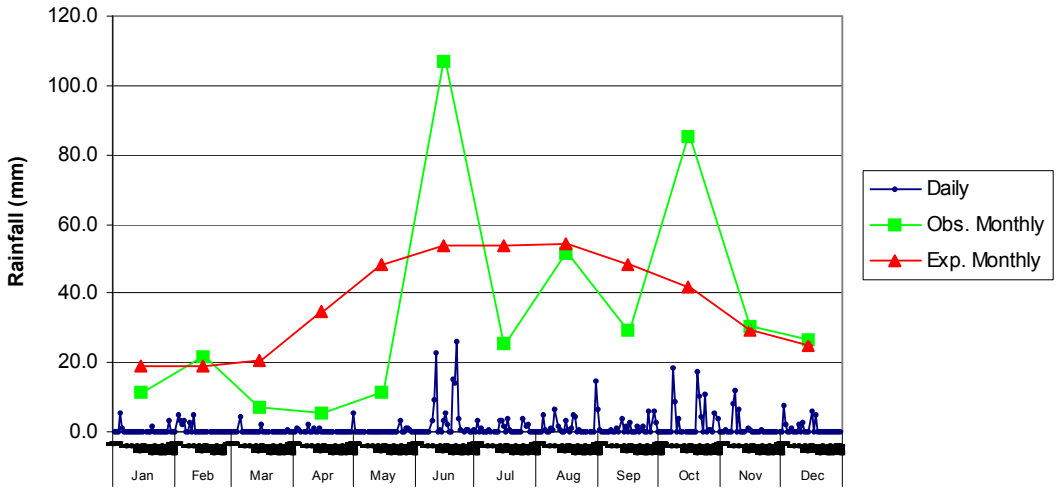


Figure 3H: The daily, and monthly observed and expected rainfall for Coonalpyn in 2005.

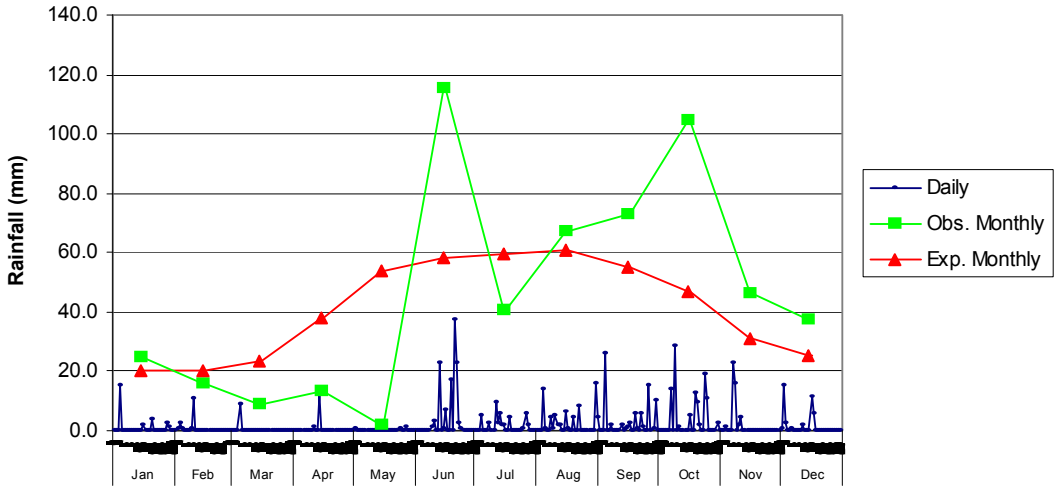


Figure 3I: The daily, and monthly observed and expected rainfall for Kapunda in 2005.

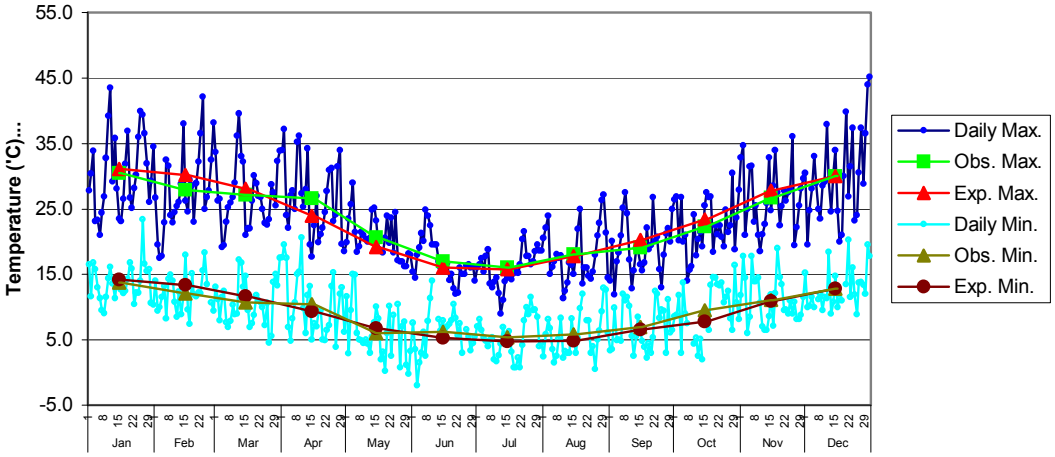


Figure 3J: The daily, and monthly observed and expected maximum and minimum temperatures for Karoonda in 2005 (Claypans, Angas Valley).

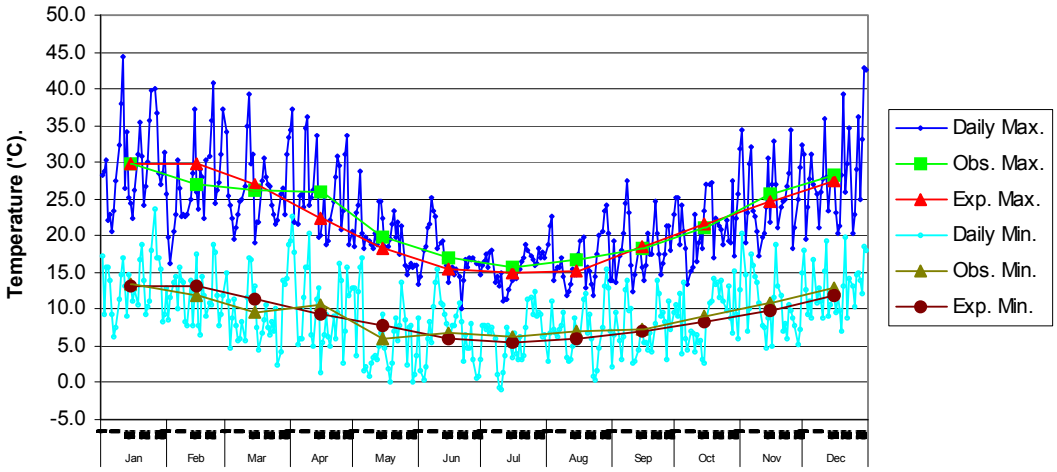


Figure 3K: The daily, and monthly observed and expected maximum and minimum temperatures for Keith in 2005 (Coonalpyn).

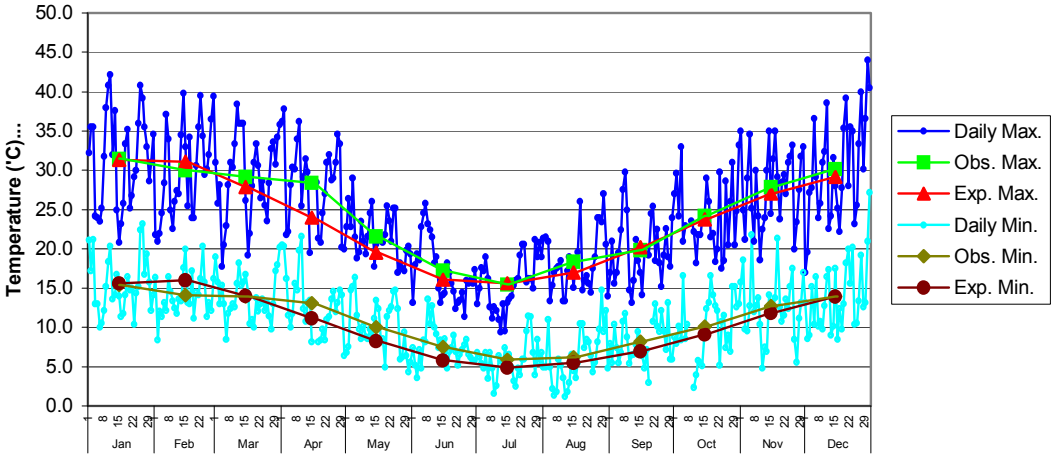


Figure 3L: The daily, and monthly observed and expected maximum and minimum temperatures for Kimba in 2005 (Buckleboo).

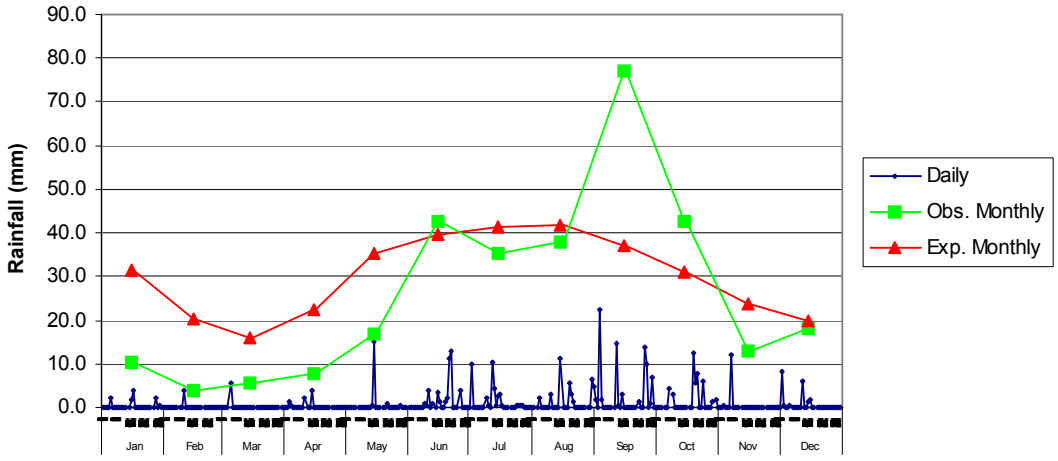


Figure 3M: The daily, and monthly observed and expected rainfall for Kimba in 2005.

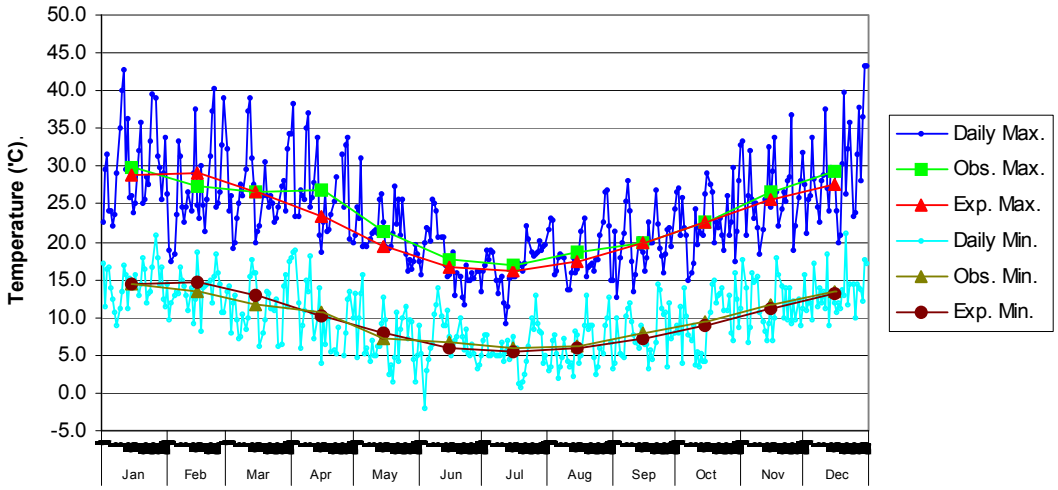


Figure 3N: The daily, and monthly observed and expected maximum and minimum temperatures for Murray Bridge in 2005 (Angas Valley).

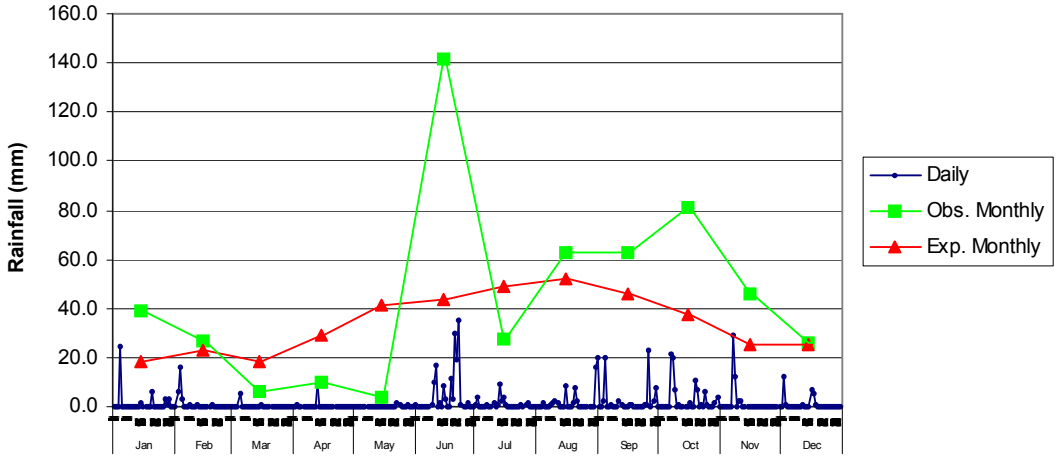


Figure 3O: The daily, and monthly observed and expected rainfall for Palmer in 2005 (Angas Valley).

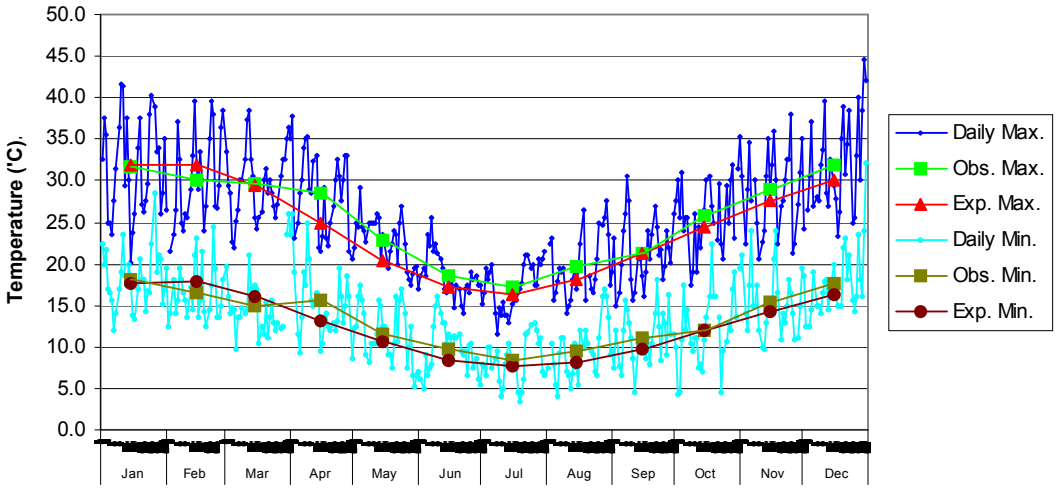


Figure 3P: The daily, and monthly observed and expected maximum and minimum temperatures for Port Pirie in 2005 (Wanderah).

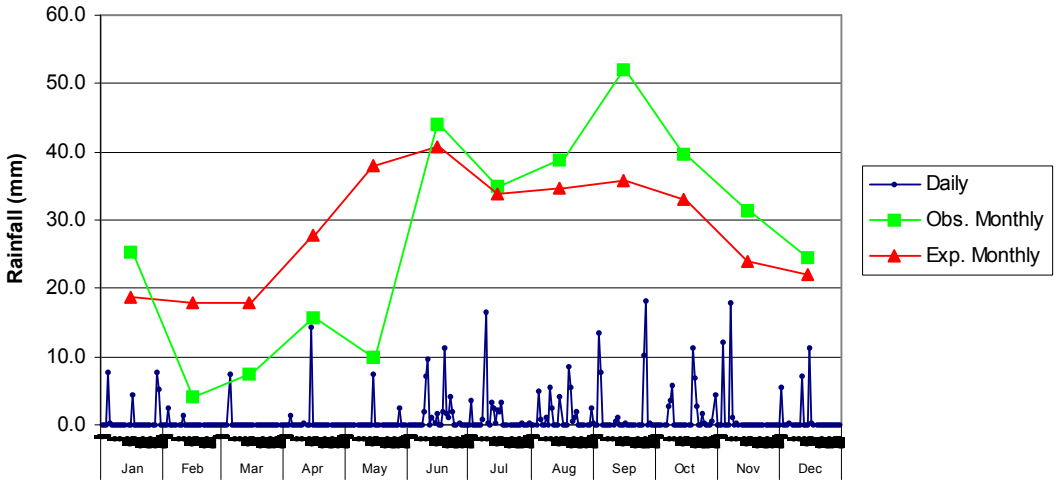


Figure 3Q: The daily, and monthly observed and expected rainfall for Port Pirie in 2005.

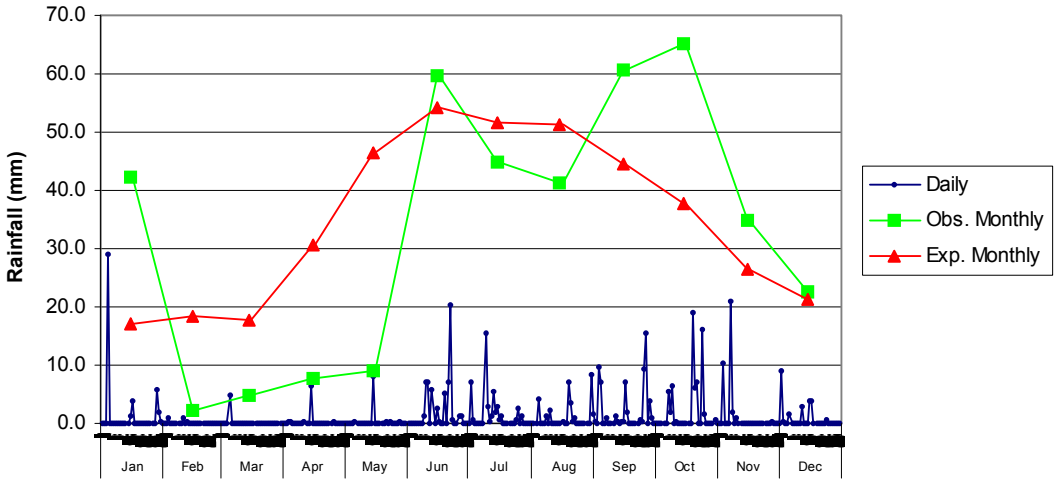


Figure 3R: The daily, and monthly observed and expected rainfall for Redhill in 2005.

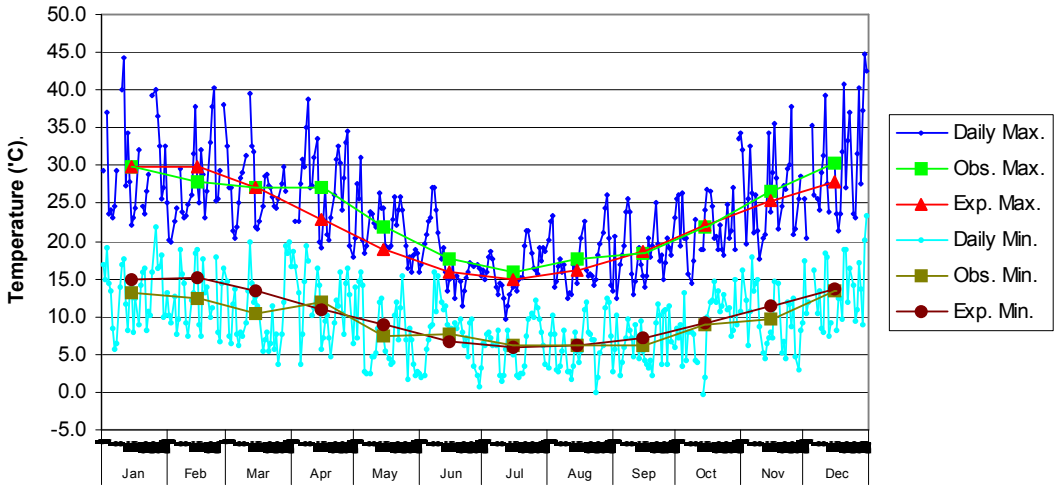


Figure 3S: The daily, and monthly observed and expected maximum and minimum temperatures for Roseworthy in 2005.

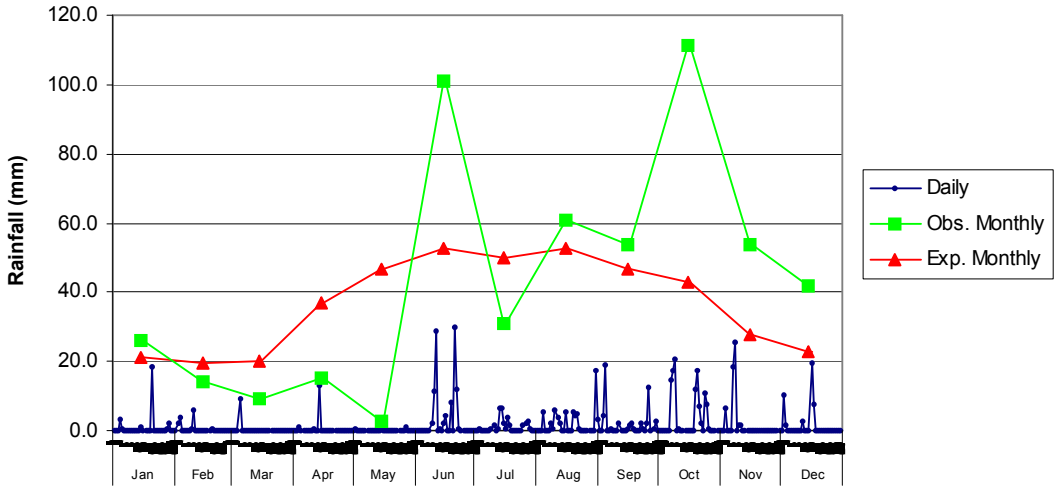


Figure 3T: The daily, and monthly observed and expected rainfall for Roseworthy in 2005.

Appendix 4: Mean root length (mm) in bicarbonate solution (pH 9.2) of durum landraces.

AUS No.	Country	Root length	AUS No.	Country	Root length
15216	Afganistan	121.33	15214	Afganistan	78.71
17501	Afganistan	117.56	14789	Afganistan	78.13
14740	Afganistan	115.63	7811	Afganistan	77.13
14794	Afganistan	104.44	17779	Pakistan	76.52
4717	Pakistan	103.46	9739	Afganistan	73.67
14735	Afganistan	101.69	4745	Pakistan	73.42
14837	Afganistan	100.78	14619	Afganistan	72.94
7808	Afganistan	100.07	14805	Afganistan	72.89
13237	Afganistan	98.50	14785	Afganistan	71.75
7809	Afganistan	95.67	14554	Afganistan	70.36
14555	Afganistan	93.44	14571	Afganistan	67.70
15337	Afganistan	92.25	26498	Pakistan	67.31
14772	Afganistan	91.33	13244	Afganistan	66.83
14734	Afganistan	89.75	14748	Afganistan	65.25
9819	Afganistan	89.04	14739	Afganistan	65.08
14806	Afganistan	84.33	26498	Pakistan	63.56
9731	Afganistan	84.25	14460	Afganistan	59.28
15215	Afganistan	83.25	14729	Afganistan	59.13
9724	Afganistan	82.50	14836	Afganistan	56.92
14618	Afganistan	81.92	15273	Afganistan	56.38
13308	Afganistan	80.22			

Figure 4A: Mean root length of durum landraces originating from Afgansitan and Pakistan.

AUS No.	Country	Root length	AUS No.	Country	Root length
26609	Spain	110.75	5348	Portugal	75.97
26517	Morocco	101.89	5339	Portugal	75.02
5641	Spain	98.42	26611	Spain	75.00
26376	Tunisia	96.12	5364	Portugal	74.93
26612	Spain	95.38	26375	Tunisia	74.09
26354	Algeria	95.35	26660	Tunisia	73.75
26610	Spain	91.38	5688	Tunisia	73.50
26524	Morocco	89.93	26373	Algeria	72.12
1238	Egypt	89.90	4999	Morocco	71.63
5687	Tunisia	89.08	26523	Morocco	71.55
5695	Tunisia	88.72	5066	Morocco	71.43
26532	Morocco	88.57	26635	Egypt	70.78
26521	Morocco	88.10	26352	Algeria	70.58
26634	Egypt	87.56	5062	Morocco	70.54
26353	Algeria	86.67	1227	Egypt	70.38
26661	Tunisia	86.21	5048	Morocco	70.24
26356	Tunisia	86.13	26663	Tunisia	70.16
26377	Tunisia	86.08	5018	Morocco	69.13
26596	Spain	85.71	26664	Tunisia	68.92
26515	Morocco	85.35	5053	Morocco	68.19
5314	Portugal	85.21	5693	Tunisia	68.17
26662	Tunisia	84.30	5727	Spain	67.83
5633	Spain	83.70	5007	Morocco	66.75
26613	Spain	83.42	5678	Tunisia	66.30
1235	Egypt	80.47	5293	Portugal	65.73
5694	Tunisia	80.41	26665	Tunisia	63.66
5304	Portugal	79.28	5329	Portugal	62.92
26519	Morocco	78.44	26351	Algeria	62.75
26355	Algeria	78.32	5390	Portugal	62.01

26511	Spain	78.01	5002	Morocco	60.88
5679	Tunisia	77.84	5041	Morocco	55.95
9899	Tunisia	77.75	5009	Morocco	53.92
5081	Morocco	77.69	5356	Portugal	53.43
4982	Morocco	77.38	5377	Portugal	53.12
26666	Tunisia	76.87	5696	Tunisia	44.30
4989	Morocco	76.20			

Figure 4B: Mean root length of durum landraces originating from North Africa, Spain and Portugal.

AUS No.	Country	Root length	AUS No.	Country	Root length
4594	Greece	124.71	27883	Italy	79.00
4600	Greece	117.74	4593	Greece	78.33
26699	Ukraine	109.63	2945	Malta	76.38
26422	Greece	109.53	2942	Malta	74.00
26425	Greece	101.70	26415	Greece	72.83
26594	Romania	94.63	26715	Bulgaria	71.88
26417	Greece	94.33	26413	Greece	70.77
26697	Ukraine	94.08	26420	Greece	69.25
26696	Ukraine	93.89	26423	Greece	68.94
26418	Greece	86.63	26631	Italy	66.29
26514	Malta	86.47	5617	Greece	66.00
26698	Ukraine	84.25	5626	Greece	65.75
26421	Greece	82.40	2944	Malta	64.75
2943	Malta	82.39	4603	Greece	62.47
5624	Greece	82.33	27876	Italy	62.25
2941	Malta	81.46	5616	Greece	58.81
26503	Italy	81.44	26700	Ukraine	57.13
26513	Malta	80.12	4605	Greece	56.28
26512	Malta	79.48	5613	Greece	55.71
26424	Greece	79.11			

Figure 4C: Mean root length of durum landraces originating from Eastern Europe.

AUS No.	Country	Root length	AUS No.	Country	Root length
20204	Turkey	116.34	26637	Turkey	77.11
20147	Turkey	107.44	26647	Turkey	77.11
20156	Turkey	105.50	26636	Turkey	76.78
20135	Turkey	104.13	20198	Turkey	76.56
20148	Turkey	103.28	26400	Azerbaijan	76.33
5649	Turkey	97.67	20153	Turkey	75.22
20146	Turkey	97.56	5662	Turkey	75.10
5653	Turkey	96.22	20201	Turkey	73.29
26405	Azerbaijan	92.34	19209	Turkey	72.52
26474	Georgia	91.56	20149	Turkey	72.00
20142	Turkey	90.13	13765	Turkey	71.69
26473	Georgia	90.00	20203	Turkey	71.37
20200	Turkey	89.37	20199	Turkey	71.00
28752	Turkey	88.11	26403	Azerbaijan	70.88
26652	Turkey	88.00	26401	Azerbaijan	70.79
26476	Georgia	87.04	26472	Georgia	70.56
26404	Azerbaijan	86.92	20155	Turkey	69.44
26402	Azerbaijan	86.71	19213	Turkey	68.50
26651	Turkey	85.17	20137	Turkey	67.13
20191	Turkey	84.67	26644	Turkey	67.11
20193	Turkey	84.44	26477	Georgia	65.84
20194	Turkey	84.33	20141	Turkey	65.63
20145	Turkey	83.10	20154	Turkey	65.44
5661	Turkey	82.12	26639	Turkey	63.67

26398	Azerbaijan	81.06	20152	Turkey	63.39
26475	Georgia	80.83	26653	Turkey	62.11
5651	Turkey	79.78	20162	Turkey	60.67
26397	Azerbaijan	79.49	20195	Turkey	59.89
20197	Turkey	79.44	20139	Turkey	59.83
26645	Turkey	79.00	20202	Turkey	57.17
26399	Azerbaijan	78.76	26388	Azerbaijan	55.95
26646	Turkey	77.94	20138	Turkey	54.33
20159	Turkey	77.22	26638	Turkey	54.00
26650	Turkey	77.22	20151	Turkey	51.44
20192	Turkey	77.22	20136	Turkey	46.00
19105	Turkey	77.22			

Figure 4D: Mean root length of durum landraces originating from Turkey, Azerbaijan, and Georgia.

AUS No.	Country	Root length	AUS No.	Country	Root length
28048	Cyprus	102.38	5176	Palestine	75.57
26437	Cyprus	97.13	5173	Palestine	75.52
26443	Cyprus	96.63	5161	Palestine	74.80
16466	Palestine	95.78	26393	Lebanon	73.00
26441	Cyprus	95.51	5174	Palestine	72.89
4209	Lebanon	94.69	5162	Palestine	72.54
26553	Palestine	94.56	5177	Palestine	71.26
5171	Palestine	92.88	26392	Lebanon	71.17
26555	Palestine	89.67	5172	Palestine	70.60
26440	Cyprus	87.26	5170	Palestine	66.68
5175	Palestine	83.81	4208	Lebanon	65.11
5169	Palestine	83.71	26435	Cyprus	64.48
26439	Cyprus	83.62	5167	Palestine	64.35
5164	Palestine	83.41	5166	Palestine	63.75
26442	Cyprus	82.78	4212	Lebanon	61.54
26436	Cyprus	81.47	4211	Lebanon	57.03
26444	Cyprus	81.13	5165	Palestine	54.91
26391	Lebanon	78.94			

Figure 4E: Mean root length of durum landraces originating from Palenstine, Lebanon, and Cyprus.

AUS No.	Country	Root length	AUS No.	Country	Root length
17132	Ethiopia	99.77	17131	Ethiopia	70.32
26733	Ethiopia	93.90	17145	Ethiopia	67.23
6266	Ethiopia	87.17	17136	Ethiopia	65.50
26729	Ethiopia	86.65	6264	Ethiopia	64.48
26469	Ethiopia	84.95	6268	Ethiopia	61.84
26470	Ethiopia	81.17	17134	Ethiopia	61.08
17144	Ethiopia	81.05	17142	Ethiopia	60.90
17126	Ethiopia	80.58	6263	Ethiopia	60.02
17129	Ethiopia	78.34	12799	Ethiopia	56.61
26471	Ethiopia	75.19	26730	Ethiopia	55.55
6267	Ethiopia	72.61	6269	Ethiopia	54.83
26732	Ethiopia	72.31	17137	Ethiopia	52.81
17140	Ethiopia	71.38	17128	Ethiopia	52.77
26734	Ethiopia	70.88	12800	Ethiopia	46.33
17141	Ethiopia	70.38			

Figure 4F: Mean root length of durum landraces originating from Ethiopia.

AUS No.	Country	Root length	AUS No.	Country	Root length
26626	Former Soviet Union	108.75	26457	Former Soviet Union	86.50
26701	Former Soviet Union	102.75	26455	Former Soviet Union	85.58
26628	Former Soviet Union	102.68	26720	Former Soviet Union	85.00
26396	Former Soviet Union	99.48	26630	Former Soviet Union	81.21
26719	Former Soviet Union	98.63	26724	Former Soviet Union	81.13
26625	Former Soviet Union	95.00	26458	Former Soviet Union	79.53
26454	Former Soviet Union	94.50	26510	Kazakhstan	77.71
26723	Former Soviet Union	93.84	26629	Former Soviet Union	76.04
26659	Turkmenistan	90.37	26691	Former Soviet Union	73.49
26456	Former Soviet Union	89.25	26692	Former Soviet Union	71.08
26718	Former Soviet Union	88.75	26717	Former Soviet Union	69.75
26721	Former Soviet Union	87.38	26716	Former Soviet Union	68.88
26658	Turkmenistan	87.17	26690	Former Soviet Union	68.32
26722	Former Soviet Union	86.88	26693	Former Soviet Union	68.19

Figure 4G: Mean root length of durum landraces originating from the Former Soviet Union, Turkmenistan, and Kazakhstan.

AUS No.	Country	Root length	AUS No.	Country	Root length
836	India	120.88	9928	India	78.63
4668	India	115.06	7896	India	78.63
10105	India	113.67	26497	India	77.33
8912	India	110.69	10096	India	75.63
4873	India	98.75	20414	India	75.40
7823	India	97.75	10097	India	75.13
4667	India	97.17	7822	India	74.75
10110	India	97.00	8629	India	73.25
4617	India	96.57	14899	India	73.19
8860	India	95.08	8630	India	73.00
15529	India	91.75	6247	India	72.63
8859	India	90.88	6248	India	72.20
8631	India	88.88	18117	India	71.83
8628	India	88.63	8634	India	71.00
21132	India	88.44	7899	India	69.92
18119	India	88.17	7951	India	69.55
21133	India	88.10	17712	India	68.50
837	India	87.25	8627	India	66.63
8625	India	86.67	17685	India	60.96
18118	India	86.65	2840	India	56.15
18116	India	82.61	835	India	52.00
8632	India	81.92	664	India	51.67
8624	India	81.36	8913	India	49.00

Figure 4H: Mean root length of durum landraces originating from India.

AUS No.	Country	Root length	AUS No.	Country	Root length
7890	Iran	122.57	9783	Iran	76.00
7886	Iran	120.21	9790	Iran	75.67
9778	Iran	109.36	26561	Iran	75.11
9779	Iran	101.71	9670	Iran	74.96
7889	Iran	101.23	26563	Iran	74.89
9783	Iran	100.19	9817	Iran	74.40
5292	Iran	98.30	9786	Iran	73.83
5184	Iran	97.22	5248	Iran	72.90
9770	Iran	91.29	9773	Iran	70.38
9775	Iran	88.83	9785	Iran	70.13
20190	Iran	87.89	20188	Iran	69.61
9781	Iran	86.88	5284	Iran	69.50

5200	Iran	85.38	7891	Iran	69.00
9771	Iran	84.36	9655	Iran	68.77
5204	Iran	84.33	9777	Iran	68.75
9774	Iran	81.92	9782	Iran	68.63
26560	Iran	81.50	26557	Iran	67.78
9767	Iran	79.00	9772	Iran	61.75
9788	Iran	77.57	20187	Iran	56.50
9668	Iran	76.02	5209	Iran	52.11

Figure 4I: Mean root length of durum landraces originating from Iran.

AUS No.	Country	Root length	AUS No.	Country	Root length
4897	Iraq	125.67	4901	Iraq	79.89
9658	Iraq	118.88	26499	Iraq	78.78
4896	Iraq	115.64	4892	Iraq	77.88
4899	Iraq	105.34	9829	Iraq	77.75
4890	Iraq	105.00	9656	Iraq	77.32
9659	Iraq	104.00	20173	Iraq	76.83
20174	Iraq	97.15	9660	Iraq	76.78
20176	Iraq	96.44	4891	Iraq	76.00
11817	Iraq	93.83	20181	Iraq	75.88
26502	Iraq	92.44	26500	Iraq	75.11
26501	Iraq	90.00	20180	Iraq	74.44
20179	Iraq	89.00	9830	Iraq	73.88
20178	Iraq	87.00	9828	Iraq	73.73
4900	Iraq	85.75	20172	Iraq	73.58
20175	Iraq	84.83	20177	Iraq	72.19
4932	Iraq	84.63	4934	Iraq	70.72
20182	Iraq	84.00	20183	Iraq	67.44
16118	Iraq	83.10	4894	Iraq	65.89
4933	Iraq	81.83	4898	Iraq	62.44

Figure 4J: Mean root length of durum landraces originating from Iraq.

AUS No.	Country	Root length	AUS No.	Country	Root length
4362	Syria	97.72	4140	Syria	75.89
4349	Syria	93.89	20163	Syria	75.00
26447	Syria	91.56	4126	Syria	74.83
26344	Syria	89.61	26450	Syria	73.78
20171	Syria	89.11	13784	Syria	72.55
4350	Syria	89.04	4136	Syria	72.50
4347	Syria	88.85	26451	Syria	72.44
26452	Syria	86.78	4364	Syria	72.25
20167	Syria	84.39	26350	Syria	72.11
20168	Syria	83.06	4354	Syria	71.83
26449	Syria	83.00	26348	Syria	71.83
20169	Syria	82.83	26445	Syria	71.56
26448	Syria	82.56	26346	Syria	71.22
20166	Syria	81.22	4355	Syria	70.63
4365	Syria	80.17	4128	Syria	70.06
4138	Syria	80.00	26347	Syria	67.11
20170	Syria	80.00	4346	Syria	64.88
4363	Syria	79.81	4348	Syria	61.40
20164	Syria	79.17	20196	Syria	55.67
4356	Syria	76.25	4120	Syria	55.17
4351	Syria	75.98	20165	Syria	54.83
21583	Syria	75.89	4127	Syria	52.83

Figure 4K: Mean root length of durum landraces originating from Syria.

Appendix 5: Correlation between replications (different hydroponic trays) for the six F_3 durum populations in the bicarbonate and control treatments.

Carbonate

Rep	1	2	3	4
1	*	*	*	*
2	0.424***	*	*	*
3	0.372***	0.417***	*	*
4	0.558***	0.526***	0.377***	*

Control

Rep	1	2	3	4
1	*	*	*	*
2	0.682***	*	*	*
3	0.673***	0.689***	*	*
4	0.664***	0.631***	0.682***	*

ns – not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

Figure 5A: Correlation between replications for the bicarbonate and control treatments for the AUS4897 x Tamaroi population.

Carbonate

Rep	1	2	3	4
1	*	*	*	*
2	0.524***	*	*	*
3	0.401***	0.395***	*	*
4	0.384***	0.470***	0.504***	*

Control

Rep	1	2	3	4
1	*	*	*	*
2	0.689***	*	*	*
3	0.697***	0.642***	*	*
4	0.575***	0.620***	0.502***	*

ns – not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

Figure 5B: Correlation between replications for the bicarbonate and control treatments for the AUS4897 x Kalka population.

Carbonate

Rep	1	2	3	4
1	*	*	*	*
2	0.558***	*	*	*
3	0.555***	0.570***	*	*
4	0.548***	0.553***	0.551***	*

Control

Rep	1	2	3	4
1	*	*	*	*
2	0.727***	*	*	*
3	0.654***	0.761***	*	*
4	0.703***	0.691***	0.724***	*

ns – not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

Figure 5C: Correlation between replications for the bicarbonate and control treatments for the AUS4897 x Na49Kalka#4 population.

Carbonate

	Rep	1	2	3	4
Rep	1	*	*	*	*
	2	0.605***	*	*	*
	3	0.439***	0.735***	*	*
	4	0.593***	0.655***	0.721***	*

Control

	Rep	1	2	3	4
Rep	1	*	*	*	*
	2	0.758***	*	*	*
	3	0.716***	0.782***	*	*
	4	0.698***	0.716***	0.673***	*

ns – not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

Figure 5D: Correlation between replications for the bicarbonate and control treatments for the AUS7890 x Na49Kalka#4 population.

Carbonate

	Rep	1	2	3	4
Rep	1	*	*	*	*
	2	0.352***	*	*	*
	3	0.314**	0.268***	*	*
	4	0.402***	0.494***	0.425***	*

Control

	Rep	1	2	3	4
Rep	1	*	*	*	*
	2	0.459***	*	*	*
	3	0.550***	0.537***	*	*
	4	0.571***	0.557***	0.582***	*

ns – not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

Figure 5E: Correlation between replications for the bicarbonate and control treatments for the R622Sh x Na49Kalka#4 population.

Carbonate

	Rep	1	2	3	4
Rep	1	*	*	*	*
	2	0.563***	*	*	*
	3	0.467***	0.658***	*	*
	4	0.379***	0.461***	0.522***	*

Control

	Rep	1	2	3	4
Rep	1	*	*	*	*
	2	0.442***	*	*	*
	3	0.437***	0.567***	*	*
	4	0.367***	0.619***	0.606***	*

ns – not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

Figure 5F: Correlation between replications for the bicarbonate and control treatments for the C8MM x Na49Kalka#4 population.

Carbonate

		Rep 1	Rep 2	Rep 3	Rep 4
Rep	1	*	*	*	*
	2	0.410***	*	*	*
	3	0.378***	0.466***	*	*
	4	0.198*	0.433***	0.471***	*

Control

		Rep 1	Rep 2	Rep 3	Rep 4
Rep	1	*	*	*	*
	2	0.581***	*	*	*
	3	0.656***	0.609***	*	*
	4	0.585***	0.574***	0.514***	*

ns – not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

Figure 5G: Correlation between replications for the bicarbonate and control treatments for the Na49Kalka#4 x Tamaroi population.

Appendix 6: Correlation between replications (different hydroponic trays) for the bread and durum wheat populations in the bicarbonate and control treatments.

Carbonate

	Rep	1	2	3	4
	1	*	*	*	*
Rep	2	0.685***	*	*	*
	3	0.707***	0.681***	*	*
	4	0.569***	0.635***	0.680***	*

Control

	Rep	1	2
	1	*	*
Rep	2	0.682***	*

ns – not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

Figure 6A: Correlation between replications for the bicarbonate and control treatments for the RAC875 x Cascades (I) population.

Carbonate

	Rep	1	2	3	4	5	6
	1	*	*	*	*	*	*
	2	0.317**	*	*	*	*	*
Rep	3	0.219*	0.122^{ns}	*	*	*	*
	4	0.274**	0.087^{ns}	0.292**	*	*	*
	5	0.337***	0.241**	0.296**	0.210*	*	*
	6	0.256**	0.196*	0.087^{ns}	0.099^{ns}	0.367***	*

ns – not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

Control

	Rep	1	2	3	4	5	6
	1	*	*	*	*	*	*
	2	0.380***	*	*	*	*	*
Rep	3	0.344***	0.103^{ns}	*	*	*	*
	4	0.420***	0.243**	0.293**	*	*	*
	5	0.123^{ns}	0.277**	0.219**	0.160^{ns}	*	*
	6	0.435***	0.463***	0.267**	0.293**	0.400***	*

ns – not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

Figure 6B: Correlation between replications for the bicarbonate and control treatments for the RAC875 x Cascades (II) population.

Carbonate

Rep	1A	2A	3A	4A	1B	2B	3B	4B
1A	*	*	*	*	*	*	*	*
2A	0.554***	*	*	*	*	*	*	*
3A	0.550***	0.348***	*	*	*	*	*	*
4A	0.550***	0.434***	0.430***	*	*	*	*	*
1B	*	*	*	*	*	*	*	*
2B	*	*	*	*	0.456***	*	*	*
3B	*	*	*	*	0.543***	0.606***	*	*
4B	*	*	*	*	0.538***	0.453***	0.501***	*

ns – not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

Control

Rep	1A	2A	3A	4A	1B	2B	3B	4B
1A	*	*	*	*	*	*	*	*
2A	0.348***	*	*	*	*	*	*	*
3A	0.255***	0.349***	*	*	*	*	*	*
4A	0.268***	0.416***	0.500***	*	*	*	*	*
1B	*	*	*	*	*	*	*	*
2B	*	*	*	*	0.523***	*	*	*
3B	*	*	*	*	0.389***	0.432***	*	*
4B	*	*	*	*	0.546***	0.586***	0.616***	*

ns – not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

Figure 6C: Correlation between replications for the bicarbonate and control treatments for the Wk/TmWLYY9//WLYY9Tm population.

Carbonate

Rep	1A	2A	3A	4A	1B	2B	3B	4B
1A	*	*	*	*	*	*	*	*
2A	0.406***	*	*	*	*	*	*	*
3A	0.343***	0.642***	*	*	*	*	*	*
4A	0.589***	0.621***	0.647***	*	*	*	*	*
1B	*	*	*	*	*	*	*	*
2B	*	*	*	*	0.276***	*	*	*
3B	*	*	*	*	0.319**	0.353***	*	*
4B	*	*	*	*	0.561***	0.488***	0.357***	*

ns – not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

Control

	Rep 1A	2A	3A	4A	Rep 1B	2B	3B	4B
1A	*	*	*	*	*	*	*	*
2A	0.208***	*	*	*	*	*	*	*
3A	0.112^{ns}	0.194^{ns}	*	*	*	*	*	*
Rep 4A	0.384***	0.036^{ns}	0.078^{ns}	*	*	*	*	*
1B	*	*	*	*	*	*	*	*
2B	*	*	*	*	0.278**	*	*	*
3B	*	*	*	*	0.257*	0.247*	*	*
4B	*	*	*	*	0.287**	0.224*	0.291**	*

ns – not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

Figure 6D: Correlation between replications for the bicarbonate and control treatments for the Frame/Yarralinka//Pugsley population.

Carbonate

	Rep 1A	2A	3A	4A	Rep 1B	2B	3B	4B
1A	*	*	*	*	*	*	*	*
2A	0.478***	*	*	*	*	*	*	*
3A	0.542***	0.507***	*	*	*	*	*	*
Rep 4A	0.707***	0.602***	0.670***	*	*	*	*	*
1B	*	*	*	*	*	*	*	*
2B	*	*	*	*	0.632***	*	*	*
3B	*	*	*	*	0.664***	0.807***	*	*
4B	*	*	*	*	0.628***	0.813***	0.765***	*

ns – not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

Control

	Rep 1A	2A	3A	4A	Rep 1B	2B	3B	4B
1A	*	*	*	*	*	*	*	*
2A	0.585***	*	*	*	*	*	*	*
3A	0.640***	0.585***	*	*	*	*	*	*
Rep 4A	0.617***	0.532***	0.653***	*	*	*	*	*
1B	*	*	*	*	*	*	*	*
2B	*	*	*	*	0.584***	*	*	*
3B	*	*	*	*	0.616***	0.639***	*	*
4B	*	*	*	*	0.722***	0.593***	0.617***	*

ns – not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

Figure 6E: Correlation between replications for the bicarbonate and control treatments for the Berkut/Krichauff population.

Carbonate

Rep	1	2	3	4
1	*	*	*	*
2	0.532**	*	*	*
3	0.505**	0.675***	*	*
4	0.650***	0.689***	0.594***	*

Control

Rep	1	2	3	4
1	*	*	*	*
2	0.615***	*	*	*
3	0.457***	0.588***	*	*
4	0.630***	0.477**	0.685***	*

ns – not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

Figure 6F: Correlation between replications for the bicarbonate and control treatments for the Kukri x RAC875 population.

Carbonate

Rep	1	2	3	4
1	*	*	*	*
2	0.433*	*	*	*
3	0.480**	0.511**	*	*
4	0.390*	0.671***	0.490***	*

Control

Rep	1	2	3	4
1	*	*	*	*
2	0.532**	*	*	*
3	0.573***	0.399*	*	*
4	0.354*	0.175^{ns}	0.246^{ns}	*

ns – not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

Figure 6G: Correlation between replications for the bicarbonate and control treatments for the Kukri x Excalibur population.

Carbonate

Rep	1	2	3	4
1	*	*	*	*
2	0.618***	*	*	*
3	0.338^{ns}	0.348^{ns}	*	*
4	0.505**	0.191^{ns}	0.395*	*

Control

Rep	1	2	3	4
1	*	*	*	*
2	0.717**	*	*	*
3	0.798***	0.807***	*	*
4	0.733***	0.592***	0.800***	*

ns – not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

Figure 6H: Correlation between replications for the bicarbonate and control treatments for the Meering x Yitpi population.

Carbonate

		Rep 1	2	3	4
Rep	1	*	*	*	*
	2	0.402*	*	*	*
	3	0.595***	0.608***	*	*
	4	0.600***	0.657***	0.770***	*

Control

		Rep 1	2	3	4
Rep	1	*	*	*	*
	2	0.123^{ns}	*	*	*
	3	0.335^{ns}	0.206^{ns}	*	*
	4	0.030^{ns}	0.470**	0.014^{ns}	*

ns – not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

Figure 6I: Correlation between replications for the bicarbonate and control treatments for the Meering x 90072 population.

Carbonate

		Rep 1	2	3	4
Rep	1	*	*	*	*
	2	0.330^{ns}	*	*	*
	3	0.225^{ns}	0.214^{ns}	*	*
	4	0.667***	0.268^{ns}	0.389*	*

Control

		Rep 1	2	3	4
Rep	1	*	*	*	*
	2	0.735***	*	*	*
	3	0.528**	0.533**	*	*
	4	0.280^{ns}	0.449*	0.253^{ns}	*

ns – not significant, *(P<0.05), **(P<0.01), ***(P<0.001)

Figure 6J: Correlation between replications for the bicarbonate and control treatments for the Stylet x Westonia population.

Appendix 7: Cumulative rainfall data from 2002 to 2008.

Table 7A: Monthly rainfall (mm) for Kimba from 2002 to 2008 compared to the long-term average (88 years).

	Year							Mean	Long-term
	2002	2003	2004	2005	2006	2007	2008	2002-08	Average
January	12.4	11.0	7.8	10.2	12.0	40.0	1.6	13.6	17.0
February	0.2	62.6	2.4	3.8	16.2	0.2	2.6	12.6	20.2
March	1.6	0.4	3.2	5.4	13.6	26.8	1.0	7.4	16.0
April	5.8	18.2	10.6	7.8	30.4	37.0	23.0	19.0	22.3
May	66.2	60.4	10.8	16.8	15.0	27.0	33.0	32.7	35.3
June	38.2	33.0	43.4	42.8	24.8	16.0	25.0	31.9	39.6
July	40.4	22.7	39.6	35.4	29.4	33.4	40.4	34.5	41.3
August	22.6	64.8	47.2	37.8	3.2	18.2	64.0	36.8	42.1
September	11.2	14.4	28.2	77.0	2.4	11.8		24.2	36.9
October	8.0	40.4	0.2	42.6	0.2	10.6		17.0	30.9
Novermber	23.8	35.0	15.0	12.8	17.2	36.2		23.3	23.7
December	6.0	19.2	15.8	18.2	36.0	46.4		23.6	19.8

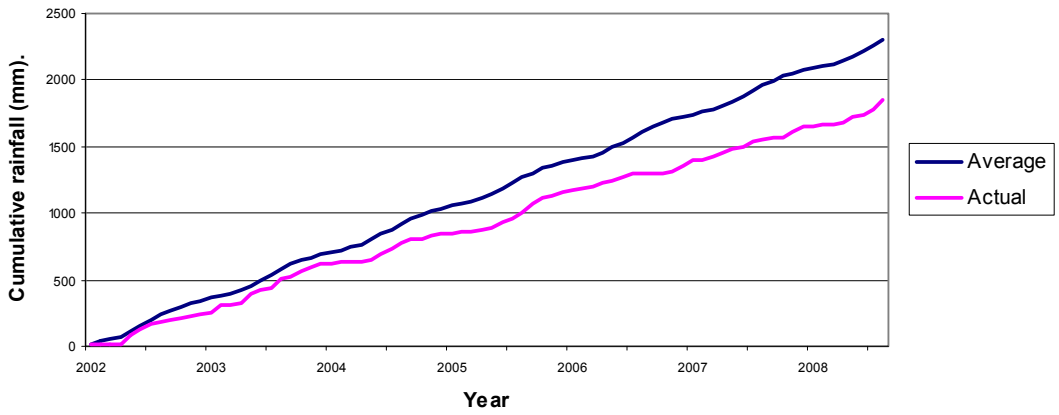


Figure 7A: Cumulative rainfall (mm) for Kimba from 2002 to 2008 compared to the cumulative average (88 years).

Table 7B: Monthly rainfall (mm) for Murray Bridge from 2002 to 2008 compared to the long-term average (123 years).

	Year							Mean	Long-term
	2002	2003	2004	2005	2006	2007	2008	2002-08	Average
January	26.2	4.4	7.5	23.4	28.8	32.5	5.0	18.3	16.5
February	2.4	45.4	5.8	40.4	14.7	1.2	2.3	16.0	18.2
March	26.6	6.7	6.1	8.6	36.4	19.2	4.5	15.4	20.0
April	12.3	14.0	5.0	4.2	32.5	86.3	11.2	23.6	28.7
May	42.3	56.6	21.5	5.4	21.6	21.3	41.0	30.0	34.9
June	15.4	52.0	54.3	158.3	14.3	31.9	29.3	50.8	37.9
July	34.2	26.0	45.0	27.2	29.7	30.1	29.6	31.7	35.1
August	29.1	71.5	47.1	36.1	5.3	7.3	62.5	37.0	37.0
September	27.0	28.8	30.8	53.7	22.3	27.5		31.7	36.3
October	15.2	56.0	7.2	76.6	0.4	17.3		28.8	34.1
Novermber	32.1	15.4	33.4	40.9	15.0	27.5		27.4	25.3
December	6.8	25.9	111.7	23.9	20.2	26.6		35.9	23.1

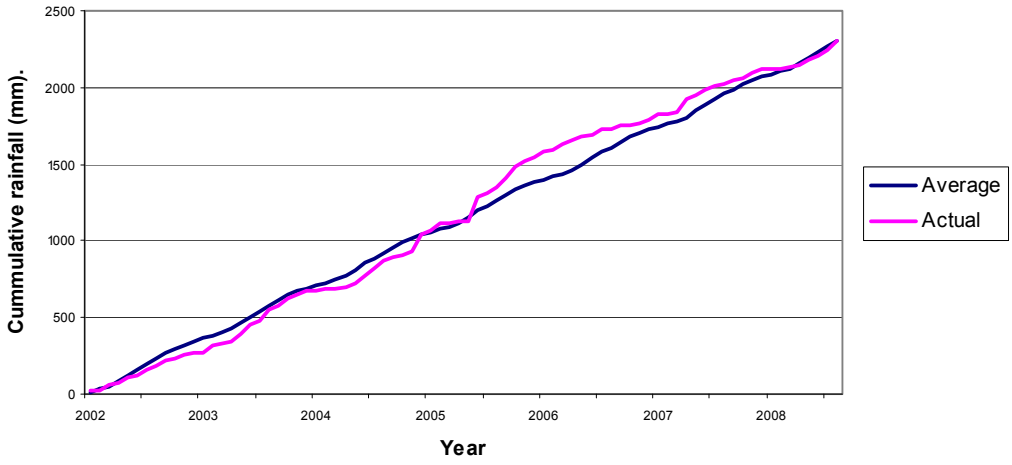


Figure 7B: Cumulative rainfall (mm) for Murray Bridge from 2002 to 2008 compared to the cumulative average (123 years).

Table 7C: Monthly rainfall (mm) for Port Pirie from 2002 to 2008 compared to the long-term average (132 years).

	Year							Mean	Long-term
	2002	2003	2004	2005	2006	2007	2008	2002-08	Average
January	16.3	13.6	22.0	25.2	2.6	130.5	0.0	30.0	18.8
February	0.0	50.8	5.6	4.0	26.0	1.1	1.7	12.7	17.8
March	4.8	2.4	9.6	7.4	28.6	61.5	25.4	20.0	17.9
April	0.8	9.6	6.4	15.8	43.4	18.3	33.1	18.2	27.7
May	40.0	69.0	23.6	9.9	13.4	17.2	26.5	28.5	38.1
June	29.1	19.0	90.6	44.0	13.8	13.5	25.5	33.6	40.6
July	25.9	9.4	41.2	35.0	14.3	23.6	45.9	27.9	33.9
August	11.2	49.0	42.0	38.8	29.1	14.4	63.2	35.4	34.9
September	17.0	4.4	29.7	52.0	6.0	21.0		21.7	35.7
October	10.0	28.2	8.6	39.7	0.8	20.4		18.0	33.1
Novermber	34.0	12.0	28.0	31.4	22.4	38.4		27.7	23.9
December	21.1	23.4	57.2	24.6	21.0	27.8		29.2	22.1

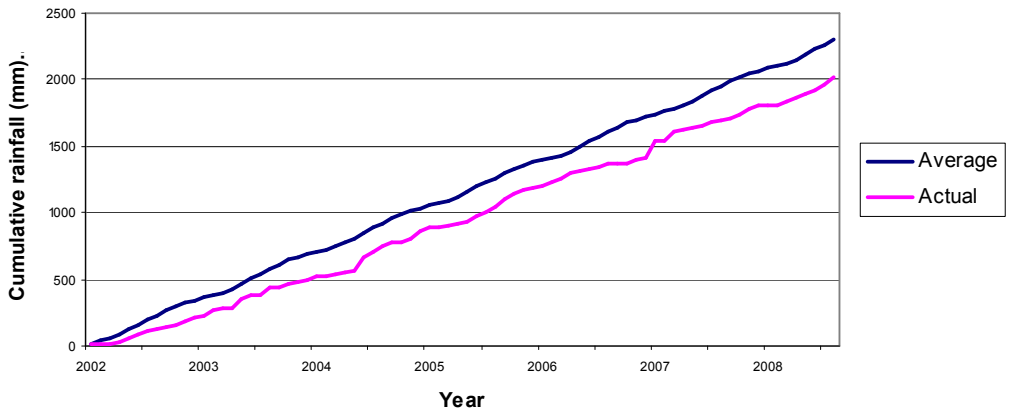


Figure 7C: Cumulative rainfall (mm) for Port Pirie from 2002 to 2008 compared to the cumulative average (132 years).