A genetic dissection of drought and heat tolerance related traits in bread wheat (*Triticum aestivum* L.)

By

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Abstract

This study was conducted with the aim of improving our understanding of the genetic basis of the superior grain yield of an elite bread wheat breeding line, RAC875, under drought and heat stressed Mediterranean-type climates in southern Australia. Here, these abiotic stresses present a significant barrier to production. Kukri is a locally adapted variety which achieves acceptable grain yield under more favourable conditions, but relatively low grain yields under severe stress. A cross between the two lines resulted in an F_1 derived doubled haploid population consisting of 368 individuals.

The population was initially used for the genetic dissection of time to ear emergence and flag leaf glaucousness, with the latter trait hypothesised to explain a significant proportion of RAC875's relative drought and heat tolerance. Whilst parents of the population achieved similar time to ear emergence, segregation for *Ppd-B1* and *Ppd-D1a* created large variation for this trait within the population. Two novel minor loci were detected for time to ear emergence (*Q.Eet.aww-1A* and *Q.Zad.aww-4A*), in addition to another eight known, minor loci. Five novel loci were detected for flag leaf glaucousness (*Q.W.aww-3A, Q.W.aww-3B, Q.W.aww-3D, Q.W.aww-4D* and *Q.W.aww-5B*), with one in particular (*Q.W.aww-3A*) accounting for up to 52 percent of the genetic variance for this trait.

Sixteen field experiments were sown across southern Australia between 2006 and 2010, where average site grain yields ranged from 314 to 5275 kg ha^{-1}. Kernels per square metre was the trait most correlated with grain yield, while spikelet fertility, which had a significant positive correlation with grains per square metre in all experiments and the subsequently derived environment clusters, was also related to grain yield. Nine loci were detected for grain yield independent of time to ear emergence and plant height. Five of these loci co-located with loci for kernels per square metre and only one of these nine loci were associated with any of the loci for flag leaf glaucousness and this genetic effect was opposite (i.e. Kukri allele resulting in large glaucousness value and lower grain yield). The RAC875

allele at QTL on chromosomes 1B and 7A (*Q.Yld.aww-1B* and *Q.Yld.aww-7A-2*) was associated with greater grain yield, kernels per spikelet and kernels per square metre. These two loci were detected in environment clusters where heat stress was a differentiating factor and it was concluded that these may therefore be associated with heat stress tolerance. Another QTL of large effect was consistently detected on chromosome 6A (*Q.Tkw.aww-6A*), with the RAC875 allele positively affecting grain size, flag leaf width and stem water soluble carbohydrate content but resulting in lower kernels per spikelet and therefore kernels per square metre.

Experiments were also sown to assess the performance of the population in north-west Mexico under well watered, high yield potential conditions, as well as drip irrigated drought treatment and late planted but well watered conditions to expose the experiments to heat stress. This resulted in three very distinctive treatments and subsequently detected different genetic regions controlling grain yield. Two distinct QTL were detected for grain yield and canopy temperature on chromosome 3B, under irrigated (Q.Yld.aww-3B-1) and irrigated, drought and heat stressed treatments (Q.Yld.aww-3B-2). The latter QTL accounted for up to 22 percent of the genetic variance for grain yield and 20 percent of the genetic variance for canopy temperature under the heat stress treatment. However, all three treatments failed to detect any major QTL of common effect to southern Australia.

This study highlighted the complex genetic basis of grain yield and physical grain quality in drought and heat stressed conditions, as well as the importance of conducting QTL dissection in the target environment. However, key loci detected offer potential for marker development and deployment of marker assisted selection within wheat breeding programmes targeting southern Australia. In the longer term, this should help improve the rate of genetic gain for grain yield, increasing production by growers in the Mediterranean type climate of southern Australia.

Declaration

I certify that 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. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Chapter 1

Chapter 1. Literature review

1.0 Introduction

Bread wheat (*Triticum aestivum* L.) was first domesticated approximately 6000 years ago in the Fertile Crescent, which stretches through the countries now known as Egypt, Israel, Jordan, Lebanon, Syria and Iran (Dvorak *et al.* 1998). Since then, it has become widely grown across the world and is now the third most important grain crop for human nutrition worldwide (FAO, 2010). Whilst total grain production in Australia is comparatively small relative to other nations, exports of bread wheat contribute significantly to international trade of this commodity (FAO, 2010). Across southern Australia, where the climate is predominantly Mediterranean, bread wheat production can account for more than a quarter of national production in some seasons but is more often than not constrained by abiotic stresses such as heat and drought during the growing season. This literature review aims to summarise current knowledge of the impact of drought and/or heat stress on bread wheat production; particularly in Mediterranean climates, at a plant physiology level, the traits that offer potential to maintain or improve production in these environments and the current state of knowledge of the genetics behind these traits.

1.1 The Mediterranean climate and the bread wheat growth cycle

In regions where a Mediterranean climate is dominant, including southern Australia, California, and regions surrounding the Mediterranean Sea, rainfall is winter dominant, where it is in excess of crop demands due to mild temperatures, low evaporation rates and slow growth rates (Loss and Siddique 1994). As a result, severe drought during the winter is rare. Leading into spring through to summer, cyclical drought is commonly experienced, with rainfall events increasingly less frequent and therefore the plant water demand:supply ratio increasing. At the same time, average daily maximum temperatures are increasing, with soil moisture severely depleted by the time the crop matures (Figure 1.1-1). Terminal drought varies in timing from year to year depending on spring rains, temperatures, soil type and crop growth (Loss and Siddique 1994).
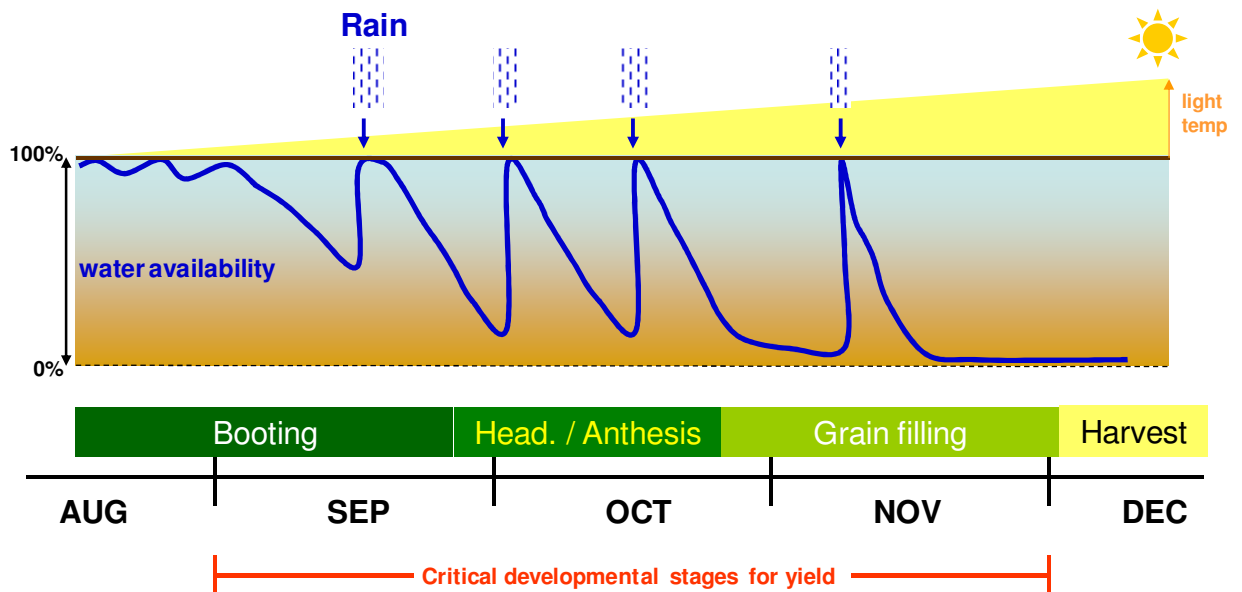
Figure 1.1-1. Typical conditions during spring and early summer in Mediterranean style climate, highlighting increasingly strong cyclical water stress during ear emergence and into grain filling, with terminal drought often ending the growing season before physiological maturity is naturally reached (Figure courtesy of T. Schnurbusch). Across southern Australia, these cyclical droughts vary in timing during the growing season, length and intensity.

At different growth stages, abiotic stress can impact different yield components that are being set at that stage, reducing yield potential. Figure 1.1-2 illustrates the phasic growth and development of the lifecycle of wheat. A basic knowledge of this cycle can help with understanding of how drought and/or heat stress would affect yield at a certain stage of growth. Commonly, in a Mediterranean type environment, water and heat stress can have an impact from as early as anthesis, increasing in intensity and frequency as time goes on.

Over the summer period, there may be no significant rainfall for two to five months, limiting crop production to the winter and spring months. Although the Mediterranean type climate is characterized by this rainfall pattern, water availability may not be the only limitation to crop production. Temperature, both high and low, can have an impact on crop growth and has been shown to be closely related to solar radiation (Loss and Siddique 1994), which can also effect evaporation.
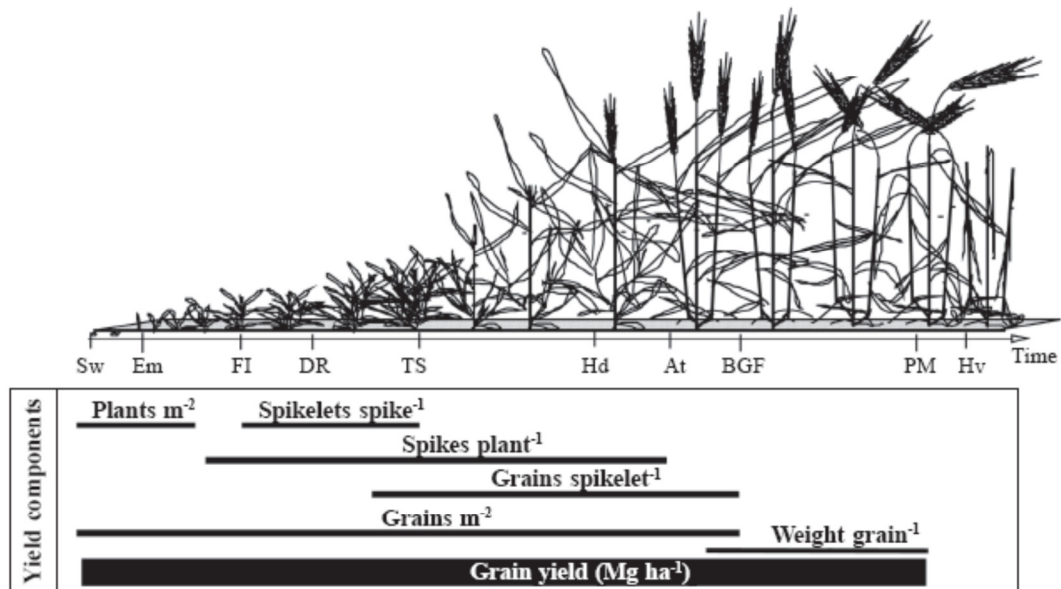
Figure 1.1-2. The growth and development of wheat from sowing (Sw) through emergence (Em), floral initiation (FI), initiation of the first double ridge (DR), terminal spikelet (TS) initiation, heading (Hd), anthesis (At), beginning of grain fill period (BGF), physiological maturity (PM) and harvest (Hv). The bars show timing when different components of grain yield are determined. Reproduced from Slafer and Rawson (1994).

In Australia, many soils in these Mediterranean type climate regions are poor, with frequent nutrient deficiencies and toxicities. Due to the geologically old age of the soils in these regions, they are generally weathered and relatively infertile. Problems associated with poor nutrient availability or toxicity cause further limitations to crop production, as well as causing significant genotype by environment (GxE) interactions when trying to interpret multi-site field data.

1.2 Agronomic manipulation of production in water limited environments

Agronomic options for drought tolerance improvement generally revolve around improving crop water use efficiency by maximizing soil water storage and minimizing soil evaporation. Sauer *et al.*(1996) found that keeping plant residues on the soil surface reduced soil evaporation by up to 50 percent, in addition to potentially increasing soil organic matter. However, plant residues can allow certain diseases to persist in subsequent seasons by acting as a source of inoculum (Bailey 1996; Krupinsky *et al.* 2004).

Tillage practices can have a significant effect on water infiltration and soil water storage by breaking up surface crusts, subsequently influencing plant available water and encouraging plant root growth. However, tillage is also associated with exposing moist soil, resulting in greater evaporative losses (Moret *et al.* 2007), which may exceed the increased level of soil infiltration, as well as frequently resulting in significant physical soil loss (Hatfield *et al.* 2001). So, whilst there are options for agronomic manipulation to assist water conservation, their implementation needs to be considered in light of other detrimental effects on plant growth and development.

1.3 The concepts of plant tolerance and resistance to abiotic stress and their relevance today

Levitt (1972) suggested the general ability of a plant to survive under water stress should be viewed as resistance to drought. This author went on to suggest that within this resistance there are two key mechanisms, drought avoidance and drought tolerance. Drought avoidance refers to the ability of a plant to be able to reduce its exposure to low water potential conditions through modifying its environment (for example, canopy temperature depression to reduce heat stress) or expressing a modified growth cycle (for example, early maturity to ensure crucial reproductive phases occur before severe stress is likely to occur). Drought or heat tolerance could therefore be viewed as the ability of a plant to maintain physiological function under low water potential or high temperatures. However, there is currently no universally recognized definition for drought resistance or tolerance (Specht *et al.* 2001).

Attempting to categorize specific traits under these definitions is also difficult, so attention is now turning to the concept of water productivity (i.e. 'more crop per drop'; the Passioura Identity) (Passioura 2006). This allows quantitative measurement of improvement, therefore encouraging more rapid progress. One such measurement is water use efficiency, which from a physiological point of view, refers to the accumulated dry matter (biomass or grain yield) divided by the water used for the same period (Abbate *et al.* 2004; Fageria *et al.* 2006). However, Blum (2009) argued that this would lead to selection of genotypes with reduced yield and that rather effective use of water (EUW) would result in improved plant water status. This implies maximising transpiration through increased soil water capture by the plant. This literature review will use drought tolerance as a term implying greater relative grain yield

under water limited conditions (or improved efficiency of water use) relative to a lower yielding, intolerant (or less efficient) variety.

1.4 Quantifying the impact of drought and heat stress on bread wheat production

Whilst it is relatively easy for plant physiologists and molecular biologists to identify negative impacts of abiotic stress on various plant processes, translation of this into the level of positive impact that growers would see in their fields is more difficult. A careful review of the literature to date on this subject highlights a number of areas where this knowledge is lacking. Many authors have reported the impact of drought on grain yield as compared to an otherwise similar, well watered environments and the subsequent average reduction in grain yield using the same set of germplasm. The reported effects have ranged from 5 percent through to 65 percent reduction in average grain yield (Dencic *et al.* 2000; Kumar *et al.* 2007; Ozturk and Aydin 2004; Pinto *et al.* 2010). However, there remains a distinct lack of detail as to the exact stage of the growth cycle these experiments were at when significant water stress was imposed, limiting the capacity to directly compare between studies. Numerous reports also indicated experiments were rainfed and were therefore lower yielding than the comparable, irrigated experiments, but generally did not specify timing or the level of intensity of water stress that the particular experiment experienced. These factors would lead different authors to reach differing conclusions about the relative value of specific traits for achieving greater grain yields under water stress conditions and where genetic dissection is conducted, potentially different genomic regions would be identified.

For heat stress, a number of studies have taken the approach of utilising a late time of sowing, with supplementary irrigation, to expose experiments to the hotter temperatures of summer in an otherwise similar environment to timely sown experiments. Here, authors have identified average reductions between 9 and 52 percent in grain yield in comparison to well watered but cooler experiments (Pinto *et al.* 2010; Rane *et al.* 2007; Tahir *et al.* 2006). However, Ferris *et al.* (1998) and Tewolde *et al.* (2006) investigated the effect of temperature during specific growth phases and identified a significant

correlation between grain yield and temperature, post anthesis, with grain yield reduced by between 287 and 488 kg/ha for each degree Celsius rise in average temperature. Kuchel *et al.* (2007c) went as far as identifying that each day greater than 30 degrees Celsius during the growing season reduced grain yield by an average 75.8 kg/ha. There do not appear to be any reports in the literature of attempts to investigate the impact of drought and heat stress as separate factors, nor the impact of the two stresses combined within one study.

1.4.1 The physiological impact of pre-anthesis drought and heat stress

In a Mediterranean type environment, plants are not generally exposed to severe drought or heat stress before anthesis (Figure 1.1-1). Wang *et al.* (2011) and Wang *et al.* (2012) identified that pre-anthesis heat stress can actually have a positive effect by acclimating wheat to post-anthesis stress, with no negative effects where no post anthesis stress was experienced. However, Robertson and Giunta (1994) highlighted that pre-anthesis drought stress resulted in reduced early biomass production, reducing total radiation interception by plants, further reducing biomass production, as well as shortening the vegetative growth stage and ultimately negatively affecting grain yield potential. The importance of pre-anthesis biomass production for support of grain yield in Mediterranean type climates has been highlighted by numerous authors previously (Palta and Fillery 1995; Robertson and Giunta 1994).

Further to this, gametophyte development can be significantly impaired when cereals experience a water deficit during meiosis, during the young microspore stage (Sheoran and Saini 1996). Saini and Aspinall (1981) imposed a water stress treatment on wheat, causing male sterility in around 40 percent of the florets, yet female fertility was unaffected, as the ovule could still be fertilized with pollen from unstressed plants. Similar results were observed in oats (Skazkin and Lukomskaya 1962) and in corn (Moss and Downey 1971), leading Saini and Westgate (2000), Oshino *et al.* (2007) and Ji *et al.* (2010) to conclude that the development and fertility of cereal pollen has greater sensitivity to drought than the ovule.

1.4.2 The physiological effect of drought and heat stress during anthesis

At this stage, spikes can be inhibited from fully emerging from the flag leaf sheath by water deficit, which delays flowering (Craufurd and Peacock 1993) and provides a physical barrier to pollen extrusion. In addition, abiotic stress at this stage can cause a reduction in the number of anthers that dehisce, reducing the amount of pollen shed. This in turn causes a lower rate of fertilization and subsequently, lower grain set, which given the strong correlation between grain yield and grain number per unit area, results in a negative effect on grain yield.

1.4.3 The physiological effect of drought and heat stress post anthesis

Water deficit following ovule fertilization primarily affects kernel sink potential. During the period 5 to 15 days after pollination, the number of cells and starch granules initiated in the endosperm are set, determining the sink potential of the developing grain (Brooks *et al.* 1982; Gleadow *et al.* 1982; Nicolas *et al.* 1984). At this stage of cell division, the number of endosperm cells is sensitive to environmental conditions, particularly drought and heat stress (Bhullar and Jenner 1983; Brooks *et al.* 1982) and starch synthase enzyme activity can also be interrupted by heat stress (Hawker and Jenner 1993). Therefore, a reduction in yield at this stage can be attributed to a reduction in the number of endosperm cells and also a reduced capacity of the endosperm to accumulate starch (Sheoran and Saini 1996).

Once this sink potential has been set, drought can limit final kernel size by reducing the rate and duration of the deposition of starch and protein reserves. Drought and the frequently accompanying high temperatures can desiccate plant tissues, inducing the early onset of physiological maturity (Saini and Westgate 2000) and causing a premature end to grain-fill. For each degree Celsius rise above 15 during grain-fill, grain-fill duration is reduced by approximately three days under field conditions (Wiegand & Cuellar 1981 and references therein). This resulted in a 2.8 mg/degree Cecius reduction in kernel weight, greater than the 1.5 mg/degree Celcius reduction they identified from the data of previous studies. This was supported by evidence that grain-fill is limited by a shorter grain-fill duration induced by heat stress, both in terms of calendar days and degree days (Stone and Nicolas 1994; Viswanathan and Khanna-Chopra 2001; Wardlaw and Moncur 1995). These authors all concluded that the lines

identified as more heat tolerant were those able to maintain their grain fill duration despite the onset of heat stress. In contrast, Dias and Lidon (2010), who argued that a shorter and more rapid rate of grain fill duration should be the aim, particularly under terminal heat stress. In reality, a combination of these two strategies would probably be beneficial to production under drought and heat stressed conditions.

1.4.4 The effect of abiotic stress on photosynthesis

When water deficit occurs, many plants respond by closing their stomata, to prevent further water loss by transpiration. However, since the stomata also provide the primary route for the exchange of CO_2, water deficit affects the availability of CO_2 for carboxylation. The closure of stomata presents a barrier to the diffusion of CO_2 and causes the amount of CO_2 at the sites of carboxylation to be lower than that in the atmosphere. This therefore reduces CO_2 assimilation (photosynthesis) below its potential level (Farquhar and Sharkey 1982). Under severe water stress, Rubisco synthesis may also be limited due to ATP deficiency, caused by the limitation of CO_2 (Lawlor and Cornic 2002).

Under heat stressed conditions, constraints on photosynthesis can limit plant growth and thylakoid function. An ability to maintain photosynthesis during and after a heat stress event is therefore a good indicator of heat tolerance as this will correlate with plant growth (Al-Khatib and Paulsen 1990). A more recent study by Zhao et al.(2007) investigated the effect of high temperature (34oC/22oC day/night), post-anthesis, on leaf senescence and grain fill in wheat. They found that, compared to optimum, high temperature treatment reduced soluble protein concentrations and SPAD values (chlorophyll content) in flag leaves.

Liu and Huang (2000) presented results showing that the loss of chlorophyll is usually attributable to membrane damage and leaf senescence. This was possibly due to the production of highly reactive active oxygen species (Camejo et al. 2006), which act on membrane lipids, causing loss of function, a typical stress response in plants (Levitt 1972). Weigand & Cuellar (1981) noted that persistence of chlorophyll in leaves, awns and glumes was mainly affected by temperature, resulting in temperature

control over grain fill duration. Similar results were found by Zhao *et al.*(2007), who concluded that reduced kernel weight due to heat stress treatment was likely the result of a shorter grain filling period.

Chlorophyll fluorescence is the ratio of variable fluorescence to maximum fluorescence (F_v/F_m), which Yamada *et al.* (1996) found to be a physiological parameter correlating with heat tolerance in the leaves of 23 different tropical and one temperate fruit tree species. F_v is a measure of PSII activity (Harding *et al.* 1990), with Camejo *et al.* (2005) identifying this process was greatly reduced or even ceased under high temperatures, leading Kadir *et al.* (2007) to conclude that changes in F_v/F_m ratio under heat stress are potentially a good indicator for screening heat tolerant cultivars of grapes. Increasing chlorophyll α-fluorescence, an indicator of increasing heat stress and its relationship to chlorophyll content was examined by Ristic *et al.* (2007). These authors identified a strong correlation between these two traits, concluding that the measurement of chlorophyll loss, using a hand held SPAD meter, could be a rapid and reliable indicator of heat stress susceptibility in wheat under these conditions.

1.4.5 The effect of elevated night temperatures

A further topic that has received little research effort to date is the impact that high night temperatures can potentially have on bread wheat production. During the night, wheat plants respire, a process essential for utilising carbon fixed during daylight through photosynthesis, to release energy for normal cellular processes. However, respiration rapidly increases with increasing temperature and carbon metabolism can exceed fixation (photosynthesis), resulting in reduced plant growth, slower or ceased grain fill, reduced spikelet fertility due to reduced sugar availability and ultimately lower grain yield (Prasad *et al.* 2008).

1.4.6 Combined effects of drought and heat stress on wheat

A close review of the literature related to the identification of drought related traits reveals few authors consider high temperatures (i.e. greater than 25ºC) to be a factor when analysing data. However, there

has recently been work conducted aiming to separate the effect of heat and drought on various plant and grain development processes, as well as to investigate the effect of the two combined factors.

Hassan (2006) investigated the effect of heat and water stress on net photosynthetic rate, stomatal conductance, chlorophyll fluorescence and some yield components. Heat and drought stress and the two stresses combined when applied to wheat plants grown in pots, reduced net photosynthesis by up to 66% compared to the control. Stomatal conductance and subsequent leaf CO_2 concentrations were also reduced significantly compared to the control. Nicolas *et al.* (1984) also found that when combined with drought treatment, heat stress could impact yield by reducing the storage capacity for starch within the grain, as well as shortening the duration of the grain fill period, the latter possibly being advantageous for drought escape (Wardlaw 2002).

1.5 Key physiological traits conferring tolerance of wheat to heat and drought stressed conditions

1.5.1 Osmotic adjustment

Osmotic adjustment (OA), also referred to as osmoregulation, is the maintenance of cell osmotic potential, by accumulation of solutes, despite declining water potentials outside of the cell (Hellebust 1976). OA maintains cell turgor, allowing normal stomatal operation. Consequently, transpiration and photosynthetic processes are not disrupted and the plant is able to function despite the onset of water stress. As such, OA prevents premature leaf senescence, subsequently allowing greater translocation of assimilates during grain fill and improved yield (Blum *et al.* 1999). However, it should be noted that if cell turgor is maintained under water limiting conditions, there is the potential that stomatal closure and other water loss preventative measures by the plant may be delayed. The result would be increased water loss and as such, a greater loss of plant water potential. Flower *et al.*(1990) suggested that OA within roots and root tips would be more beneficial than in leaves, allowing deeper root penetration in order to exploit water stored further down the profile. This is in contrast to most studies where OA has been studied in a variety of above ground plant components.

Serraj and Sinclair (2002) concluded, following a review of published research, that there was a significant positive effect of OA on yield. However, these authors suggested that the drought stresses imposed to identify this advantage was so severe that the yields were too low to be of any value in a commercial farming situation. However, in a severe drought situation, this yield, although very low, would be better than complete crop failure. There is also some evidence that osmoregulation may allow a plant to respond more rapidly to water availability following water stress (Nayyar 2003). In addition, it could also be a useful trait for preventing anther desiccation and therefore maintaining fertility under stress conditions (Morgan 1980a).

1.5.2 Transpiration efficiency

Approximately 1% of carbon in atmospheric CO_2 exists as the carbon isotope ^{13}C, which is heavier than the prevalent form, ^{12}C. Plants discriminate in favour of $^{12}CO_2$, resulting in relatively less ^{13}C in plant dry matter (Condon *et al.* 1987). The level of discrimination is determined by the intercellular (controlled by stomata) and atmospheric partial pressures of CO_2 and the ratio (Δ) of $^{13}C/^{12}C$ in plant material, relative to the value of the $^{13}C/^{12}C$ ratio in the air that plants use in photosynthesis. It is therefore considered as a measure of the efficiency of transpiration and photosynthesis (Richards *et al.* 2001). A small Δ value is associated with high transpiration efficiency resulting from more closed stomata and can be seen as an indicator of improved water status (Araus *et al.* 2002; Araus *et al.* 2003). The value of Δ correlates negatively with transpiration efficiency and positively with stomatal conductance, which is also associated with transpiration, reflecting the water status of the plant. Under water stress conditions, this reflects the relative ability of the plant to access and utilize scarce soil water (Slafer *et al.* 2005). Recently, Sinclair (2012) argued that the measurement of transpiration efficiency, through Δ, was the culmination of many plant processes, much like grain yield and that the focus should instead be on traits contributing to greater transpiration efficiency.

1.5.3 Non structural carbohydrates

The basic units for stem storage of carbohydrates are often analysed as total non structural (TNS) or water soluble carbohydrates (WSC). These refer to carbohydrates, ranging from simple sugars such as glucose and sucrose through to more complex, high molecular weight oligosaccharides like fructans (Wardlaw and Willenbrink 1994), which accumulate in the stem during pre-anthesis growth. The amount present can be highly dependent upon the growing conditions experienced at this stage. Stem reserve remobilization is a constitutive trait, but under drought or heat stress conditions, it has a greater relative contribution to grain filling (Blum 1998; Wardlaw and Willenbrink 1994). Under droughted conditions, Virgona and Barlow (1991) found that the proportion of simpler sugars to fructans increased, particularly in the peduncle, indicating that more sucrose and readily available sugars were being mobilised to the developing grain. Wardlaw and Willenbrink (1994) found that most storage occurred in the peduncle and the penultimate internode.

The contribution of stem reserves to final kernel weight is determined by sink size, environment and genotypic factors (Blum 1998). As such, different authors are going to reach different conclusions about the relative usefulness of stem reserve mobilization based upon the analysis of different cultivars and their exposure to varying degrees of abiotic stress. Despite these differences, many authors have concluded that stem reserve mobilization is an important trait in increasing or maintaining kernel weight and therefore grain yield in cereals under stress conditions (Blum 1998; Blum *et al.* 1994; Dreccer *et al.* 2009; Gebbing and Schnyder 1999; McIntyre *et al.* 2010; Ruuska *et al.* 2006; Tahir and Nakata 2005; Yang *et al.* 2004).

1.6 Key hormonal responses of wheat to heat and drought stressed conditions

1.6.1 Abscissic acid

The hormone abscissic acid (ABA) plays a role in drought avoidance for almost all plants, being an important part of the regulation of plant water relations. It is rapidly accumulated in the leaves following a fall in turgor pressure of leaf and root cells and directs stomatal closure, thereby reducing transpiration and photosynthesis and slowing further water loss (Gupta 2005). However, at growing points it can

inhibit cell division and expansion, reducing growth, whilst accumulation in the ear between meiosis and anthesis can significantly affect pollen viability and subsequently, grain set (Ji *et al.* 2011; Morgan 1980b; Morgan and King 1984). Loss and Siddique (1994) therefore suggested that high ABA lines can have a negative effect on yield, suggesting greater pollen tolerance to ABA should be found and exploited. Ji *et al.* (2011) found that a number of genes related to a more rapid rate of ABA metabolism had increased expression in response to drought stress, improving spikelet fertility, further implicating the role of ABA in the drought response.

1.6.2 Ethylene

Ethylene is involved with a number of plant pathways, including leaf and flower senescence, root hair growth and seed germination. However, under heat and drought stress, ethylene production can be inhibited (Narayana *et al.* 1991), permitting accumulation of 1-amino-cyclopropane-1-carboxylic acid (ACC), the precursor to ethylene production. However, Narayana *et al.* (1991) only noted the decrease in ethylene production under stress conditions and did not measure ACC levels, leading them to conclude that the role of ethylene in the wheat plants' response to these conditions had previously been overstated. Larkindale and Huang (2005) presented results illustrating how creeping bent grass' ethylene production was inhibited at high temperatures but recommenced upon recovery. Different species exhibit different threshold levels for this – wheat ethylene production was severely inhibited at 40oC, while in soy bean, production was severely inhibited at 45oC (Arshad & Frankenberger, 2002). Upon return to cooler temperatures, this accumulation of ACC is converted to ethylene, resulting in a two to five fold increase in levels of ethylene (Balota *et al.* 2004), causing damage to plant structures and the induction of premature senescence.

Hays *et al.* (2007) investigated ethylene production during and after a heat stress event, identifying that the heat susceptible cultivar studied had a 6 fold increase in ethylene production within 4 hours of commencing heat stress (38oC/25oC day/nights). In contrast, the heat tolerant line did not experience a significant increase in ethylene production at any stage during or after heat treatment. This response in the heat susceptible cultivar resulted in a significantly lower kernel weight and kernel number per spike.

To prove the role of ethylene in these findings, the authors applied an ethylene inhibitor, which prevented the significant reduction in kernel weight and grain number in the susceptible cultivar. Numerous other authors have also taken this approach to highlight the role of ethylene in heat stress recovery (Balota *et al.* 2004; Chen *et al.* 2002; Narayana *et al.* 1991), all with similar results to Hays *et al.* (2007).

1.7 Key morphological traits of wheat conferring tolerance to heat and drought stressed conditions

1.7.1 Glaucousness

Glaucousness refers to the production of epicuticular wax on leaf surfaces of a plant, often causing a white to blue-white colour. In glaucous genotypes of wheat, the amount of wax on leaves increases rapidly from seedling emergence through to flag leaf emergence on sheaths and just prior to anthesis on leaves (Richards *et al.* 1986). Glaucousness increases radiation reflectance and reduces leaf temperatures and transpiration thereby increasing leaf survival under water stress and improving WUE (Febrero *et al.* 1998; Richards *et al.* 1986). However, the precise value of this trait on biomass production and grain yield remains somewhat uncertain, with Clarke *et al.* (1993) identifying high levels of flag leaf glaucousness not necessarily being correlated with physical wax content on the leaf surface. Further to this, Simmonds *et al.* (2008) found that the viridescence (absence of visible epicuticular wax) phenotype can be higher yielding than a glaucous phenotype in certain environments and Merah *et al.* (2000) identified lower biomass production in high-glaucous genotypes, creating some uncertainty around the precise value of this trait.

1.7.2 Canopy structure

The primary function of the leaf is to intercept radiation to drive photosynthesis and other metabolic processes (Fageria *et al.* 2006). In addition to glaucousness, there are a number of other leaf traits that can potentially lower the radiation load on the leaf surface, in addition to lowering the rate of evapotranspiration and the chance of photoinhibition occurring (Reynolds *et al.* 2006). Craufurd *et*

al.(1999) found that thicker leaves had greater photosynthetic capacity due to higher concentrations of chlorophyll per unit leaf area.

Leaf rolling and erect plant posture both increase total leaf area exposed to light (Innes and Blackwell 1983). Erect leaves allow for a greater penetration of light through the canopy and therefore the capacity to maintain plant photosynthetic capacity, whilst reducing radiation loads on individual leaves (Reynolds *et al.* 2006). Fageria *et al.* (2006) suggested that erect leaves appear to be a pleiotropic effect as a result of the adoption of dwarfing genes. It is therefore a highly heritable trait, so visually easy to score.

Leaf size is directly related to leaf angle, with shorter leaves being easier to support than longer leaves, which tend to droop. Fageria *et al.* (2006) found that shorter leaves were also more evenly distributed through the canopy, resulting in less shading and more efficient use of available sun light. Further to this, narrower leaves are more desirable than wider ones, again allowing more even distribution through the canopy.

1.7.3 Canopy temperature

Utilisation of canopy temperature measurement in wheat using infrared thermometry was first realised as a prediction of crop water status in the late 1970s (Blad and Rosenberg 1976; Idso *et al.* 1977; Jackson *et al.* 1977; Millard *et al.* 1978). However, Blum *et al.* (1982) first recognised the potential that this technique had as a screening methodology to identify cultivars with improved water stress tolerance in wheat. However, it is only relatively recently that developments in infrared thermography technology have permitted feasible and cost effective screening equipment and methodology to be developed (Amani *et al.* 1996; Munns *et al.* 2010). However, there does not appear to be any reports of selection for this trait in commercial breeding programs to date.

Reynolds *et al.* (2007) and Saint Pierre *et al.* (2010) found that genotypes able to maintain a cooler canopy under drought and heat stress conditions were those that had increased rooting depth and were

therefore able to access greater amounts of the limited soil water. Measurement of canopy temperature or the canopy temperature depression from ambient has therefore been proposed as an efficient selection criterion for drought and heat tolerance. However, few authors appear to acknowledge the conditions required to measure this trait accurately. Olivares-Villegas *et al.* (2007) highlighted the difficulties in measuring this trait in field trials in the Mediterranean type climate of southern Australia, citing relatively low soil moisture levels, gusty winds and cloud cover as issues preventing accurate measurement. However, there is no doubt that this trait is able to account for significant proportions of the variance for grain yield in certain environments (Pinto *et al.* 2010).

1.7.4 Spike photosynthesis

Where extreme drought stress has induced senescence within the leaf canopy of a cereal plant, spike photosynthesis can contribute up to 38 percent of total carbon fixation under drought conditions (Bort *et al.* 1994). Bort *et al.* (1994) also found that covering the flag leaf of barley during grain fill did not have a significant effect on yield; yet covering the spike resulted in a significant reduction. Maydup *et al.* (2010) found that the glumes, lemmas and awns of bread wheat were able to maintain higher relative water content under water and heat stress conditions, allowing photosynthesis to continue functioning. Reynolds *et al.* (2006) suggested that greater levels of spike photosynthesis could be a highly complementary trait to stem reserve remobilization under post anthesis water stress, as both traits are constitutive and directly contribute carbon to grain filling.

1.8 Genetic improvement of wheat for abiotic stress tolerance

1.8.1 Genetic markers

The use of markers within plant breeding is not a new principle; indeed as early as 1923, researchers were aware that selection for one trait could improve another (Sax 1923). This and other similar works fall into the category of morphological markers, where selection for one phenotype results in the improvement of another due to linkage between the genes controlling the two phenotypes. These markers have been utilized for a relatively long time but recent advances have been in the development

of DNA or molecular markers. This refers to a unique genetic marker associated with a specific genomic location.

Molecular markers can be a useful tool within breeding programs as they can be used to indirectly select genes controlling a trait of interest. To achieve this, the marker must be linked to the gene and able to discriminate for the desired allele i.e. polymorphic. This enables the gene/s controlling a trait to be directly selected for, instead of relying on phenotypic assays. Applying this selection strategy (marker assisted selection; MAS) to a breeding program can be useful for improving the rate of genetic gain in situations where a trait:

- is of low heritability;

- requires larger quantities of grain to phenotype (such as quality), which is not always available in early generations or a trait is particularly expensive to phenotype;

- requires special conditions to express the presence of a gene (such as disease inoculation); and/or

- is recessive

MAS has previously been found to be an economically viable method of improving the rate of genetic gain for quantitatively inherited traits (Dubcovsky 2004; Howes *et al.* 1997; Kuchel *et al.* 2007a), particularly now that DNA assays are generally cheaper than phenotypic assessment.

1.8.2 Quantitative Trait Loci analysis

Quantitative traits refer to those phenotypes which have measureable continuous in variation, are under polygenic control and therefore do not follow a Mendelian pattern of inheritance. Quantitative trait loci (QTL) are regions of the genome controlling such a trait, the alleles of which contribute to variation. To maximise the chance of detecting such regions, appropriate populations need to be developed, segregating for genes controlling the trait of interest, including bi-parental doubled haploid or recombinant inbred populations. A map of polymorphic markers covering a majority of the genome also needs to be established, with markers forming a linkage map. The phenotypic performance of the

genotypes within the population is then used to statistically detect genomic regions associated with that trait.

Table 1.8.2-1. The number assigned to each study used in Figure 1.3.1-1, including reference and species investigated. Full citations can be found in the references.

Study number	Reference	Species
1	Carter *et al*. (2011)	Bread Wheat
2	Cutherbert *et al*. (2008)	Bread Wheat
3	Golabaldi *et al*. (2011)	Durum Wheat
4	Groos *et al.* (2003)	Bread Wheat
5	Habash *et al*. (2006)	Bread Wheat
6	Huang *et al*. (2006)	Bread Wheat
7	Kuchel *et al*. (2007)	Bread Wheat
8	Kumar *et al*. (2007)	Bread Wheat
9	Marza *et al*. (2006)	Bread Wheat
10	Mason *et al*. (2010)	Bread Wheat
11	Mason *et al*. (2011)	Bread Wheat
12	McCartney *et al*. (2005)	Bread Wheat
13	McIntyre *et al*. (2010)	Bread Wheat
14	Naruoka *et al*. (2012)	Bread Wheat
15	Peleg *et al*. (2009)	Durum Wheat
16	Pinto *et al*. (2010)	Bread Wheat
17	Rebetzke *et al*. (2008)	Bread Wheat
18	Rebetzke - personal communication	Bread Wheat
19	Sun *et al*. (2009)	Bread Wheat
20	Sun *et al*. (2010)	Bread Wheat
21	Tsilo *et al*. (2010)	Bread Wheat
22	Tuberosa *et al*. (2008)	Durum Wheat
23	Vijayalak *et al*. (2010)	Bread Wheat
24	Wu *et al*. (2011)	Bread Wheat
25	Yang *et al*. (2007)	Bread Wheat
26	Zhang *et al*. (2010)	Bread Wheat

1.8.3 Genomic regions associated with grain yield, yield components and physiological traits under drought and heat stress across multiple studies

QTL mapping has been rapidly adopted as an approach to dissect the genetic basis of quantitative or continuous class traits. To date, there have been many reports of QTL across the bread wheat genome associated with the expression of the drought related traits discussed in the previous sections, as well as other yield components. Figure 1.3.1-1 outlines a summary of 26 studies (listed in Table 1.8.2-1) that

have dissected grain yield, yield components and drought related traits by QTL analysis (full list of traits in Table 1.8.3-1). These clusters of QTL have been assembled based on the markers associated with each locus within each study and their estimated position according to the consensus molecular marker map of Somers *et al.* (2004). In a number of studies, some QTL were located near markers not published by Somers *et al.* (2004), linkage maps developed using wholly unpublished markers or markers were not named under the same convention. Therefore, their approximate consensus position could not be identified and they were not included in this summary. However, of particular interest are those regions where there are no previous reports of height reducing or ear emergence time genes, such as the Rht, Ppd and Vrn series of genes (indicated in Figure 1.3.1-1). These can have a pleiotropic effect on many traits, particularly when measured under conditions suffering from abiotic stress.

1.8.3-1 Group 1 chromosomes

Numerous loci were reported on chromosome 1A to be influencing grain yield although most of these were in single studies. The exception was around 65 centi Morgans (cM) distal to the end of the short arm, where three studies detected QTL for grain yield, kernels per spike, WSC and heat stress index of kernel weight (Marza *et al.* 2006; Mason *et al.* 2010; Yang *et al.* 2007). On chromosome 1B, a region around 30 cM distal to the end of the short arm harboured a grain yield QTL in 3 different studies (Kuchel *et al.* 2007b; Marza *et al.* 2006; Tsilo *et al.* 2010), where QTL have also been detected for grain fill duration (Mason *et al.* 2010; Yang *et al.* 2002), grain size (Sun *et al.* 2010), as well as spikes/m^2 and biomass in durum (Golabadi *et al.* 2011; Peleg *et al.* 2009). Cuthbert *et al.* (2008) detected a minor time to ear emergence (EET) locus in this region, but the studies where QTL were detected for grain yield had measured this trait and did not detect QTL for EET there. Kuchel *et al.* (2007c) indicated that the yield QTL they detected was responsive to heat stress, implicating this locus as important for production under abiotic stress. A second locus on 1B, at approximately 60 cM distal to the end of the short arm, Wu *et al.* (2011) detected a QTL for grain yield and other studies detected QTL for traits such as grain size, heat stress index of grain size, spikes per plant, spikes/m^2 and kernels per

Table 1.8.3-1. Trait abbreviations used in Figure 1.3.1-1

Abbreviation	Trait
AnthBio	Anthesis Biomass
Bio	Biomass at harvest
CT	Canopy Temperature
DELTA	Carbon isotope discrimination
Ears/plant	Spikes per plant
EET	Ear emergence time (heading)
FLL	Flag leaf length
FLW	flag leaf width
Fv/Fm	Chlorophyll flouresence ratio
GFD	Grain fill duration
Wax	Glaucousness
GPM	Grains per metre sq
GrainWt/plant	Grain weight per plant
GrainWt/spk	grain weight per spike
Green	Green leaf area duration
HI	Harvest Index
HSIGFD	Heat stress index grain fill duration
HSIKern	Heat stress index kernel number per spike
HSITKW	heat stress index thousand grain weight
HT	height
KernWt/Pl	kernel weight per plant
Kern/spk	kernels per spike
Kernel D	kernel diameter
Kernel L	kernel length
Kernel W	kernel width
KPM	kernels per metre sq

Abbreviation	Trait
MAT	physiological maturity
M-Yld	MET QTLs
MaxSen	Maximum senescence rate
NDVI	NDVI
OsP	Osmotic potential
Ped	peduncle length
Prot	grain protein content
SCR	Grain screenings percent
SHAT	shattering score
SL	spike length
Spk/m2	spikes per metre sq
SpkHI	spike harvest index
Spklts/Spk	spikelets per spike
SpkWt	spike weight
SSIheat	stress susceptibility index heat
TKW	Thousand grain weight
TPP	tillers per plant
TWT	Test weight
WSC	Water soluble carbohydrates (various measures)
YLD	Grain yield (various units)

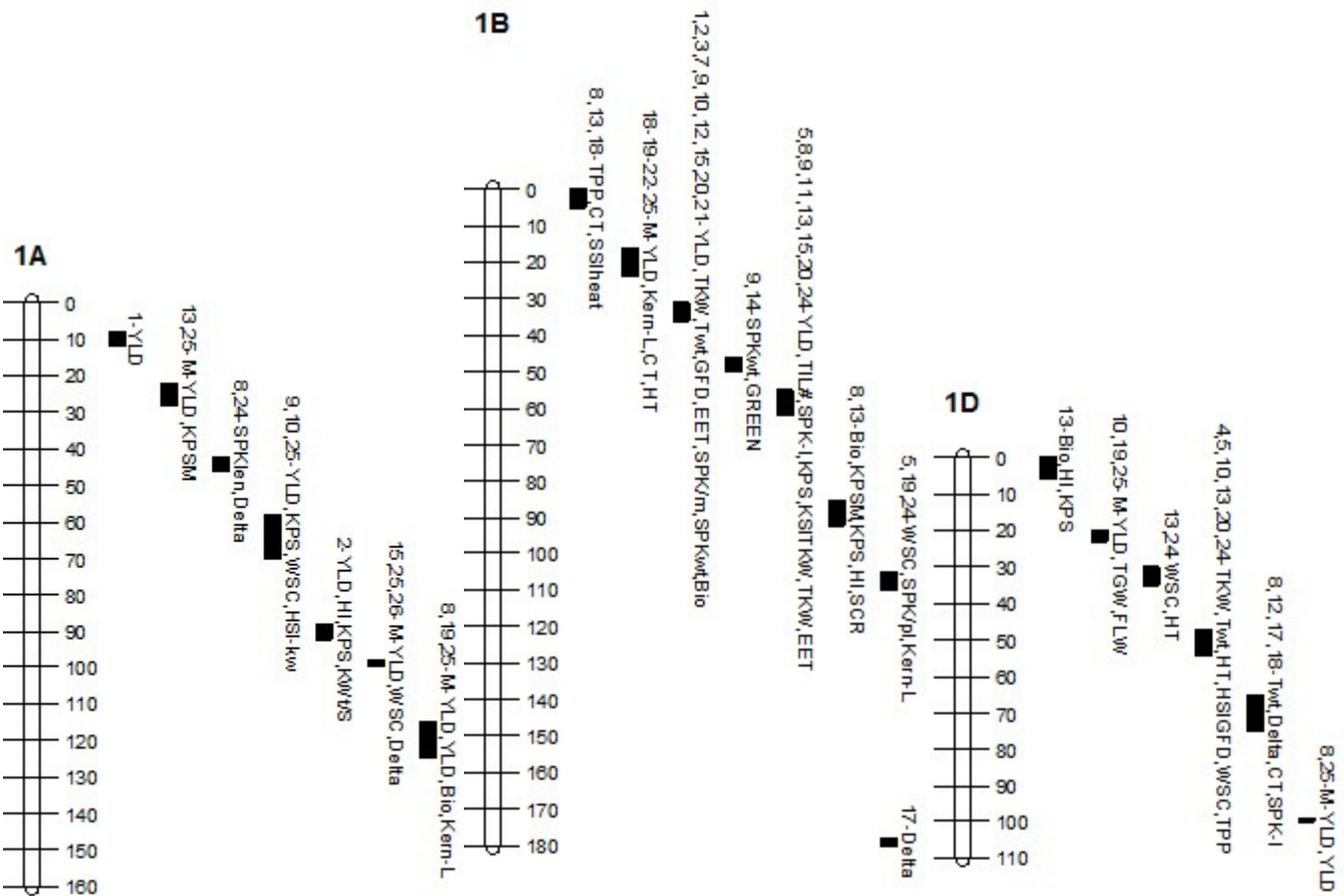**Figure 1.3.1-1 Positioning of QTL for a wide variety of traits within wheat (abbreviations and full list of traits in Table 1.8.3-1), detected by 26 different studies (see Table 1.8.2-1), according to the consensus map of Somers *et al*. (2004). Chromosome length (left hand side) is in centiMorgans and the 'name' of each region consists of the numbers corresponding to the studies where QTL for the subsequent traits were detected.**

per spike (Habash *et al.* 2007; Marza *et al.* 2006; Mason *et al.* 2011; McIntyre *et al.* 2010; Sun *et al.* 2009). Peleg *et al.* (2009) detected a minor EET locus but again, this effect was not observed by any other studies.

1.8.3-2 Group 2 chromosomes

The photoperiod sensitivity loci are located on the group 2 chromosomes, implicating them as important regions for adaptation and drought avoidance. The alternate semi dwarf gene *Rht-8* is also located close to *Ppd-D1a* on chromosome 2D and unless time to ear emergence or plant height have been measured and discounted as possible causes, these should be considered like factors. Chromosome 2A contained interesting QTL cluster around 50 cM distal to the end of the short arm for grain yield, CID, WSC and tillers per plant (McCartney *et al.* 2005; Peleg *et al.* 2009; Wu *et al.* 2011; Yang *et al.* 2007), but this is in a similar region to Ppd-A1 and may explain these observations. Another interesting region was on the long arm of the same chromosome, where Vijayalakshmi *et al.* (2010) detected many QTL for accelerated flag leaf senescence rates under heat stress and Peleg *et al.* (2009) detected a locus for Osmotic potential, a trait that has been hypothesised to minimise flag leaf senescence under stress conditions.

Chromosome 2B contains many rust resistance genes and so field based studies could potentially interpret resistance or susceptibility to rust as loci of interest due to the pleotropic effects on most traits. All studies considered in this review generally maintained disease free experiments and therefore, this should not have been an issue. With these potentially confounding factors, it is not surprising that numerous QTL of minor effect were reported in many regions along chromosomes 2B and 2D, but none appear to have been consistently detected.

2A

4,10,15,26-M-YLD,Prot,OsP,GFD,HSI,KKPS

8,22-YLD,Bio,EET

2B

3,5,9,13,15-YLD,SPK/m,HI,Gwtbpl,GwtbSpk,OsP

1,3,5,6,8-TKW,HI,LeafCol,SpkVSpk,Spk/pl

9,10,12,13,17,25-M-YLD,YLD,TKW,FLW,Delta,EET

8,12,15-Twt,KPS,Pdl

4,8,9,13,15,21,25-M-YLD,TKW,Prot,Twt,HT,Bio

5,8,16,19-TKW,Bio,CT,Kern-L

8,22,25-M-YLD,KPS,EET

15-YLD,HI,Bio,EET

9,22-HT

22-YLD

17,18,24,25-WSC,Delta,CT,TPP

12,15,24,25,26-M-YLD,Delta,WSC,TPP

15,18-HI,CT

17,25,26-M-YLD,WSC,Delta

8,10-KPS,HSITkw

19-Twt,TKW,Kern-W

15,23-LeafSen,OsP

2D

4,9,11-TKW,HSI,Tkw,SpkWt

1,6,8,9,11,25-M-YLD,KPS,TGW,Twt,HSI,Tkw,HI,HT,EET

10,11,14,21-GFD,Green,EET

24,25-M-YLD,WSC

2,8,12-YLD,GFD,HI,HT

2,9,24,25-M-YLD,KPS,TKW,WSC,EET

1,8,17,18,24,25,26-M-YLD,WSC,TKW,CT,Delta

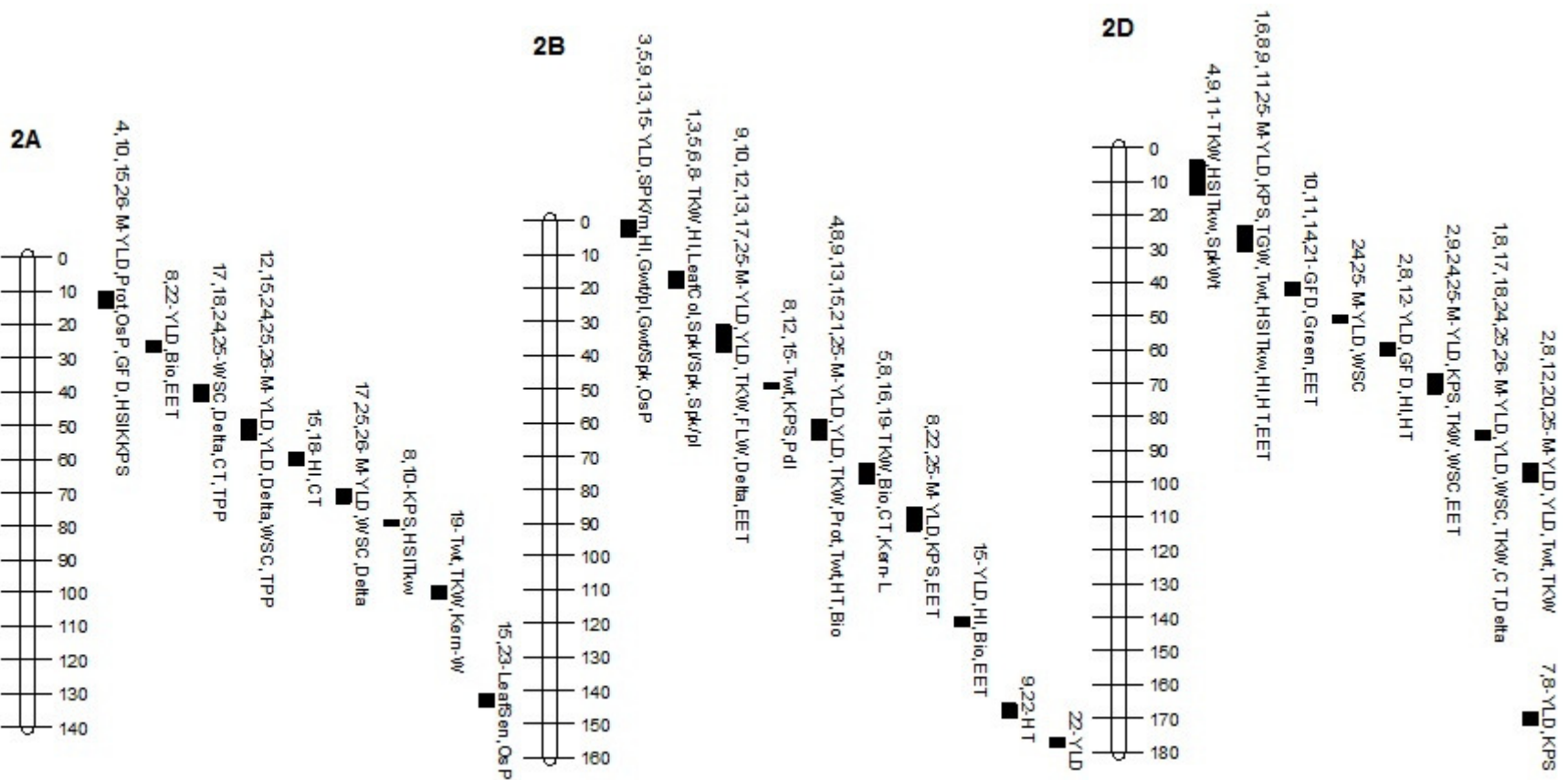2,8,12,20,25-M-YLD,YLD,Twt,TKW

7,8-YLD,KPS

Figure 1.3.1-1. (cont.)

1.8.3-3 Group 3 chromosomes

A number of rust genes have been detected on this group but far fewer than have been identified in the group 2 chromosomes. The short arm of chromosome 3B also contains the alternate semi dwarf gene Rht-5, which appears to have been detected by Carter *et al.* (2011), Maccaferri *et al.* (2008) and Marza *et al.* (2006). However, there is a region on 3B distal to this, where three different studies detected QTL for kernels per spike (Habash *et al.* 2007; Marza *et al.* 2006), and notably under heat stress (Mason *et al.* 2010), as well as a QTL for kernel weight (Golabadi *et al.* 2011), which could be expected to have an inverse relationship to greater kernels per spike. Zhang *et al.* (2010) also detected two MET-QTL in this region for grain yield, indicating that it is important region across studies for grain yield. A second region of interest on this chromosome is between 50 and 60 cM distal to the end of the short arm (Figure 1.3.1-1), where QTL for a range of traits were detected. Chromosome 3D contained 2 loci for grain yield but these were detected in single studies.

1.8.3-4 Group 4 chromosomes

Chromosomes 4B and 4D contain the major height reducing genes *Rht-B1* and *Rht-D1* (Ellis *et al.* 2002), respectively, and given the major effect that these genes have on plant height, they can be expected to exert significant pleiotropic or more likely, directly related effects over a range of traits, where they are segregating in a mapping population. Located on the short arm of these chromosomes, large numbers of studies detected QTL for many different traits, particularly the 15-40 cM region on 4B and 10-20 cM region on 4D (Figure 1.3.1-1). On chromosome 4A, a number of regions contained multiple QTL for grain yield and one region on the short arm was co-located with QTL for WSC and carbon isotope discrimination. Pinto *et al.* (2010) detected a second major QTL on this chromosome for grain yield and canopy temperature under heat stressed conditions but due to the marker types on that region of their linkage maps, could not be anchored on the consensus map. The long arm of chromosome 4D contained two regions where QTL for grain yield were detected (Habash *et al.* 2007; Kumar *et al.* 2007), although no physiological or yield characteristics co-located here.
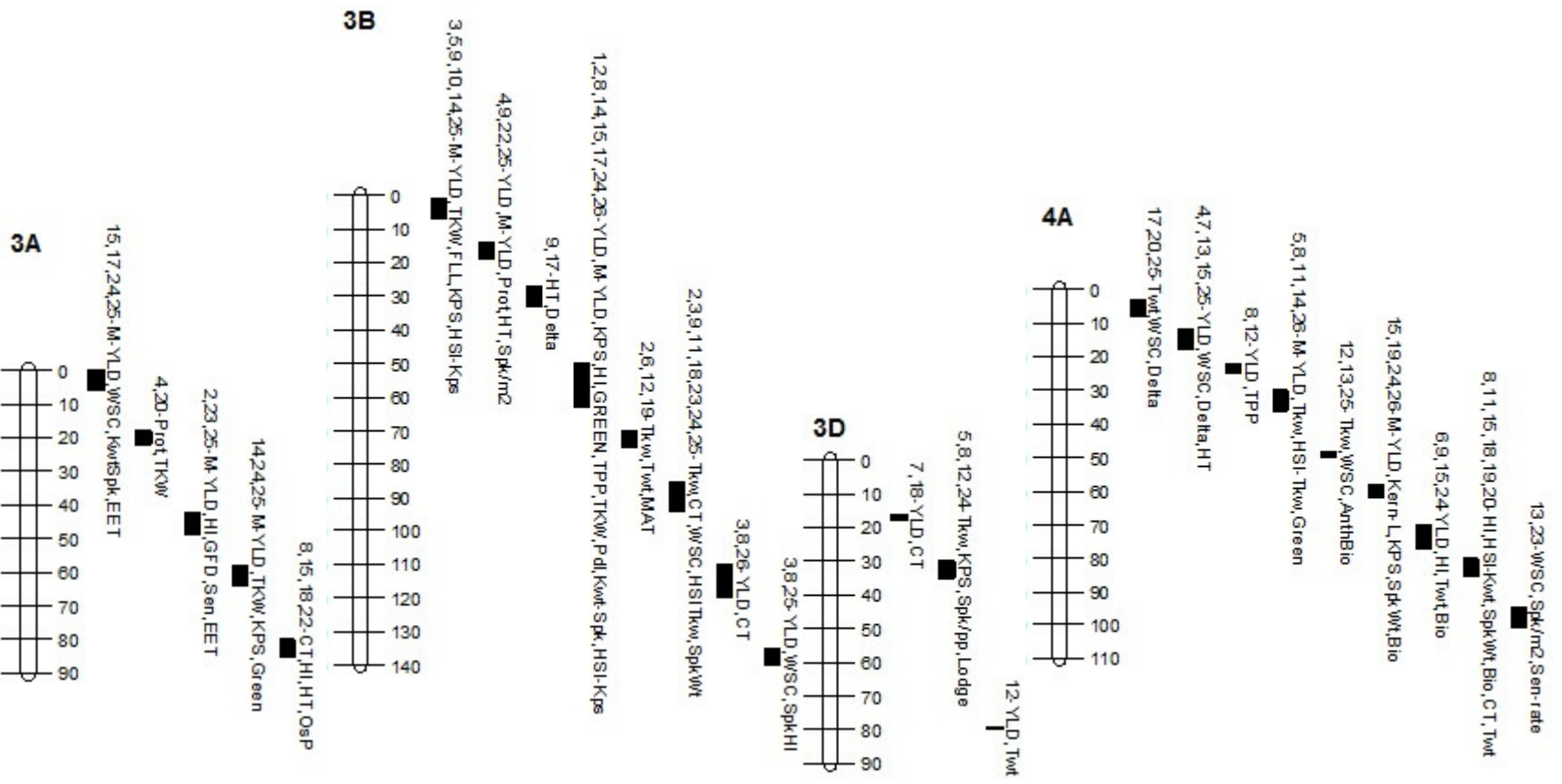
Figure 1.3.1-1. (cont.)

1.8.3-5 Group 5 chromosomes

The group 5 chromosomes are home to of the major vernalization (Vrn) series genes (Yan *et al.* 2006; Yan *et al.* 2004) and like the *Ppd* and *Rht* genes, can exert major effects when segregating in a mapping population, particularly under abiotic stress conditions (Richards *et al.* 2010). Additionally, chromosome 5A has two alternate semi dwarf genes, *Rht-9* and *Rht-12*; but none of the studies considered in this review reported QTL for height here. On chromosome 5A, around 40 cM distal to the end of the short arm, Zhang *et al.* (2010) detected a MET-Yield QTL and Marza *et al.* (2006) and Peleg *et al.* (2009) detected QTL for grain yield, although the QTL detected by Marza *et al.* (2006) could potentially be explained by the presence of a QTL for shattering. Nonetheless, Yang *et al.* (2007) detected QTL for WSC in a similar region and two further studies detected QTL for carbon isotope discrimination (Peleg *et al.* 2009; Rebetzke *et al.* 2006), around 100 cM distal to the end of the short arm; although there have been no reports of yield effects there. On chromosome 5B, at approximately 50 cM, Marza *et al.* (2006) detected a QTL for grain yield, which was in a similar region to the canopy temperature QTL detected by G Rebetzke (personal communication) and for carbon isotope discrimination (Rebetzke *et al.* 2008a), as well as QTL for HI, kernel weight/spike and spikes/square metre (Cuthbert *et al.* 2008). Approximately 70 cM distal to the end of the short arm, Kuchel *et al.* (2007b) detected a QTL for grain yield, although in an earlier study, also detected a minor earliness per se QTL (Kuchel *et al.* 2006). Interestingly though, QTL have been detected in a similar region for osmotic potential, canopy temperature and WSC, without detecting the same EPS effect (Peleg *et al.* 2009; Rebetzke *et al.* 2008b; Yang *et al.* 2007).
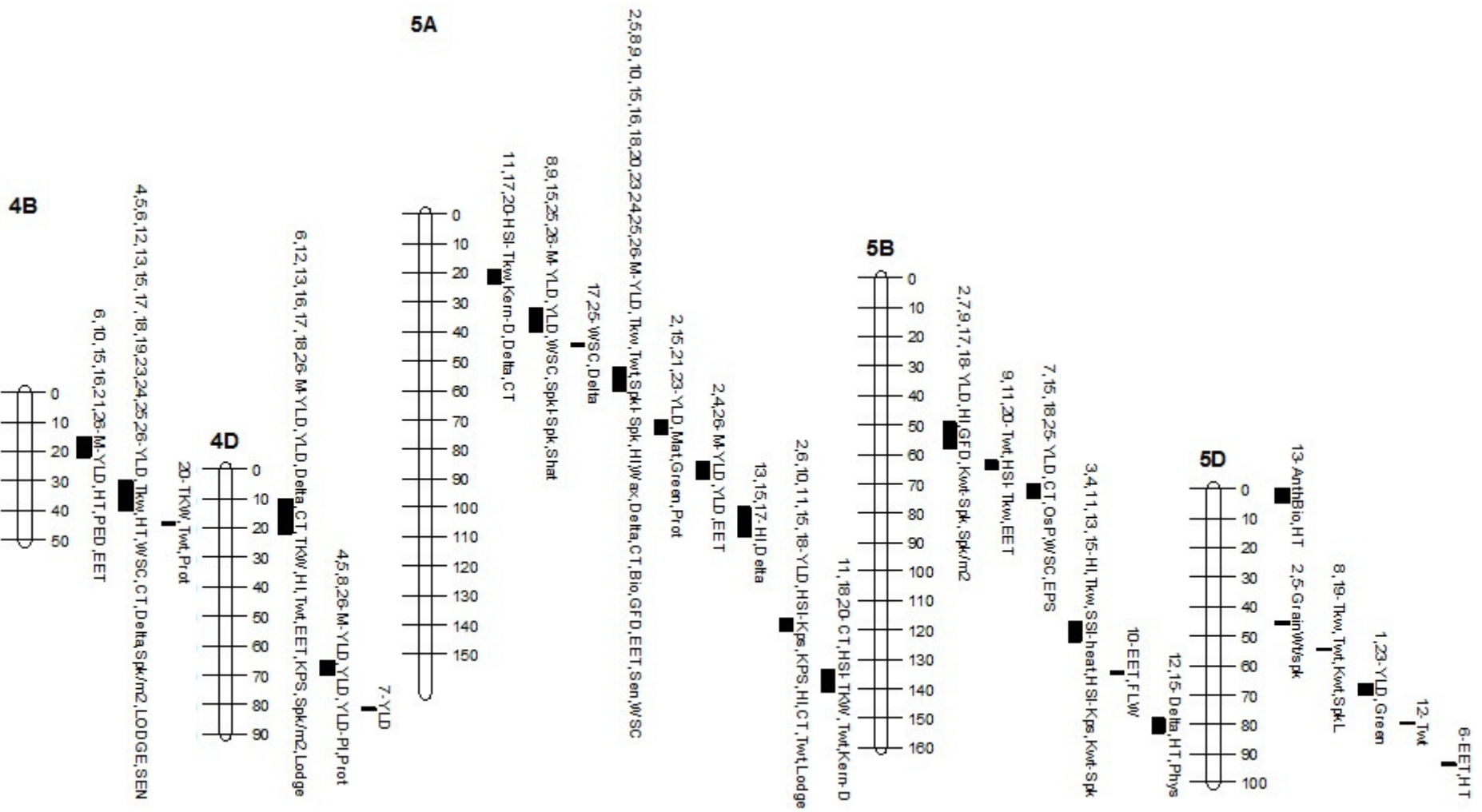
Figure 1.3.1-1. (cont.)

1.8.3-6 Group 6 chromosomes

On the short arm of chromosome 6A, there have been numerous reports of loci influencing WSC, screenings (small grains) and test weight (McIntyre *et al.* 2010; Sun *et al.* 2010; Sun *et al.* 2009; Yang *et al.* 2007). One area of great interest though is around 50 cM distal to the end of the short arm, where five different studies reported a QTL affecting kernel size (Groos *et al.* 2003; Huang *et al.* 2006; McIntyre *et al.* 2010; Sun *et al.* 2010; Wu *et al.* 2011) and subsequently, an influence on grains per square metre (McIntyre *et al.* 2010) and grain protein (Groos *et al.* 2003). Zhang *et al.* (2010) also reported a MET yield QTL in this region, making this region even more interesting. Su *et al.* (2011) reported a gene in this region for grain size (*TaGW2*) and this is potentially the gene segregating in the populations used in the above studies. However, the perfect marker developed by these authors would need to be screened across these populations to validate this hypothesis. A second region of interest is on the long arm of 6A, where 2 studies have detected QTL for harvest index (Kumar *et al.* 2007; Peleg *et al.* 2009) and one further QTL of large effect for WSC (Yang *et al.* 2007). Both Marza *et al.* (2006) and Wu *et al.* (2011) detected QTL for grain yield in the region around 40 cM on chromosome 6B, in a similar region to QTL for carbon isotope discrimination (Rebetzke *et al.* 2008a), Leaf senescence (Vijayalakshmi *et al.* 2010), kernel weight per plant and spikes per plant (Habash *et al.* 2007). Cuthbert *et al.* (2008) did report a QTL for ear emergence time and physiological maturity in the region, but this does not appear to have been detected by the former authors, indicating the presence of a further common region of interest. The final region of interest on the group 6 chromosomes is on 6D, again on the end of the short arm, where a yield QTL was detected by McIntyre *et al.* (2010) in two METs and grain size and heat stress index of grain size QTL were detected (Habash *et al.* 2007; Mason *et al.* 2010), indicating another potentially heat responsive region.
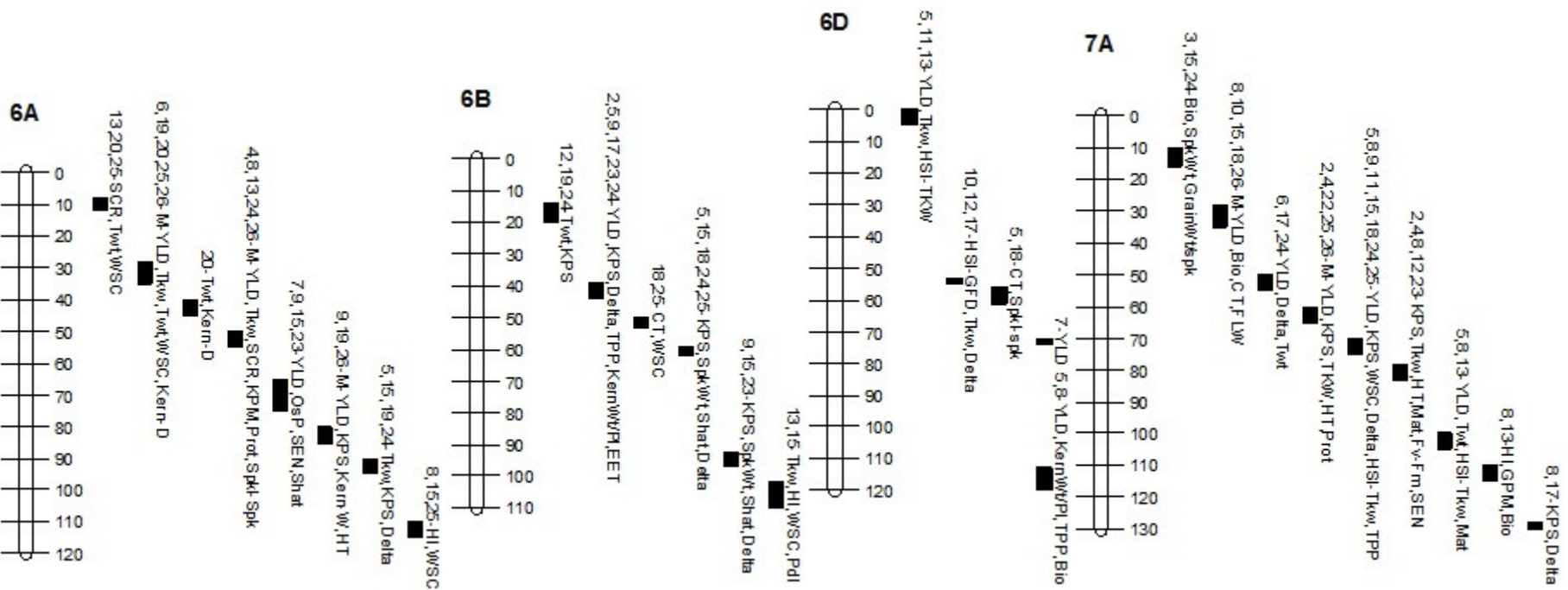
Figure 1.3.1-1. (cont.)

1.8.3-7 Group 7 chromosomes

In the region around 50 cM on chromosome 7A, Rebetzke *et al.* (2008a) reported QTL for carbon isotope discrimination in two separate populations and Wu *et al.* (2011) detected a QTL for the same trait in another population, indicating a common genetic region for this trait. Huang *et al.* (2006) detected a QTL for grain yield here also but it is also known that a Flowering Time locus (*FT*), previously known as a *VRN3* gene, is located in this region (Yan *et al.* 2006) and yet again, the pleiotropic effect of ear emergence time can probably explain the above observations. A second region around 70 cM, in a similar region to the centromere of chromosome 7A, QTL have been detected for grain yield, carbon isotope discrimination, WSC, kernels per spike, heat stress index of grain size and tiller per plant, all potentially related traits. Chromosome 7B also contains a FT gene on the short arm and this is highlighted by the detection of multiple ear emergence time QTL here, with pleiotropic effects on multiple traits including grain yield. Chromosome 7D contained multiple QTL for grain yield, with multiple time to ear emergence QTL detected on the short arm. One region around 100 cM distal to the end of the short arm contained a MET-Yield QTL (Zhang *et al.* 2010) and grain yield (Groos *et al.* 2003), with
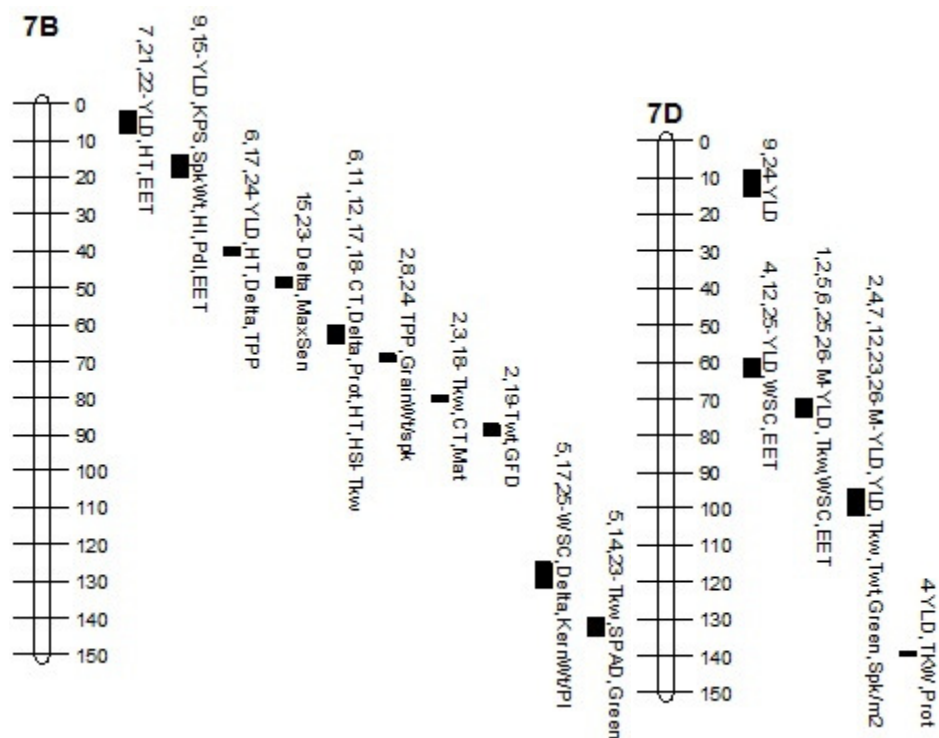
Figure 1.3.1-1. (cont.)

QTL for grain size, test weight, leaf senescence and spikes/square metre (Cuthbert *et al.* 2008; Kuchel *et al.* 2007b; McCartney *et al.* 2005; Vijayalakshmi *et al.* 2010), implicating a key region for grain yield and physical grain quality.

1.8.3-8 Summary of QTL mapping to date

In general, the genomic regions for grain yield common between multiple studies obviously arose from segregation in the respective populations for major loci influencing plant height and/or ear emergence time. The significance of this observation should not be dismissed; it highlights the importance of achieving the appropriate maturity in the target environment, particularly where water availability or heat stress is expected to be a limiting factor for production. Nonetheless, breeders have excellent understanding of the effect of most of these major genes and therefore, manipulation is relatively straight forward.

The interest in QTL analysis lies in identifying those regions not associated with these factors and shedding light on just how complex the genetic regulation of a given trait may be. Therefore, key regions of importance for grain yield, not associated with plant height or ear emergence time, appear to be on the group 1 and 2 chromosomes and chromosomes 3B, 4A, 4D, 5A, 6A, 6D, 7A and 7D. The trait with QTL most commonly co-located with yield QTL was WSC, further illustrating the importance of this trait for supporting grain yield under abiotic stress conditions (Blum *et al.* 1994; Rebetzke *et al.* 2008b; Yang *et al.* 2007).

However, failing to detect grain yield effects in regions where common genetic effects were found between studies and probably different germplasm should not be considered a negative result. The first factor that could have confounded the value of a specific locus is the complexities around the timing of and drought or heat stress in many studies, as discussed earlier in this literature review. The result may have been that the relative importance of a given trait at a specific growth stage can differ and lead

different studies to come to different conclusions about the value of that specific trait. In addition, the expression of specific QTL are known to have differing relative values depending on the genetic background that they are being expressed in (Pankova *et al.* 2008).

Different studies are also going to have varying ability to detect QTL of minor effect due to the heritability of grain yield (or any other measured trait) any experiments achieve. The heritability of a trait, or the proportion of the total variance for a trait that is attributable to the genetic variance is going to dictate the proportion of the genetic variance that any detected QTL can account for. Further compounding this problem is the fact that the total variance accounted for by QTL detected at a significance level of $p < 0.05$ or greater in most studies for grain yield and most related components is only around 40 percent or less (Breseghello and Sorrells 2007; Cuthbert *et al.* 2008; Kuchel *et al.* 2007b; Pinto *et al.* 2010).

1.9 Conclusions and priority topics for research

Bread wheat has historically been an important crop for human nutrition, with production across a range of climates. Locally, it is grown under frequently water limited and often heat stressed conditions, which limit productivity. However, it is clear that there is significant genetic variation present within bread wheat to maintain grain yield under drought and/or heat stressed conditions faced by plants in the southern Australian Mediterranean style climate. Many traits have illustrated value in bread wheat production under drought and heat stress, including water soluble carbohydrates, ethylene and abscisic acid metabolism, leaf and stem glaucousness, spikelet fertility and photosynthetic performance, among others. However, the variation for these; and their value under the southern Australian Mediterranean climate is not well understood. The development of a mapping population, with parents contrasting for yield performance under drought and heat stressed environments, but otherwise similar yield potential in optimum conditions should permit (i) determination of the actual value of a specific traits in increasing or maintaining superior grain yield under southern Australian production conditions and (ii) provide the framework for dissecting grain yield and any associated traits genetically, by QTL analysis. By reviewing

QTL dissection studies conducted to date, we will be able to compare the genetic basis of the same traits measured in this proposed study to that of the same trait investigated previously. The broad aim of this study is therefore to dissect and improve our understanding of the genetic basis of grain yield, physical grain quality and any specific traits of interest utilising field experiments across southern Australia, as well as in the managed environments of the International Centre for Wheat and Maize improvement (CIMMYT), Mexico.

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Chapter 2

Chapter 2. Research articles

Identification of novel quantitative trait loci for days to ear emergence and flag leaf glaucousness in a bread wheat (Triticum aestivum L.) population adapted to southern Australian conditions

Ear emergence time is a key stage in wheat development, as it is the key point of transition from vegetative to reproductive growth. In the Mediterranean type climate of southern Australia, the timing of this transition is very important, to minimise the risk of exposure to frost during anthesis and dry and hot conditions during anthesis and reproductive growth. Whilst the major genes controlling this trait are well understood, these do not explain all genetic variation for time to ear emergence. In addition to ensuring correct flowering time to maximise grain yield potential, numerous traits have been hypothesised to confer tolerance of wheat to drought and heat stress. One of these, flag leaf glaucousness, shows a high expression in RAC875 and was expected to be a key trait contributing to this elite genotypes' superior grain yield under drought and heat stressed conditions. This paper presents the genetic dissection of ear emergence time and flag leaf glaucousness in the 'RAC875/Kukri' doubled haploid population and the identification of the loci contributing to the variation for these traits within this population.

Identification of novel quantitative trait loci for days to ear emergence and flag leaf glaucousness in a bread wheat (Triticum aestivum L.) population adapted to southern Australian conditions

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Identification of novel quantitative trait loci for days to ear emergence and flag leaf glaucousness in a bread wheat (Triticum aestivum L.) population adapted to southern Australian conditions

Theoretical and Applied Genetics 2012; 124:697-711

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Phenotyped population in 2007 and 2008, performed statistical analysis on all data, interpreted data, wrote manuscript and acted as corresponding author

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Bennett, D., Izanloo, A., Edwards, J., Kuchel, H., Chalmers, K., Tester, M., Reynolds, M., Schurbusch, T. & Langridge, P. (2012) Identification of novel quantitative trait loci for days to ear emergence and flag leaf glaucousness in a bread wheat (*Triticum aestivum* L.) population adapted to southern Australian conditions.
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Chapter 2. Research articles

Genetic dissection of grain yield and physical grain quality in bread wheat (Triticum aestivum L.) under water limited environments.

In the past, wheat breeders have achieved reasonable rates of genetic gain for grain yield, which is a genetically and morpho-physiologically complex trait. In southern Australia, grain yield is frequently limited by dry and hot conditions, particularly after flowering, which negatively impact production. However, there is considerable genetic variation for grain yield under these conditions. RAC875 is a breeders' line that has been identified as able to achieve above average grain yield under droughted conditions, without penalty in more favourable conditions. By dissecting the genetic basis of this tolerance, key genomic regions can be tagged with molecular markers, utilised in marker assisted selection and improve rates of genetic gain for grain yield. With the loci controlling ear emergence time reported in the previous paper, it is possible to look for loci controlling grain yield *per se.* This paper reports a QTL dissection of grain yield, yield components and physical grain quality traits, under the challenging environmental conditions of southern Australia.

Genetic dissection of grain yield and physical grain quality in bread wheat (Triticum aestivum L.) under water-limited environments

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Chapter 2. Research Articles

Detection of two major grain yield QTL in bread wheat (Triticum aestivum L.) under heat, drought and high yield potential environments.

Heat and drought stress is not just a problem for just southern Australia, but also an affliction for much of the world's wheat production zones. However, one of the major issues in breeding for improved production in these climates is that drought and heat stress are variable in both timing and intensity. The International Wheat and Maize Improvement Center (CIMMYT) has developed distinct, repeatable field assay systems to aid improvement of wheat production under drought (timely sown into a minimal moisture profile and irrigated with drip irrigation) and heat stressed (late sowing to expose to high summer temperatures, with supplementary irrigation) environments. By dissecting the genetic basis of yield under these conditions, further loci contributing to improved grain production may be identified. The value of the loci identified in the southern Australian experiments could be further validated, under controlled and repeatable droughted and heat stressed conditions. This paper presents the genetic dissection of grain yield and a number of traits hypothesised to influence grain yield in the 'RAC875/Kukri' under these climatic conditions.

Detection of two major grain yield QTL in bread wheat (Triticum aestivum L.) under heat, drought and high yield potential environments

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Bennett, D., Reynolds, M., Mullan, D., Izanloo, A., Kuchel, H., Langridge, P. & Schnurbusch, T. (2012) Detection of two major grain yield QTL in bread wheat (*Triticum aestivum* L.) under heat, drought and high yield potential environments.
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Chapter 3

Chapter 3. General Discussion

3.1 Applying results from the current study to grain yield improvement in water limited and heat stressed environments

The aim of the present study was to detect QTL associated with grain yield in drought and heat limited production environments of southern Australia and North-Western Mexico. The number of loci identified just for grain yield, as well as the fact that many were only identified in specific environments, highlights a complex genotype by environment interaction system for grain yield production within southern Australia. The differential detection of most loci between Australia and Mexico emphasises the importance of breeding and selection in the target environment.

The challenge now is to develop a strategy to apply these research outcomes for improving productivity under water and heat stress. There are a number of steps that can be taken from here, which include:

- Investigating the frequency of various marker alleles at key loci, such as Q.Yld.aww-2D-2, Q.Yld.aww-3B-2 and Q.Yld.aww-4D, in more genetically diverse but still locally adapted germplasm. This will determine whether marker development for a given locus would enable more effective selection. The efficacy of the different alleles in different genetic backgrounds could be established if assayed in a germplasm set that has already been grown extensively in the field.

- Following the inheritance of key loci through breeding and selection in the pedigrees of key adapted germplasm, particularly those known to be derived from RAC875. Whilst largely of academic interest, it has previously been found that empirical breeding does not necessarily result in the theoretically optimal combination of alleles (Kuchel *et al*. 2007).

- Prioritise key QTL for fine mapping, with the aim of developing closely linked or diagnostic markers for those loci of high value. The recombinant inbred population derived from the same

RAC875/Kukri cross (Fleury *et al.* 2010) can be utilised to identify individuals with recombination events in the region/s of interest and form the basis of this marker development.

- Some loci may already have diagnostic markers developed, as appears to be the case at *Q.Tkw.aww-6A.* Su *et al.* (2011) characterised a gene called *TaGW2*, and developed a single nucleotide polymorphism (SNP-) based marker. This marker appears to be closely linked to this QTL. However, this would require further validation but if adopted, would be one of the first examples of marker assisted selection being applied to a yield component within a breeding program in wheat.

- A BC_1F_4 population could be achieved in two years, using a rapid cycle program backcrossing program to pyramid key QTL detected in this study into more elite backgrounds, unrelated to the donors of RAC875 and Kukri. This would restore 93.7% of the recurrent parents' genetic background on average and determine if a level of stress tolerance can be achieved above that of RAC875.

- A similar backcrossing strategy could be utilised for a 'defect elimination' program. QTL detected for a specific trait or traits could be incorporated into otherwise acceptable genetic backgrounds where, say, grain size is prone to unacceptable levels of screenings in certain environments, to rapidly improve otherwise acceptable germplasm.

These strategies are practical methods to deliver improved rates of genetic gain to growers for grain yield under the drought and heat stressed conditions of southern Australia.

3.2 Population development in the future

Bi-parental mapping population structures have been widely utilised for identifying genomic regions (QTL) for genes associated with the control of economically important traits (for example, Kuchel *et al.* 2007; Pinto *et al.* 2010). Segregation for off-target traits, such as phenology and plant height, which are largely controlled by single or few major genes, can confound attempts to characterise the genetic

control of target traits. In the RAC875/Kukri population, this could have been avoided by using strategies such as:

- After creating the cross, growing a small number of the F_2 individuals to ear emergence, which would have identified the segregation for maturity. Doubled haploid development could have been abandoned at this stage or strategies put in place to phenotypically select individuals for reduced variation for time to ear emergence for inclusion in the population.

- Utilising diagnostic molecular markers, which have since been developed for many of the major ear emergence time and plant height reducing genes (Beales *et al*. 2007; Ellis *et al*. 2002; Yan *et al*. 2006; Yan *et al*. 2004), which could have been used to screen the parents and identify likely segregation. In the future, when selecting parents differing in alleles for these confounding traits is unavoidable (for example, an exotic donor), these markers could be used to screen F_2 lines and select individuals with specific genotypes to fix the population for specific allele combinations.

Alternative population structures to bi-parental doubled haploid crosses could also be considered in the future. More recent designs have incorporated the use of multi-parent crosses, such as 'nested association mapping' populations (Yu *et al*. 2008), which has been successfully applied to QTL detection in maize (Buckler *et al*. 2009) or Multi parent Advanced Generation Inter-Cross (MAGIC), being developed in bread wheat (Cavanagh *et al*. 2008). These approaches utilise multiple parents, with relatively complex crossing strategies and combines the advantages of both traditional linkage and association mapping.

However, two potential drawbacks over traditional bi-parental doubled haploid populations are the number of lines generated during population development being relatively large and the resources and time required to produce sufficient quantities of seed for field experiments also being limiting. The resources required to phenotype these populations for a trait such as grain yield, could also be large since yield is generally only moderately heritable, subject to large genotype by environment interactions and therefore should be studied over multiple sites and years. The upside is that the larger number of

individuals increases phenotypic repetition at any given allele, improving confidence in any genetic effects detected. With more than two parents, it is also possible that more than two different alleles could be identified at one locus.

The rapidly decreasing costs associated with genotyping such a population also means that the potential advantages for dissection of genomic regions controlling complex traits such as grain yield therefore may outweigh these phenotyping costs. A further advantage is that during the development of the recombinant inbred lines that constitute these population designs, molecular markers could be used to fix the population for potentially confounding traits, as discussed above. However, consideration would need to be given to whether fixing populations for specific genotypes would reduce the power of detection around these loci, potentially masking important linked genomic regions.

3.3 Phenotyping technologies and applications for the future

In the present study, field experiments were conducted in environments where they were exposed to severe water and heat stress, in addition to numerous other biotic and abiotic stresses. This presented a variety of issues including:

- Large number of plots required to grow the population, accurately capture variation between experiments and achieve replication within each field experiment;
- Variation for time to ear emergence effectively exposing different maturity groups to different levels of stress at different growth stages; and
- Time and costs required to travel to each field experiment, as well as time required to accurately phenotype each experiment.

These factors therefore affected the final range of traits that could be effectively assessed. To alleviate some of these constraints, new technologies for rapid phenotyping should be embraced, such as image analysis. Mullan and Reynolds (2010) highlighted how early vigour/early canopy closure can be

estimated using digital photography and basic image analysis software. Further promising technologies could include:

- Simultaneous infrared photos of experiments to estimate canopy temperatures at one time point;

- Near infrared reflectance (NIR) at different wavelengths to detect specific traits such as chlorophyll content, biomass, crop water status and leaf area (for example, Gutierrez *et al.* 2010; Tucker 1979);

- Secondary, 'simpler' traits could also be measured using aerial photography methodologies, particularly foliar biotic stress infections, potentially adding value to the investment in any equipment.

Unfortunately, many of these technologies are out of the realms of application to commercial breeding programs for a number of possible reasons:

- Large capital expense and often the need to extensively train technicians in its use or large labour requirement for measurement. Within a breeding programme, this would have to come at the expense of measuring another proven trait or extra yield plots;

- Any proposed trait or phenotyping technology should be able to accurately, quickly and cheaply measure on rows, head hills or small plots and shift population genotypes during segregating generations. This is the stage within a breeding programme where such phenotyping will be of greatest value. In addition, if a trait is genetically correlated, yet of higher heritability than yield, selection for such a trait or the use of multivariate analysis would improve selection for grain yield (Falconer 1989).

Traits and phenotyping technologies often lack validation of their yield value across germplasm pools and environments. This reduces breeders' confidence in the usefulness of measuring such a trait. However, much like genotyping technologies, these costs continue to fall and in the near future, they may be applicable in early to mid generation selection in a breeding program. Studies such as the one

presented in this thesis, are therefore key to improving breeders' understanding of the value of specific traits and introducing new phenotyping methodologies, such as canopy temperature and water soluble carbohydrates, since locally adapted germplasm relevant to the local wheat breeders' gene pool has been studied in the target environment.

3.4 Other developments for genomic manipulation in bread wheat

The literature review highlighted the complexity of genetic control of grain yield, particularly under drought and heat stressed conditions and especially when considering the interaction between growth stage, stress intensity and timing of stress. In addition, QTL mapping with bi-parental populations and association panels usually lacks the resolution to detect the many loci of minor effect contributing to the trait of interest. However, genomic selection, as initially proposed by Meuwissen *et al.* (2001), could overcome these limitations. With few exceptions (Heffner *et al.* 2010; Lorenz *et al.* 2012; Rutkoski *et al.* 2011), genomic selection is largely theoretical in application to plant breeding at this stage.

However, it has the potential to accelerate the breeding cycle and therefore increase the rate of genetic gain for quantitative traits such as grain yield. With genotyping costs rapidly falling, whole genome marker panels can be used to develop a 'training set' population. This set of germplasm contains well phenotyped and genotyped individuals for the trait of interest and the combination of marker alleles for each individual used to develop a model that can be applied to a 'validation set'. The validation set contains individuals, typically derived from crosses between individuals in the training set, that have not yet been phenotyped but are given a 'genomic estimated breeding value' (GEBV) based on their genotype and its value in the training set – a similar concept to developing and utilising a calibration equation for near infrared spectroscopy.

The benefit is that selection can occur for individuals with estimated high value for the trait of interest when seed quantities required for extensive field phenotyping are unavailable, particularly earlier in the breeding cycle. These individuals are progressed to field validation, to ensure that they express a

superior phenotype. As a result, this approach has the ability to not only increase the rate of genetic gain (note, of both the trait of interest *and* undesired traits), but also the efficient use of breeders' limited resources by limiting phenotyping to superior individuals. A further advantage is that positive alleles for all loci exerting an effect on the trait of interest can potentially be selected. Unlike relying on marker assisted selection, which requires a locus to have been identified (for example through QTL detection), all loci affecting a trait, even the many minor effects unable to be detected by QTL analysis, may be selected for with superior GEBVs.

However, a number of issues would need to be addressed if considering the application of genomic selection to, for example, improving rates of genetic gain for grain yield under abiotic stress. These include:

- Achieving highly heritable phenotypes of traits in the training set. Given that GEBVs are generated from the relationship between genotypic and phenotypic values, the accuracy of such values will be reduced if field trials of low heritability are used. However, even low accuracy GEBVs appear to be able to more than double the rate of genetic gain able to be achieved by marker assisted selection alone (Heffner *et al*. 2010). In addition, a larger training population size may be able to compensate for some of the loss of phenotyping accuracy (Heffner et al. 2011);

- Secondly, under abiotic stress, traits under the control of few major genes, such as ear emergence time, are likely to be exerting significant influence over a trait such as grain yield. Great care would need to be taken in deriving GEBVs from such data, which could simply result in a shift in ear emergence time in the validation set;

- Finally, without knowing the physical location of genes controlling the trait of interest, it is not possible to actively pursue allelic diversity at these loci in a targeted approach.

3.5 Conclusion

This study highlighted the complexity of the Mediterranean type climate experienced in southern Australia, which was demonstrated through the different water availability patterns. The result was a physiologically complex response, with many traits of small effect contributing to grain yield in this water limited and heat stressed environment. Therefore, the genetic basis of grain yield in these conditions was complex, with different QTL expressed both across environments (Mexico and southern Australia) and within the target environment. Key QTL, such as *Q.Yld.aww-2D-2*, *Q.Yld.aww-3B-2* and *Q.Yld.aww-4D* should therefore form the basis of further research, specifically, fine mapping to develop perfect markers. In the medium term, with appropriately validated tools, such as markers linked to grain yield QTL, breeders' are likely to adopt them to implement marker assisted selection for grain yield, related components and/or physical grain quality under the drought and heat stressed conditions of southern Australia. This will ultimately aid in improving the rate of genetic gain for grain yield in this challenging environment.

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Appendix

Appendix 1. Supplementary material from research articles

Table 1 Number of markers per chromosome, chromosome length centiMorgans (cM) and average cM between markers per chromosome of a genetic linkage map for the RAC875/Kukri doubled haploid population. From research article titled 'Identification of novel quantitative trait loci for days to ear emergence and flag leaf glaucousness in a bread wheat (*Triticum aestivum* L.) population adapted to southern Australian conditions'.

Chromosome		Total markers	SSRs	DArTs	Length (cM)	Average interval
1A		36	14	22	201.2	5.59
1B		37	18	19	192.8	5.21
1D		25	11	14	180.4	7.22
	TOTAL	**98**	**43**	**55**	**574.4**	**6.01**
2A		21	12	9	167.4	7.97
2B		23	4	19	140.7	6.12
2D		17	5	12	135.4	7.96
	TOTAL	**61**	**21**	**40**	**443.5**	**7.35**
3A		24	14	10	179.9	7.50
3B		41	20	21	226.1	5.51
3D		19	13	6	189.1	9.95
	TOTAL	**84**	**47**	**37**	**595.1**	**7.65**
4A		22	9	13	186.8	8.49
4B		18	8	10	151.4	8.41
4D		5	3	2	8.1	1.62
	TOTAL	**45**	**20**	**25**	**346.3**	**6.17**
5A		11	8	3	200.7	18.25
5B		19	10	9	144.7	7.62
5D		6	3	3	120.1	20.02
	TOTAL	**36**	**21**	**15**	**465.5**	**15.29**
6A		19	4	15	150.3	7.91
6B		29	6	23	164.9	5.69
6D		7	6	1	81.7	11.67
	TOTAL	**55**	**16**	**39**	**396.9**	**8.42**
7A		42	23	19	229.6	5.47
7B		20	9	11	128.8	6.44
7D		15	10	5	192.3	12.82
	TOTAL	**77**	**42**	**35**	**550.7**	**8.24**
	AVE	21.71	14.00	16.93	161.50	8.50
	SUM	456	210	246	3391.5	

Table 2. Summary of all QTL detected for three traits (Days to ear emergence, Zadok score and flag leaf glaucousness) in the RAC875/Kukri doubled haploid and recombinant inbred line populations, in five field trials in South Australia (BOL06, MIN06, RACMET), Northern Mexico (CIMD07, CIMI07) and one glasshouse experiment. The position of each QTL on their respective chromosomes, flanking markers, additive effect at that locus (negative indicates RAC875, positive is Kukri), QTL heritability at that site and the QTL LOD score are presented. From research article titled 'Identification of novel quantitative trait loci for days to ear emergence and flag leaf glaucousness in a bread wheat (*Triticum aestivum* L.) population adapted to southern Australian conditions'.

Trait	Site	QTL	Position (cM)	Flanking markers	Effect	H2	LOD
Days to ear emergence	BOL06	QEet.aww-2D	34.7	WPT-6003-PPD-D1	6.9	0.49	74.1
		QEet.aww-2B	36.1	WPT-7757-BARC0013A	-3.8	0.18	38.4
		QEet.aww-7A-3	139.3	BARC0049-DUPW0254	-1.2	0.03	15.1
		QEet.aww-7A-1	79.7	WPT-5153-CFA2028	-2.4	0.04	8.3
		QEet.aww-5B	79.1	WPT-4936-WPT-3457	1.9	0.05	6.5
		QEet.aww-7B	52.8	WPT-4230-WMC0517B	-1.8	0.06	6.3
		QEet.aww-5A	106.0	GWM0186-WPT-1370	1.2	0.01	4.0
	MIN06	QEet.aww-2D	37.0	PPD-D1-WPT-0330	8.1	0.48	103.6
		QEet.aww-2B	37.1	WPT-7757-BARC0013A	-5.1	0.24	50.6
		QEet.aww-7A-3	141.3	BARC0049-DUPW0254	-1.3	0.02	8.4
		QEet.aww-7A-1	95.2	WPT-5153-CFA2028	-1.9	0.02	3.9
		QEet.aww-5A	132.3	GWM0186-WPT-1370	1.5	0.01	3.3
	RACMET	QEet.aww-2D	37.0	PPD-D1-WPT-0330	7.4	0.50	105.1
		QEet.aww-2B	37.1	WPT-7757-BARC0013A	-5.0	0.25	59.1
		QEet.aww-5B	96.9	WPT-4936-WPT-3457	1.7	0.05	4.4
		QEet.aww-7A-3	141.3	BARC0049-DUPW0254	-0.4	0.01	4.0
		QEet.aww-7A-1	95.2	WPT-5153-CFA2028	-2.0	0.01	3.8
		QEet.aww-4B	27.9	GWM0495-GWM0149	-1.0	0.01	3.6
		QEet.aww-1A	16.1	WPT-6709-GDM0033A	-0.9	0.01	3.4
		QEet.aww-5A	135.3	GWM0186-WPT-1370	1.2	0.01	2.9
	CIMD07	QEet.aww-2D	39.0	WPT-6003-PPD-D1	5.9	0.36	53.5
		QEet.aww-2B	38.1	WPT-7757-BARC0013A	-3.9	0.15	23.6
	CIMI07	QEet.aww-2D	37.0	PPD-D1-WPT-0330	7.9	0.43	85.4
		QEet.aww-2B	37.1	WPT-7757-BARC0013A	-5.2	0.24	23.6
		QEet.aww-5B	16.0	GWM0234B-WPT-8604	1.1	0.02	3.1
		QEet.aww-7A-2	115.5	BARC0282-BARC0108	-1.5	0.01	5.1
		QEet.aww-5A	138.3	GWM0186-WPT-1370	1.5	0.01	2.8
Zadoks score	BOL06	QZad.aww-2D	33.7	WPT-6003-PPD-D1	-6.7	0.44	65.8
		QZad.aww-2B	35.1	WPT-7757-BARC0013A	4.1	0.19	32.1
		QZad.aww-7A-2	106.4	BARC1004-BARC0174	2.6	0.04	12.9
		QZad.aww-7B	55.8	WPT-4230-WMC0517B	1.9	0.05	4.0
		QZad.aww-5A	106.0	GWM0186-WPT-1370	-1.2	0.01	3.7
		QZad.aww-5B	79.1	WPT-4936-WPT-3457	-1.5	0.04	3.1
	MIN06	QZad.aww-2D	35.0	PPD-D1-WPT-0330	-8.0	0.40	74.7
		QZad.aww-2B	26.8	WPT-9644-WPT-5672	5.9	0.25	46.2
		QZad.aww-7A-3	139.3	BARC0049-DUPW0254	1.1	0.02	5.1
		QZad.aww-7A-2	109.4	BARC1004-BARC0174	0.8	0.01	5.2
	RACMET	QZad.aww-2D	35.0	PPD-D1-WPT-0330	-5.6	0.49	121.0
		QZad.aww-2B	35.1	WPT-7757-BARC0013A	2.4	0.26	58.6
		QZad.aww-7A-1	91.2	CFA2028-WMC0083A	1.1	0.01	5.5
		QZad.aww-5B	79.1	WPT-4936-WPT-3457	-0.9	0.04	3.0
		QZad.aww-1A	12.1	WPT-3870-WPT-6122	0.8	0.02	2.8
		QZad.aww-4A	14.1	BARC0106-DUPW0328	-0.5	0.01	3.0
		QZad.aww-7A-3	139.3	BARC0049-DUPW0254	0.4	0.01	5.6
Glaucousness	BOL06	QW.aww-2D	38.0	PPD-D1-WPT-0330	-0.5	0.22	16.7
		QW.aww-3A	94.3	WMC0264-CFA2193B	0.3	0.07	4.8
		QW.aww-6A	71.5	WPT-7599-BARC0118	0.3	0.09	4.9
		QW.aww-4D	8.0	WPT-2379-WPT-0431	-0.2	0.06	4.6
		QW.aww-3B	33.9	BARC0102-WMC0043	0.2	0.04	3.2
	MIN06	QW.aww-2D	38.0	PPD-D1-WPT-0330	-1.1	0.52	94.5
		QW.aww-2B-1	16.6	WPT-1489-WPT-9644	-0.6	0.13	27.6
		QW.aww-3D	79.6	WMC0533-WPT-6262	0.2	0.01	4.2
		QW.aww-3A	94.3	WMC0264-CFA2193B	0.2	0.05	3.1
		QW.aww-6A	77.5	WPT-7599-BARC0118	0.2	0.04	2.7
	RACMET	QW.aww-3A	92.3	WMC0264-CFA2193B	0.7	0.35	41.0
		QW.aww-2D	27.7	WPT-6003-PPD-D1	-0.6	0.21	29.6
		QW.aww-2B-1	12.6	WPT-1489-WPT-9644	-0.2	0.02	5.5
		QW.aww-1D	98.5	WPT-8854-CFD0027	-0.1	0.01	2.0
		QW.aww-4D	6.3	BARC0288-WPT-2379	-0.1	0.01	2.0

	QW.aww-6A	75.5	WPT-7599-BARC0118	0.1	0.03	4.1
	QW.aww-7D	112	BARC0058-GWM0428	0.1	0.01	2.4
	QW.aww-5B	124.5	WPT-3030-BARC0275	0.1	0.03	2.1
	QW.aww-3D	88.1	WPT-6262-WPT-7894	0.1	0.01	2.8
CIMI07	*QW.aww-3A*	90.3	WMC0264-CFA2193B	0.5	0.49	71.0
	QW.aww-2D	20.7	WPT-6003-PPD-D1	-0.3	0.14	16.0
	QW.aww-5B	55.4	BARC0216-BARC0004B	0.1	0.04	3.0
	QW.aww-1D	90.5	WPT-8854-CFD0027	-0.1	0.02	2.7
CIMD07	*QW.aww-3A*	89.5	WPT-8658-WMC0264	0.6	0.42	51.3
	QW.aww-2B-2	37.1	WPT-7757-BARC0013A	0.3	0.09	11.9
Glasshouse (RILs)	*QW.aww-3A*	93.1	WMC0264-CFA2193B	0.4	0.35	23.8

Table 3. Quantitative trait loci detected for yield, yield components and various physiological traits in a subset of the RAC875/Kukri population of 260 doubled haploid lines. QTL name, environment cluster that it was detected in, position of the QTL peak (in cM) and marker interval on the respective chromosome of the population linkage map (as described by Bennett *et al.* 2011), effect (positive indicated Kukri allele, negative indicated RAC875 allele), QTL heritability and LOD score of detection are also presented. From research article titled 'Genetic dissection of grain yield and physical grain quality in bread wheat (*Triticum aestivum* L.) under water limited environments'.

QTL	ENV CLUSTER	POSITION (cM)	FLANKING MARKERS	EFFECT	H2	LOD
QScr.aww-1A	ENV1	73.8	WPT-2872-GWM0164	0.20	0.06	3.35
QKps.aww-1A	ALL EXPTS	100.4	WPT-6074-WMC0093	-0.71	0.04	4.98
QKpsm.aww-1A	ENV2	182.4	WPT-0864-WPT-6754	-99.14	0.07	3.37
QKpsm.aww-1A	ENV1	182.4	WPT-0864-WPT-6754	-31.83	0.11	3.96
QYld.aww-1A	ALL EXPTS	186.4	WPT-0864-WPT-6754	-18.14	0.04	3.16
QKpsm.aww-1A	ENV2-1	198.8	WPT-6568-CFE0242B	-120.84	0.08	3.78
QScr.aww-1B	ENV3	0	WPT-1560-PSP3000	0.5182	0.05	3.12
QScr.aww-1B	ENV3-2	0	WPT-1560-PSP3000	0.6402	0.05	3.50
QTwt.aww-1B	ENV3-1	7.9	WPT-2052-WPT-1139	-0.3594	0.07	3.80
QTwt.aww-1B	ENV3	4.9	WPT-2052-WPT-1139	-0.3164	0.07	4.63
QTwt.aww-1B	ENV3-2	4.9	WPT-2052-WPT-1139	-0.3196	0.06	3.48
QHi.aww-1B	PID08	45.2	WMC0338B-GWM0264	-0.004	0.08	4.59
QTn.aww-1B	PID08	61.9	BARC0008-GWM0413	0.0196	0.05	2.99
QKpsl.aww-1B	ENV2	91.6	WMC0216-GWM0131B	-0.0353	0.09	4.65
QKpsl.aww-1B	ENV1	132.3	WMC0147-BARC0256	-0.0424	0.06	3.03
QYld.aww-1B	ENV2	164.7	BARC0256-WPT-9809	-24.3464	0.06	3.46
QYld.aww-1B	ENV2-2	165.7	BARC0256-WPT-9809	-21.1811	0.04	3.10
QKps.aww-1B	ALL EXPTS	179.7	STM0658A-WPT-1313	-0.8239	0.10	6.41
QHt.aww-1D	ALL EXPTS	92.5	CFD0065B-BARC0210	0.50	0.08	4.98
QTkw.aww-1D	ALL EXPTS	115.8	CFD0027-WPT-1799	-0.22	0.03	3.12
QTpa.aww-1D	NUN08	114.8	CFD0027-WPT-1799	0.61	0.04	3.38
QTwt.aww-1D	ENV3	144.4	WPT-1799-WPT-1263	0.38	0.04	3.48
QScr.aww-2A	ENV3-1	79.6	WPT-7306-WPT-4201	0.16	0.07	3.05
QYld.aww-2A	ENV3-1	94.5	WPT-7285-WPT-7056	55.02	0.03	3.51
QTwt.aww-2A	ENV2	110.6	GWM0312-WMC0181B	0.45	0.10	5.15
QTwt.aww-2A	ENV3	111.6	GWM0312-WMC0181B	0.26	0.06	3.49
QTwt.aww-2A	ENV3-1	128	WMC0181B-GWM0382B	0.32	0.05	3.31
QHi.aww-2A	ALL EXPTS	140	WMC0181B-GWM0382B	-0.004	0.02	1.56
QEv.aww-2B	ENV2	43.2	BARC0013A-GWM0271A	-0.12	0.03	14.90
QFll.aww-2B	ALL EXPTS	41.2	BARC0013A-GWM0271A	-7.09	0.05	17.38
QScr.aww-2B-1	ENV3-2	49.7	BARC0091-WPT-0335	0.40	0.05	3.29
Qtpa.aww-2B-1	MIN07	49.7	BARC0091-WPT-0335	0.38	0.05	3.53
QTkw.aww-2-1	ALL EXPTS	53.7	BARC0013A-GWM0271A	-0.69	0.14	4.62
QFlw.aww-2B	ALL EXPTS	53.7	BARC0091-WPT-0335	-0.25	0.11	7.07
QYld.aww-2B	ENV2-2	61.3	WPT-0335-WPT-0950	-20.75	0.04	4.35
QYld.aww-2B	ALL EXPTS	63.3	WPT-0335-WPT-0950	-19.33	0.06	2.62
QTpa.aww-2B	PID07	67.3	WPT-0335-WPT-0950	0.94	0.07	3.92
QKpsm.aww-2B	ENV3-1	134	WPT-3378-WPT-7360	54.66	0.03	2.92
QScr.aww-2B-2	ENV2-2	134	WPT-3378-WPT-7360	0.64	0.11	3.35
QKpsm.aww-2B	ENV3-2	134	WPT-3378-WPT-7360	62.77	0.06	3.29
QKpsm.aww-2B	ENV3	134	WPT-3378-WPT-7360	78.49	0.06	5.76
QScr.aww-2B-2	ENV3	136.3	WPT-7360-WPT-2135	0.62	0.11	7.38
QScr.aww-2B-2	ENV3-2	136.3	WPT-7360-WPT-2135	0.65	0.12	7.57
QScr.aww-2B-2	ENV2	137.3	WPT-7360-WPT-2135	0.49	0.11	5.19
QScr.aww-2B-2	ENV2-1	138.3	WPT-7360-WPT-2135	0.29	0.06	3.78
QTkw.aww-2B-2	ALL EXPTS	139.3	CFD0050A-WPT-3378	-0.75	0.17	4.08
QTpa.aww-2B-2	MIN07	140.3	WPT-7004-CFD0050A	0.42	0.05	3.03
QKpsm.aww-2D-1	ENV3-2	25.7	WPT-6003-PPD-D1	-119.85	0.08	3.16
QEn.aww-2D	RAC08	35	PPD-D1-WPT-0330	-0.12	0.09	3.51
QFll.aww-2D	ALL EXPTS	42	PPD-D1-WPT-0330	-12.33	0.29	32.47
QEv.aww-2D	ENV1	43	PPD-D1-WPT-0330	-0.27	0.18	9.91
QEv.aww-2D	ENV3	45	PPD-D1-WPT-0330	-0.19	0.12	8.37
QEv.aww-2D	ENV2	47	PPD-D1-WPT-0330	-0.25	0.20	13.92
	ENV	POSITION	NEAREST MARKER RANGE	EFFECT	H2	LOD

QTL						
QYld.aww-2D-1	ENV3-1	50	PPD-D1-WPT-0330	81.62	0.18	4.57
QHt.aww-2D	ALL EXPTS	58.4	WPT-0330-BARC0328B	-0.40	0.09	9.35
QFlw.aww-2D	ALL EXPTS	65.4	WPT-0330-BARC0328B	-0.21	0.02	7.90
QTpa.aww-2D	NUN08	66.4	WPT-0330-BARC0328B	0.65	0.05	2.81
QYld.aww-2D	ENV1	66.4	WPT-0330-BARC0328B	7.19	0.08	4.00
QKpsm.aww-2D-2	ENV3-1	72.2	BARC0328B-WPT-6574	82.87	0.06	4.19
QYld.aww-2D	ENV3-1	74.2	BARC0328B-WPT-6574	27.75	0.13	7.06
QHt.aww-2D	ALL EXPTS	77.2	BARC0328B-WPT-6574	-0.58	0.13	5.91
QKpsm.aww-2D-2	ENV3	78.2	BARC0328B-WPT-6574	107.73	0.10	7.25
QYld.aww-2D-2	ENV2-1	78.2	BARC0328B-WPT-6574	49.89	0.18	11.19
QYld.aww-2D-2	ENV3	79.2	BARC0328B-WPT-6574	43.52	0.10	6.08
QYld.aww-2D-2	ENV3-2	79.2	BARC0328B-WPT-6574	41.92	0.15	9.59
QYld.aww-2D-2	ALL EXPTS	79.2	BARC0328B-WPT-6574	34.26	0.15	9.07
QYld.aww-2D-2	ENV2	80.2	BARC0328B-WPT-6574	31.8	0.07	4.16
QKpsm.aww-2D-2	ENV2	81.2	BARC0328B-WPT-6574	95.05	0.07	4.65
QWsc-c.aww-2D	ALL EXPTS	82.2	BARC0328B-WPT-6574	-4.24	0.08	4.89
QScr.aww-2D	ENV2	82.2	BARC0328B-WPT-6574	0.36	0.03	4.63
QKpsm.aww-2D-2	ENV2-2	83.2	BARC0328B-WPT-6574	152.29	0.06	3.37
QScr.aww-2D	ENV2-2	105.9	WPT-0021-WPT-4559	0.52	0.04	5.32
QPdl.aww-3A	ALL EXPTS	51.1	WPT-0714-GWM0002	2.73	0.06	4.84
QKps.aww-3A	ALL EXPTS	56.9	GWM0002-BARC0328A	0.72	0.09	5.26
QKpsm.aww-3A	ENV1	58.9	BARC0328A-WMC0388A	23.91	0.06	5.06
QTwt.aww-3A	ENV3-2	59.9	BARC0328A-WMC0388A	-0.31	0.06	3.61
QHt.aww-3A	ALL EXPTS	61.9	GWM0002-BARC0328A	-0.77	0.15	9.42
QTwt.aww-3A	ENV2	62.6	WMC0388A-BARC0324	-0.31	0.07	3.76
QWsc-c.aww-3A	ALL EXPTS	64.9	WMC0388A-BARC0324	-6.29	0.19	9.22
QWsc-a.aww-3A	RAC07wsca	64.9	WMC0388A-BARC0324	-3.45	0.10	6.25
QWsc-t.aww-3A	RAC07wsct	67.7	WMC0388A-BARC0324	-0.02	0.09	6.17
QKpsm.aww-3B	ENV2-1	33	CFA2226B-BARC0102	103.57	0.06	3.55
QScr.aww-3B-1	ENV2	33.9	BARC0102-WMC0043	0.42	0.11	5.25
QScr.aww-3B-1	ENV2-1	33.9	BARC0102-WMC0043	0.34	0.08	5.00
QPdl.aww-3B	ALL EXPTS	55.9	BARC0102-WMC0043	-1.96	0.03	3.05
QHi.aww-3B	ALL EXPTS	84	WPT-9510-BARC0234	0.003	0.01	2.16
QHi.aww-3B	RAC07	85	WPT-9510-BARC0234	0.01	0.06	3.55
QEv.aww-3B	ENV3	108.8	GWM0131A-GWM0383A	-0.11	0.06	5.52
QFll.aww-3B	ALL EXPTS	115.6	GWM0383A-BARC0344	-2.67	0.04	8.46
QEv.aww-3B	ENV1	120.6	GWM0131A-GWM0383A	-0.11	0.04	3.68
QScr.aww-3B-2	ENV1	190.7	WPT-8021-GWM0114B	0.21	0.07	4.22
QEv.aww-3D	ENV3	6	WPT-2464-CFD0079	-0.09	0.05	3.34
Qtn.aww-3D	MIN07	11	WPT-2464-CFD0079	0.004	0.06	3.27
QBio.aww-3D	ALL EXPTS	50	CFD0079-CFD0064	-2.72	0.03	1.99
QHi.aww-3D	ALL EXPTS	116.6	GWM0664-GWM0383B	0.004	0.02	1.56
QTkw.aww-3D	ALL EXPTS	136.8	CFD0223B-BARC0071	-0.23	0.02	3.00
QTwt.aww-4A	ENV3	11.9	WPT-7001-BARC0106	0.31	0.06	3.12
QTn.aww-4A	RAC08	15.1	STM0092TCTG-WPT-7001	-0.03	0.06	3.12
QTwt.aww-4A	ENV3-1	15.1	BARC0106-DUPW0328	0.34	0.06	3.66
QFll.aww-4A	ALL EXPTS	13.9	WPT-7001-BARC0106	2.17	0.03	5.06
QTkw.aww-4A	ALL EXPTS	119.7	WPT-5694-WPT-7939	0.41	0.04	4.65
QEn.aww-4A	RAC08	122.7	WPT-5694-WPT-7939	0.08	0.05	2.90
QKpsl.aww-4B	ENV2	31.5	WMC0047-BARC0114	0.03	0.05	3.53
QTpa.aww-4B	PID07	150.4	WPT-1272-WPT-0246	-0.82	0.07	2.90
QScr.aww-4D	ENV3-1	5.5	WMC0457-BARC0288	-0.14	0.05	3.37
QTwt.aww-4D	ENV2	5.5	WMC0457-BARC0288	0.28	0.03	3.37
QKpsm.aww-4D	ENV2	6.3	BARC0288-WPT-2379	-92.17	0.07	3.13
QKpsm.aww-4D	ENV2-2	6.3	BARC0288-WPT-2379	-171.81	0.09	3.71
QScr.aww-4D	ENV3	6.3	BARC0288-WPT-2379	-0.38	0.05	4.41
QScr.aww-4D	ENV3-2	6.3	BARC0288-WPT-2379	-0.45	0.05	4.05
QHi.aww-4D	STR08	8	BARC0288-WPT-2379	0.006	0.06	3.42
QYld.aww-4D	ENV2	8	WPT-2379-WPT-0431	-20.39	0.05	2.97
QYld.aww-4D	ENV2-2	8	WMC0457-BARC0288	-27.90	0.06	2.86

QTL	CLUSTER	POSITION	NEAREST MARKER RANGE	EFFECT	H2	LOD
QScr.aww-5A	ENV3-1	89	GWM0186-WPT-1370	0.28	0.08	3.59

QFlw.aww-5A	ALL EXPTS	163.7	CFA2141-WPT-5231	-0.21	0.05	5.75
QPdl.aww-5A	ALL EXPTS	171.7	CFA2141-WPT-5231	3.56	0.09	5.30
QHt.aww-5A	ALL EXPTS	200.2	CFA2141-WPT-5231	0.58	0.06	4.94
QScr.aww-5B	ENV2-1	77.1	WPT-4936-WPT-3457	0.28	0.05	3.16
QTkw.aww-5B	ALL EXPTS	97.9	WPT-3457-GWM0271B	-0.40	0.07	2.90
QFll.aww-5B	ALL EXPTS	95.9	WPT-3457-GWM0271B	5.84	0.13	15.30
QEv.aww-5B	ENV3	101.5	WPT-4936-WPT-3457	0.11	0.08	4.22
QEv.aww-6A	ENV1	76.5	WPT-7599-BARC0118	0.10	0.07	3.57
QKpsl.aww-6A	ENV3	80.8	BARC0118-WMC0256A	0.03	0.03	3.83
QTwt.aww-6A	ENV3	82.2	WMC0256A-GWM0169	-0.30	0.04	3.29
QKpsl.aww-6A	ENV1	84.2	WMC0256A-GWM0169	0.03	0.04	3.83
QKpsm.aww-6A	ENV3	92.2	WMC0256A-GWM0169	78.17	0.04	3.98
QFlw.aww-6A	ALL EXPTS	95.2	WMC0256A-GWM0169	-0.46	0.24	22.20
QScr.aww-6A	ENV3-1	97.2	WMC0256A-GWM0169	-0.20	0.04	4.28
QKpsm.aww-6A	ENV3-1	97.2	WMC0256A-GWM0169	102.33	0.07	5.56
QTkw.aww-6A	ALL EXPTS	98.2	WMC0256A-GWM0169	-0.92	0.11	6.21
Qtpa.aww-6A	PID07	101.2	BARC0118-WMC0256A	0.91	0.04	3.14
QWsc-a.aww-6A	RAC07wsca	121.5	WMC0256A-GWM0169	-2.91	0.06	3.53
QWsc-t.aww-6A	RAC07wsct	118.5	WMC0256A-GWM0169	-0.008	0.06	3.23
QScr.aww-6B	ENV3-1	93.5	GWM0626-BARC0223	0.18	0.06	3.96
QTkw.aww-6B	ALL EXPTS	118.7	BARC0223-BARC0247	0.36	0.009	3.94
QKpsm.aww-6D	ENV3-2	47.2	BARC0301-BARC0054	-112.64	0.04	4.30
QYld.aww-6D	ENV3-2	84.1	BARC0301-BARC0054	-23.83	0.04	2.86
QYld.aww-6D	ENV2	85.1	GWM0325B-CFD0287	-18.81	0.03	2.86
QKps.aww-7A	ALL EXPTS	4	STM0671TCTG-GWM0681	0.51	0.04	2.98
QKpsm.aww-7A-1	ENV3-1	90.2	CFA2028-WMC0083A	59.25	0.05	3.70
QEv.aww-7A-2	ENV2	94.2	WPT-5153-CFA2028	-0.14	0.03	3.23
QYld.aww-7A-1	ENV2	116.6	BARC0108-WMC0603B	-24.38	0.07	4.74
QYld.aww-7A-1	ALL EXPTS	116.6	BARC0108-WMC0603B	-27.83	0.11	7.27
QEv.aww-7A-1	ENV1	117.6	BARC0108-WMC0603B	-0.14	0.03	4.62
QYld.aww-7A-1	ENV2-1	117.6	BARC0108-WMC0603B	-34.09	0.11	5.89
QYld.aww-7A-1	ENV3	117.6	BARC0108-WMC0603B	-33.01	0.07	3.74
QYld.aww-7A-1	ENV3-2	117.6	BARC0108-WMC0603B	-33.37	0.12	7.97
QScr.aww-7A	ENV3	127	WPT-4744-WPT-4796	0.58	0.09	4.85
QScr.aww-7A	ENV3-2	127	WPT-4744-WPT-4796	0.78	0.09	5.04
QTkw.aww-7A	ALL EXPTS	131	WPT-4744-WPT-4796	-0.54	0.07	2.90
QYld.aww-7A-2	ENV2-2	164.9	BARC0292-GWM0332C	-18.27	0.04	2.92
QKpsm.aww-7A-2	ENV3-2	169.7	WPT-6013-WPT-0961	-85.80	0.05	3.75
QHi.aww-7A	STR08	169.7	GWM0746-WPT-5558	-0.007	0.07	3.87
QKpsl.aww-7A	ENV3	171.7	WPT-0961-WPT-4553	-0.038	0.11	5.78
QHi.aww-7A	ALL EXPTS	175.5	WPT-4553-CFA2019	-0.004	0.02	1.47
QEv.aww-7B	ENV1	13	BARC0338-STM0671ACAG	-0.08	0.06	3.53
QKpsm.aww-7B	ENV2-1	39.1	BARC0065-BARC0137	-100.99	0.06	4.11
QKpsm.aww-7B	ENV3-2	41.6	BARC0137-WMC0396	-108.76	0.08	5.49
QFll.aww-7B	ALL EXPTS	69.8	WMC0396-WPT-4230	-2.43	0.05	3.16
QTkw.aww-7D	ALL EXPTS	59.8	GWM295-WPT-4115	-0.24	0.04	2.83
QFll.aww-7D	ALL EXPTS	109	STM0001TCAC-GWM0437	1.86	0.02	4.20

Table 4. Quantitative trait loci detected for yield, yield components and various physiological traits in a subset of the RAC875/Kukri population of 260 doubled haploid lines. QTL name, environment cluster that it was detected in, position of the QTL peak (in cM) and marker interval on the respective chromosome of the population linkage map (as described by Bennett *et al.* 2011), effect (positive indicated RAC875 allele, negative indicated Kukri allele), variance accounted for by each QTL (in the case of detection in multiple treatments, maximum variance accounted for is shown) and LOD score of detection are also presented. From research article titled 'Detection of two major grain yield QTL in bread wheat (*Triticum aestivum* L.) under heat, drought and high yield potential environments'.

QTL	Experiment	Flanking markers	Position (cM)	Effect	r2	LOD
Q.Spad.aww-1A	ALL	GWM0357-WPT6074	89.8	0.26	0.02	3.53
Q.Flw.aww-1A	Irr	WPT6074-WMC0093	104.4	0.30	0.01	3.38
Q.Phys.aww-1B	All	WPT0240-BARC0008	55.3	0.13	0.02	2.92
Q.Wsc.aww-1B	Heat	WPT3679-BARC1138B	84.6	5.08	0.05	3.35
Q.Ctgf.aww-1B	Irr, Drt	WPT-0506-WMC0147	117.1	-0.05	0.05	2.08
Q.Ndvi.aww-1B	Drt	WPT0944-WPT4129	172.7	-0.00	0.08	3.81
Q.Tkw.aww-1D	Heat	CFD0027-WPT-1799	117.8	0.59	0.08	3.16
Q.Ctgf.aww-2A	Irr	WPT-7285-WPT-7056	46.4	3.37	0.01	2.47
Q.W.aww-2A	All	WPT4201-WMC632	80.7	-0.10	0.02	2.88
Q.Phys.aww-2A	All, Heat	CFA2263-WPT6361	86.8	-0.16	0.04	4.00
Q.Ndvi.aww-2A	Drt	GWM0382B-WPT9277	148.7	-0.00	0.03	2.71
Q.Eet.aww-2B	Irr, Drt, Heat	WPT7757-BARC0013A	37.1	-2.10	0.01	6.65
Q.Tkw.aww-2B	Irr, Drt, Heat	BARC0091-WPT-0335	52.7	0.51	0.08	4.72
Q.Vig.aww-2B	Heat	WPT7200-WPT5128	75.4	0.08	0.04	2.94
Q.Flw.aww-2B	Heat, Drt	WPT7200-WPT5128	76.4	0.38	0.06	3.27
Q.Spad.aww-2B	Drt	WPT7200-WPT5128	82.4	0.49	0.10	6.34
Q.Ndvi.aww-2B	Heat	CFD0050A-WPT3378	131.1	0.01	0.08	4.16
Q.W.aww-2D	All	BARC0095-WMC0111	0	-0.14	0.05	4.68
Q.Ctveg.aww-2D	heat	WPT-6003-PPD-D1	20.7	0.14	0.09	3.64
Q.Ctgf.aww-2D	heat	WPT-6003-PPD-D1	30.7	0.21	0.05	2.79
Q.Eet.aww-2D	Irr, Drt, Heat	PPDD1-WPT0330	35	-4.37	0.39	28.01
Q.Wsc.aww-2D	Irr	PPDD1-WPT0330	49	11.89	0.11	5.74
Q.Tkw.aww-2D	Irr	WPT-0330-BARC0328B	66.4	0.57	0.05	6.36
Q.Flw.aww-2D	Irr	BARC0328B-WPT6574	70.2	0.30	0.02	2.84
Q.Vig.aww-2D	Heat, Drt	BARC0328B-WPT6574	78.2	0.11	0.07	4.33
Q.Ndvi.aww-2D	Heat	WPT0021-WPT4559	109.9	-0.01	0.04	3.66
Q.Phys.aww-2D	All	WPT6343-WPT1301	130.6	-0.17	0.03	3.98
Q.Ctveg.aww-3A	heat	WPT-0714-GWM0002	32.1	0.08	0.04	3.72
Q.Tkw.aww-3A	Drt	GWM0002-BARC0328A	56.9	0.65	0.13	7.77
Q.Yld.aww-3A	Drt	GWM0002-BARC0328A	56.9	65.57	0.03	2.79
Q.Pdl.aww-3A	All	BARC0324-WPT4077	62.9	-3.70	0.10	5.50
Q.Wsc.aww-3A	Irr, Heat	BARC0324-WPT4077	62.9	5.76	0.08	6.45
Q.W.aww-3A	All	WMC0264-CFA2193B	91.3	0.64	0.68	101.19
Q.Phys.aww-3A	Irr	WPT4725-WMC169	151.6	0.29	0.07	3.38
Q.Spad.aww-3B	All, Irr, Drt	WPT7225-WPT7984	0	0.49	0.08	4.16
Q.Ctveg.aww-3B-1	Irr	BARC0147-CFA2226B	20.8	0.02	0.02	2.12
Q.Yld.aww-3B-1	Irr, Heat	WMC0043-WPT6973	73.1	-216.08	0.14	9.18
Q.Ctgf.aww-3B-1	Irr	WMC0043-WPT-6973	74.1	2.40	0.02	1.52
Q.Kpsm.aww-3B-1	Irr	WMC0043-WPT6973	74.1	-343.08	0.06	4.18
Q.Tkw.aww-3B	Heat	WPT-6973-WPT-9510	75.8	-0.45	0.06	3.87
Q.Ndvi.aww-3B	Heat	WPT6973-WPT9510	76.8	-0.01	0.10	5.52
Q.Wsc.aww-3B	Irr	WPT4401-WPT9368	176.7	-7.04	0.06	5.97

Q.Kpsm.aww-3B-2	Irr	WPT4401-WPT9368	181.7	419.44	0.09	5.30
Q.Ctgf.aww-3B-2	Irr	WPT-4401-WPT-9368	182.7	-4.71	0.05	4.09
Q.Flw.aww-2B	Heat	WPT8021-GWM0114B	190.7	0.50	0.10	6.08
Q.Kpsm.aww-3B	Heat	WPT8021-GWM0114B	190.7	347.15	0.17	10.65
Q.Pdl.aww-3B	All	WPT8021-GWM0114B	190.7	2.81	0.07	3.03
Q.Vig.aww-3B	Heat	WPT8021-GWM0114B	190.7	0.12	0.12	4.26
Q.Yld.aww-3B-2	Heat	WPT8021-GWM0114B	190.7	131.78	0.22	11.56
Q.Yld.aww-3B-2	Irr, Drt	WPT-8021-GWM0114B	192.7	110.18	0.12	4.07
Q.Ctgf.aww-3B	Heat	WPT-8021-GWM0114B	192.7	-0.42	0.21	3.16
Q.Ctveg.aww-3B-2	Irr	WPT-8021-GWM0114B	192.7	-0.03	0.04	2.94
Q.Kpsm.aww-3D	Drt, Heat	CFD0034-WMC0533	64.9	-213.17	0.11	6.90
Q.Yld.aww-3D	Drt, Heat	CFD0034-WMC0533	64.9	-72.97	0.04	4.46
Q.Vig.aww-3D	Drt	WMC0533-WPT6262	71.6	0.12	0.06	4.61
Q.Ndvi.aww-3D-1	Drt, Heat	WPT6262-WPT7894	94.1	-0.00	0.07	3.55
Q.Ndvi.aww-3D-2	Drt	BARC0071-GWM0114A	180.4	-0.00	0.02	3.05
Q.Yld.aww-4A	Irr	STM0092TCTG-WPT7001	0	106.06	0.03	2.79
Q.Ndvi.aww-4A	Drt	WPT7001-BARC0106	12.9	-0.01	0.07	4.31
Q.Vig.aww-4A	Drt	BARC0106-DUPW0328	18.1	-0.15	0.07	3.90
Q.Vig.aww-4A	Irr	BARC0170-GWM0637A	47.9	-0.16	0.04	2.86
Q.Spad.aww-4B	All, Irr, Drt	BARC0340A-GWM0495	0	0.58	0.09	4.91
Q.Ctgf.aww-4B	Irr	GWM0495-GWM0149	3.9	-2.53	0.02	1.28
Q.Pdl.aww-4B	All	WMC0047-BARC0114	37.5	2.66	0.06	2.84
Q.Phys.aww-4B	All, Heat	BARC0114-WPT0391	52.8	0.27	0.05	4.18
Q.Ndvi.aww-4B	Heat	WPT0391-WPT3608	53.1	0.01	0.08	3.40
Q.Spad.aww-4D	All, Drt, heat	GWM0297B-WMC0457	5	-0.34	0.10	3.35
Q.Yld.aww-4D	Heat	WMC0457-BARC0288	5.5	-57.09	0.04	3.33
Q.Eet.aww-5A	Drt, Heat	GWM0186-WPT1370	110	1.40	0.01	3.79
Q.Phys.aww-5A	All	CFA2141-WPT5231	164.7	-0.28	0.08	4.24
Q.Kpsm.aww-5A	Heat	CFA2141-WPT5231	165.7	-176.91	0.05	3.29
Q.Pdl.aww-5A	All	GWM0126-STM0627ACAGB	198.2	-2.81	0.05	3.23
Q.Flw.aww-5B	Irr	WPT5914-BARC0216	47.8	0.39	0.08	3.96
Q.Ctveg.aww-5B	Irr	BARC0004B-GWM0540A	55.7	-0.03	0.04	2.81
Q.Vig.aww-5B	Drt	BARC0088-WPT4936	56.6	0.16	0.07	3.74
Q.Ndvi.aww-5B	Drt	BARC0088-WPT4936	61.6	0.01	0.09	8.14
Q.Yld.aww-5B	Irr	BARC0088-WPT4936	61.6	96.97	0.04	3.23
Q.Flw.aww-5B	Heat	BARC0088-WPT4936	68.6	0.36	0.05	2.94
Q.Wsc.aww-5B	Irr	WPT4936-WPT3457	72.1	7.01	0.06	3.74
Q.Tkw.aww-5B	Drt	WPT-4936-WPT-3457	83.1	0.60	0.13	7.84
Q.Kpsm.aww-5B	Drt	WPT3457-GWM0271B	83.9	-66.87	0.06	4.24
Q.Spad.aww-5B	All, Irr	WPT3457-GWM0271B	93.9	0.76	0.08	5.32
Q.Eet.aww-5B	Heat	WPT9103-WMC0099	144	0.62	0.03	2.90
Q.Ctveg.aww-5D	Irr	KSM0023D-WPT-5870	17	-0.02	0.02	1.80
Q.Spad.aww-6A-1	Irr, Drt	WPT2636-WPT8266	15.2	-0.67	0.06	5.74
Q.Ctveg.aww-6A	Irr	WPT-3965-WPT-7127	41.7	-0.02	0.02	2.68
Q.Flw.aww-6A	Irr	WPT7599-BARC0118	76.5	0.79	0.20	10.74
Q.Spad.aww-6A-2	All, Drt	WPT7599-BARC0118	77.5	-0.34	0.06	6.90
Q.Phys.aww-6A	All, Irr	BARC0118-WMC0256A	81.8	0.28	0.13	6.60
Q.Tkw.aww-6A	Irr	WMC0256A-GWM0169	95.2	0.51	0.08	5.67
Q.Ctgf.aww-6B	Drt	WPT-3118-WPT-2095	0	-0.05	0.02	1.75
Q.Spad.aww-6B-1	All, Drt	WPT1700-WPT3060	69.1	-0.17	0.03	4.37
Q.Phys.aww-6B	Heat	BARC0247-BARC0134	117.7	-2.96	0.06	3.48
Q.Spad.aww-6B-2	All, Irr, Heat	BARC0134-WPT5480	145.2	-0.39	0.06	3.70
Q.Tkw.aww-2B	Drt	BARC0134-WPT-5480	149.2	-0.36	0.03	3.61
Q.Vig.aww-6B	Irr	WPT9423-WPT6116	163.5	-0.19	0.07	3.40
Q.Spad.aww-6D	All	CFD0287-BARC0204	81.1	0.22	0.02	2.94

Q.Phys.aww-6D	All, Irr	BARC0204-WMC0278	96.9	0.31	0.08	5.45
Q.Yld.aww-7A-1	Heat	WPT9207-WPT4748	1	-64.66	0.04	3.01
Q.Ctveg.aww-7A	Heat	WPT-5153-CFA2028	76.7	0.08	0.02	2.75
Q.Eet.aww-7A-1	Irr, Drt, Heat	CFA2028-WMC0083A	90.2	-1.17	0.04	5.74
Q.Ndvi.aww-7A	Heat	CFA2028-WMC0083A	90.2	-0.00	0.02	3.16
Q.Eet.aww-7A-2	Irr	BARC1004-BARC0174	107.4	-1.15	0.03	5.26
Q.Yld.aww-7A-2	Drt	BARC1004-BARC0174	109.4	73.88	0.06	3.07
Q.Tkw.aww-7A	Irr	BARC0108-WMC0603B	115.6	0.46	0.08	5.35
Q.W.aww-7A	All	BARC0108-WMC0603B	115.6	0.09	0.05	4.18
Q.Kpsm.aww-7A	Irr	BARC0108-WMC0603B	115.6	-378.32	0.07	5.65
Q.Wsc.aww-7B	Heat	WPT9887-WPT0745	0	6.34	0.07	4.85
Q.Spad.aww-7B	All, Irr	WPT0745-BARC0338	3.6	0.34	0.05	3.10
Q.Eet.aww-7B	Drt	BARC0338-STM0671ACAG	7	-1.08	0.07	4.03
Q.Phys.aww-7B	All, Irr	BARC0338-STM0671ACAG	15	-0.19	0.05	3.81
Q.Eet.aww-7B	Irr, Heat	GWM0297-BARC0065	32.6	-1.82	0.11	5.54
Q.Wsc.aww-7B	Irr	GWM0297-BARC0065	32.6	7.24	0.08	3.48
Q.Flw.aww-7B	Irr, Drt	BARC0137-WMC0396	41.6	-0.45	0.12	7.21
Q.Tkw.aww-7B	Irr	WPT-4230-WMC0517B	48.8	-0.31	0.06	3.68
Q.Ctveg.aww-7B	Heat	WPT-4230-WMC0517B	48.8	0.09	0.07	2.64