# Genotypic variation in rough-seeded lupins (*Lupinus pilosus* Murr. and *L. atlanticus* Glads.) for tolerance to calcareous soils

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This thesis is dedicated to the loving memory of my mother

Julie Ann Brand

1st July 1951 - 9th January 1999

'Serenity'

In God's care

.....

**Plate 1** *Lupinus pilosus* in the field, the plant and the flower.

# Table of Contents

# Abstract

Lupins are a widely cultivated pulse crop in the southern Australian cereal cropping zone, being commonly used in rotational farming systems with cereal and oilseed crops. Varieties of the cultivated species (*Lupinus angustifolius, L. albus and L. luteus*) are well adapted to acidic to neutral soils, but not to calcareous soils, developing symptoms resembling Fe chlorosis which results in severe grain yield reductions or death. Currently the only alternative legumes on calcareous soils in lower rainfall areas are field pea or pasture, the cultivation of which are limited due to environmental and economic shortcomings. Thus two undomesticated species of lupins (*L. pilosus* and *L. atlanticus*), which have been collected from calcareous or alkaline soils in the wild are being domesticated and developed for agriculture. However, neither species has been investigated for intraspecific variation in tolerance to calcareous soil. The aims of this research were to assess the tolerance of *L. pilosus* to calcareous soils, to identify intraspecific variation through the development of screening methods and to propose physiological and genetic reasons for genotypic differences.

On a wide range soils *L. pilosus* was more broadly adapted than *L. angustifolius* and showed less chlorosis and dry weight yield reductions on calcareous soils compared with non-calcareous soils. *Lupinus pilosus* also showed similar or improved growth on the more acidic soils compared with *L. angustifolius*, indicating that this species will be particularly useful in agricultural areas where small outcrops of calcareous soil occur within a paddock.

In preliminary studies on a soil with 50% $CaCO_3$, wild types and landraces of *L. pilosus* had a range of tolerance from tolerant to intolerant. Thus soil and solution screening methods were developed that provided the greatest discrimination between genotypes. The most suitable soil screen was in a field soil (Wangary calcareous sand; 50% $CaCO_3$) with a moisture content of 90% of field capacity. The addition of nutrients to the soil and the use of inorganic nitrogen compared with inoculation did not affect the ranking of genotypes. The solution screen used 15mM of $KHCO_3$ with $CaCO_3$ as a buffer. In both soil and solution methods chlorosis recorded 21 days after sowing or transplanting appeared most indicative of likely grain yield reductions associated with intolerance.

A range of species and genotypes within species were screened in both methods. The most tolerant species was *L. pilosus*, with a number of genotypes being tolerant to moderately intolerant. *Lupinus pilosus* also showed the greatest range of variation in tolerance. Most genotypes of *L. atlanticus* appeared moderately intolerant to intolerant, although a few genotypes showed significantly less chlorosis. There were no fully tolerant genotypes of *L. albus*, although there was variation from moderately tolerant to moderately intolerant, and all genotypes of *L. angustifolius* were intolerant. To confirm glasshouse screening results, a range of *L. pilosus* genotypes were tested on structurally and chemically similar calcareous and non-calcareous soils under field conditions. Results showed that the tolerant to moderately tolerant genotypes had higher relative grain yields than moderately intolerant to intolerant genotypes.

The major factor affecting lupin growth in calcareous soils is high concentrations of bicarbonate in the soil solution. This study suggests that chlorosis in intolerant genotypes of *L. pilosus* was related to a number of factors including bicarbonate-induced Mn and Fe deficiency and Ca toxicity. Also bicarbonate may be taken up into the plant, transported to leaves and have a direct effect on chlorophyll formation. Physiological work indicated that the most probable mechanisms of tolerance in *L. pilosus* were either exclusion of bicarbonate by the roots or rapid sequestering or neutralization of the ion once inside the plant. There also appeared to be two levels at which the mechanisms function; in fully tolerant genotypes the mechanism was likely to be functioning from germination whilst in moderately tolerant to moderately intolerant genotypes the mechanism was 'switched on' in response to the soil environment. Furthermore, preliminary genetic studies indicated that tolerance is related to one or two major, partially recessive genes with many minor genes having additive effects.

In conclusion, screening of both *L. pilosus* and *L. atlanticus* genotypes is necessary in a breeding program to develop cultivars suitable for highly calcareous soils. *Lupinus pilosus* appeared to have the greater potential, and in field studies grain yields were up to 30% greater than *L. angustifolius* on non-calcareous soils and up to 100% greater on calcareous soils. However, both species appear to have suitable material within the collections that will enable the development of lupin varieties tolerant to calcareous soils.

# Declaration

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

I give consent for this thesis being made available for photocopy and loan.

J. D. Brand

Date

3/11/00

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# Publications

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# CHAPTER 1

## General introduction

Lupins are a pulse crop used in rotational farming systems with cereals and oilseeds to provide a pest and disease break (Landers, 1991) and add mineral nitrogen to the soil (Rowland, *et al.*, 1988). In the southern Australian cereal zone approximately 1.2 million hectares are sown annually. The two major species of lupin cultivated in Australia are: *Lupinus angustifolius* L. (narrow-leafed lupin) on the sandy infertile, acidic/neutral soils most common in low rainfall areas (> 350-400 mm) of Western Australia, and *L. albus* (white lupin) on the more fertile, neutral, heavier textured soils in higher rainfall areas (ABARE, 1999). Small areas of *L. luteus* are also cultivated and in some areas *L. cosentinii* is used as a self-regenerating pasture in Western Australia. All these species, except *L. cosentinii*, are poorly adapted to calcareous soils, displaying symptoms of chlorosis in the younger leaves and stunting, ultimately resulting in a severe or total yield loss.

In south-eastern Australia there are large areas of calcareous soils, particularly in low rainfall zones; in South Australia approximately 70-80% of the cropping land is classed as calcareous (D Maschmedt, pers. comm.). The most common pulse grown in these areas is field pea (*Pisum sativum*). However, this species has limitations of a sprawling growth habit conducive to high levels of leaf and stem disease and a weak stem base leaving the soil exposed to erosion after harvest. Alternatively, medic pastures can be used as a disease break in conjunction with grazing animals, but currently this option is not economically viable in many areas due to the low prices of meat and wool. Lupins are a potential alternative to peas having strong erect stems for ease of harvest and prevention of erosion, while still providing a pest and disease break resulting in substantial increased yields in subsequent cereal crops (Henderson, 1989).

Two undomesticated species (*L. pilosus* Murr. and *L. atlanticus* Glads.), found on calcareous or alkaline soils in the wild, are being developed to increase the area that can be sown to lupins in Australia. Preliminary field trials generally have indicated that the wild types and landraces of both species show less chlorosis and have higher yields on calcareous soils compared with the currently cultivated species of lupins (Egan and Hawthorne, 1994), however there had been no research identifying intraspecific variation for tolerance to calcareous soils. *L. pilosus* would be expected to show a range of tolerance for calcareous soils as wild types and landraces have been

collected throughout the circum-Mediterranean on calcareous and non-calcareous soils (Clements and Cowling, 1990). The range of variation in *L. atlanticus* is likely to be smaller as all the genotypes collected were found in the Atlas mountains of Morocco (Clements and Cowling, 1990).

To identify intraspecific variation the major limitations to growth must be identified and screening protocols developed that reliably predict grain yield responses in the field. In previous studies the major factor causing chlorosis in calcareous soils has been shown to be the elevated bicarbonate concentrations in the soil solution which induce Fe deficiency in other legume species, by either preventing its uptake or translocation (Coulombe, *et al.*, 1984a; Chaney, *et al.*, 1992a). Also calcareous soils are likely to show many nutritional deficiencies, such as Mn, Zn, P and Cu, while in South Australia most calcareous soils also have associated toxicities of B and sodicity in the subsoil (Reuter, *et al.*, 1973; Mengel and Geurtzen, 1986; Fairbanks, *et al.*, 1987; Rashid, *et al.*, 1990; Rimmer, *et al.*, 1993; Marschner, 1995; Cakmak, *et al.*, 1997).

The work reported here was modeled on the techniques developed to breed for B tolerance in wheat and bicarbonate induced Fe deficiency tolerance in soybeans. For B toxicity in wheat, Chantachume, *et al.* (1995) has developed a filter paper method and Campbell, *et al.* (1998) a solution culture method which efficiently identify breeding lines with improved tolerance. In soybean both soil and solution based screening methods have been developed that identify breeding lines more tolerant to bicarbonate-induced Fe deficiency (Byron and Lambert, 1983; Coulombe, *et al.*, 1984b; Chaney, *et al.*, 1992a). The incorporation of genes that conferred tolerance to calcareous soil into high-yielding soybean cultivars resulted in yield benefits of between 5 and 100%, depending on the initial susceptibility of the cultivar (W Fehr, pers. comm.).

The aims of this research were to:

1. identify the tolerance of *L. pilosus* and *L. angustifolius* to a range of South Australian soils.
2. develop soil and solution screening methods to identify intraspecific variation that related to rankings to field observations.
3. propose mechanisms of tolerance and identify the number of genes controlling the trait.

# CHAPTER 2

## Literature review

## 2.1 Lupins

Lupins are a pulse crop used in rotational farming systems with cereals and oilseeds to provide a pest and disease break (Landers, 1991) and add mineral nitrogen to the soil (Rowland, *et al.*, 1988). They can be harvested as a grain crop and the stubble grazed for forage. The seed is generally used in stock feed.

### 2.1.1 Species and distribution (Taxonomy)

Polhill (1976) included the genus *Lupinus* L. in the tribe Genisteae of the family Fabaceae, however Plitmann (1981) suggested that the genus represents a separate entity intermediate between the Genisteae and Thermopsideae tribes. There are approximately 200 species of *Lupinus* distributed throughout the New and Old Worlds. It is the 12 species of Old World lupins that are of most interest in agriculture because of their large seeds, erect vigorous growth and adaptation to Mediterranean climates (Table 2.1). Only one New World lupin, *L. mutabilis*, is cultivated in some South American countries for its protein and oil, but further expansion of its growing areas appears limited (Gladstones, 1994; B Buirchell, pers. comm.).

**Table 2.1.** Main differences between Old and New World Lupins. (from Plitmann, 1981).

| | Old World | New World |
|---|---|---|
| Habit | Annuals, herbaceous. | Annuals and perennials, sometimes with woody stems. |
| Leaves | 5-or more leaflets. | 1-3-5 or more leaflets. |
| Fruits | Large, usually over 3x1 cm. | Small, usually narrower or shorter. |
| Seeds | Large (> 5 mm in diam., seed sizes > 60 mg). | Small (< 5 mm in diam., seed sizes < 60 mg). |
| Alkaloids | Mostly commonly lupinine, lupanine, hydroxylupanine (and LA4, LP4). | Mostly commonly lupinine, lupanine and sparteine. |
| Chromosome number | 2n = 32,36,38,40,42,50,52. | 2n = 48 (36). |
| Reproductive biology (and hybridization) | Self-pollinated. No interspecific hybridization. | Cross pollinated and self-pollinated. Hybridization occurring. |
| Habitats | Often secondary or disturbed. | Primary and secondary. |
| Climatic zones | Mediterranean. | Mediterranean, temperate, tropical, subalpine. |

The Old World lupins are endemic to Mediterranean and Northern African countries including Turkey, Syria, Iran, Israel, Hungary, Greece, Lebanon, Jordan, and Morocco (Table 2.2) (Gladstones, 1974; Clements and Cowling, 1990). They are generally found on infertile soils and display a range of adaptation to soil physical and chemical characteristics (Clements and Cowling, 1990). Landraces and wild types of the agriculturally important species *L. angustifolius* and *L. luteus* are most commonly found on sandy acidic soils, *L. albus* on neutral, fertile loams, *L. cosentinii* on sandy calcareous soils and *L. pilosus* and *L. atlanticus* are common on loam to clay calcareous and/or alkaline soils (Clements and Cowling, 1990). Old World lupins also have broad range of adaptation to rainfall and topography being found from coastal regions to mountainous areas and in rainfall zones ranging from 100 to 1200mm (Table 2.2)(Clements and Cowling, 1990).

### 2.1.2 Morphology

In contrast to the other Old World lupins which are morphologically diverse (that is, both broad and narrow-leafed species occur and different growth habits can be observed), the rough-seeded group (*L. pilosus*, *L. atlanticus*, *L. cosentinii*, *L. digitatus*, *L. palaestinus*, *L. princei* and *L. somaliensis*) have similar characteristics. Detailed morphology of each of the species has been reviewed by Gladstones (1974), hence I will summarise here only the features that are likely to be of importance in this project.

Table 2.3 outlines the range of flower colour, flowering time, height, seed weight and number of seed per pod of the agriculturally important lupin species. Other relevant characteristics are (Gladstones, 1974):

1. all species when mature have a strong thick stem which enables easy harvesting with conventional machinery such as that used on a cereal farm.
2. *Lupinus pilosus*, *L. atlanticus* and *L. luteus* display an early rosette growth pattern compared with the erect growth pattern of *L. angustifolius*, *L. albus* and *L. cosentinii*.
3. undomesticated genotypes of all species generally have dehiscent (shattering) pods and produce bitter (high alkaloid) seeds that are impermeable to water.
4. when comparing the two rough-seeded species of interest in this thesis, *L. pilosus* flowers earlier than *L. atlanticus*, but the pods on *L. atlanticus* mature more quickly allowing it to be harvested at a similar time to *L. pilosus*.

**Table 2.2** Ecological distribution and attributes of landraces and wild types of the Old World lupins (Buirchell, 1993; Clements and Cowling, 1990; Gladstones, 1974).

| Species | 2n | Distribution | Soil pH | Rainfall (mm) | Altitude (m) | Attributes |
|---|---|---|---|---|---|---|
| *L. angustifolius* | 40 | circum-Mediterranean: Spain, Portugal, France, Italy, Morocco, Egypt, Israel, Syria, Turkey. | 5.0-8.5 | 200-1200 | 5-1800 | Narrow-leafed, high yielding, large number of pods, domesticated. |
| *L. albus* | 50 | Greece and Crete, Albania, S. Yugoslavia. | 5.0-8.0 | 450-1000 | 10-2460 | Large white seed, suited to higher rainfall areas, domesticated. |
| *L. luteus* | 52 | Western coastal region of the Iberian Peninsula. | 5.5-7.4 | 400-1100 | 10-320 | Tolerant of acidic soil, moderate yield, domesticated. |
| *L. hispanicus* | 52 | S. and Central Spain, to about 1200m. | 4.5-7.0 | 450-1500 | 150-1250 | Undomesticated, good adaptation to waterlogging. |
| *L. micranthus* | 50 | Relatively rare; circum-Mediterranean: Spain, Portugal, S.E. France, Sicily, Italy, Greece, Turkey, etc. | 5.8-8.5 | 300-1000 | 10-1200 | Undomesticated, although used as green manure on calcareous soils in Greece. |
| *L. pilosus* | 42 | E. Mediterranean, coastal to 1200m, Greece, Turkey, Syria, Israel. | 5.5-8.7 | 350-2000 | 5-1100 | Undomesticated, early flowering, early vigour, slow maturity, high yield, tolerates free lime. |
| *L. atlanticus* | 38 | Morocco, Anti Atlas and foothills of High Atlas mountains. | 6.5-9.5 | 200-650 | 450-1630 | Undomesticated large number of pods, high yielding, late flowering, rapid maturity. |
| *L. cosentinii* | 32 | Coastal areas of West Mediterranean, Morocco, Portugal. | 6.5-9.0 | 100-700 | 10-800 | Moderate yield, early vigour/maturity, tolerates free lime, fully domesticated. |
| *L. digitatus* | 36 | N. Africa sub Saharan, Egypt, Niger, Algeria Senegal. | | | | Undomesticated, desert ephemeral, drought resistant. |
| *L. palaestinus* | 42 | Central and S. Israel, Sinai Peninsula, semi-arid to desert regions. | 7.7 | 500 | | Undomesticated, low shrub, early flowering, insect pollinated. |
| *L. princei* | 38 | Equatorial, highlands of Kenya, Tanzania and S. Ethiopia. | 5.7 | 580-1000 | 2100-2460 | Undomesticated, tall, very late flowering, requires different rhizobia. |
| *L. somaliensis* | ? | Golis Range in NW Somalia. | | | | Unknown, may be extinct. |

**Table 2.3** Range of flower colour, flowering time, height, seed weight and number of seed per pod of six of the agriculturally important lupin species (domesticated and undomesticated genotypes included) (Clements and Cowling, 1991).

| Species | Flower colour[a] | Flowering time (days) | Height (cm) | 100 seed weight (g) | Number seed per pod |
|---|---|---|---|---|---|
| *L. angustifolius* | 1, 2, 5, 6, 7, 8, 9 | 75-120 | 50-110 | 5-19 | 4-6 |
| *L. albus* | 1, 2, 3, 4, 5, 6, 7, 8 | 75-120 | 80-135 | 20-60 | 5-6 |
| *L. luteus* | 8, 10, 11 | 75-150 | 45-110 | 7-15 | 4-6 |
| *L. cosentinii* | 3, 7, 8 | 85-135 | 65-100 | 6-23 | 4-6 |
| *L. pilosus* | 1, 5, 9 | 75-130 | 65-100 | 32-76 | 3-5 |
| *L. atlanticus* | 8 | 100-120 | 60-80 | ~30 | 4-6 |

[a] 1. pure white, 2. white, 3. white, blue tinge, 4. white, purple tinge, 5. pink, 6. mauve, 7, light blue, 8. blue, 9. purple, 10. light yellow, 11. golden yellow.

Root morphology was also variable among species (Clements, *et al.*, 1993). Root systems of *L. angustifolius* consisted of a prominent taproot and a relatively high number of primary lateral roots, but few secondary laterals. In *L. albus*, *L. cosentinii* and *L. luteus* there was a greater proportion of secondary lateral roots than *L. angustifolius* and in *L. pilosus* and *L. atlanticus* the primary, secondary and tertiary lateral roots were more dominant than the taproot (Clements, *et al.*, 1993). Also *L. albus*, *L. cosentinii*, *L. pilosus* and *L. atlanticus* produce proteoid roots (Section 2.3.2), which may enhance the uptake of P in deficient soils (Gardner, *et al.*, 1981). These differences in root morphology may help to explain the differences in adaptation between the species. A taproot dominant root system appears suited to deep sandy soils where penetration of the taproot enhances the extraction of water at depth within the profile (Clements, *et al.*, 1993). If penetration of the taproot is restricted, such as on loam and clay loam soils, a lower density of secondary and tertiary laterals may limit the efficient uptake of soil water and nutrients (Dracup, *et al.*, 1992; Clements, *et al.*, 1993).

### 2.1.3 Lupins in agriculture

The cultivation of *L. albus* may have been practised in Egypt as early as 2000 B.C. and was well established in classical Greek and Roman times (Gladstones, 1970). The species was recognised for its ability to grow on poor infertile soil, together with their value for soil improvement (Gladstones, 1970). The plants could be green manured and grazed for stock feed, and the seed harvested for human consumption after boiling and prolonged steeping to rid them of their bitter alkaloids (Gladstones, 1960). Until recent domestication (permeable seed coat, indehiscent pods, low-alkaloid content and early flowering), *L. albus* had evolved from a wild type into a large-seeded plant with indehiscent (non-shattering) pods and a permeable seed coat. It retained its

high alkaloid content which would have been agronomically advantageous in primitive agriculture, providing protection against grazing and some insect pests (Gladstones, 1970). All other lupin species also appear to have been cultivated at some time in history without ever becoming fully domesticated (Gladstones, 1970). For example, *L. pilosus* has extremely large seeds with relatively low alkaloids that would be unexpected in a wild plant.

Consistent with a role under primitive cultivation all annual species of lupins tend to grow in thickets, thus seeds are easily collected (Gladstones, 1970). They are known to colonize disturbed or roughly cultivated places, such as would have been found around prehistoric encampments (Gladstones, 1970). Also most lupin species are adapted to soils of low fertility on which the earliest clearing and cultivation most likely took place (Gladstones, 1970).

(Gladstones, 1970) suggests that with the rise of civilisation and the development of implements which made it possible to cultivate the heavier and more fertile soils, the cultivation of lupins declined in favour of crops more amenable to domestication and conventional agricultural practices. However, it was the ability of lupins to persist and produce seed under adverse conditions which in the last few centuries has provoked interest in their agricultural potential (Gladstones, 1970), hence the domestication of *L. angustifolius*, *L. albus*, *L. cosentinii* and *L. luteus* earlier this century and currently *L. atlanticus* and *L. pilosus*.

The expansion of modern lupin growing started in 1780, when King Frederick II of Prussia personally supervised experiments on the cultivation of *L. albus* (Gladstones, 1970). In 1841, a German farmer grew *L. luteus* and found these to be far better adapted than *L. albus*, despite their propensity to pod-shattering and their impermeable seeds (hard seededness) (Gladstones, 1970). At the end of the 18$^{th}$ century the area sown to lupins (mostly *L. luteus*) in Germany was in excess of 300,000 hectares, although outbreaks of lupinosis in sheep in the 1860's and 70's resulted in a sharp fall in the area of lupins grown for sheep feed (Gladstones, 1970). In the first decades of the 20th century the area sown to lupins declined rapidly due to the availability of inexpensive nitrogen fertilizer and a decline in the profitability of the wool industry (Gladstones, 1970). During the First World War, the need for high protein foodstuff increased, as there was a shortage of food for the soldiers. As lupins have a high protein seed, interest in developing more efficient methods of removing the alkaloids increased, so that lupin flour could be used as a protein additive in bread making (Sengbusch, 1938). Breeding of lupins led to an alkaloid free 'sweet' lupin being selected in 1928-29 (Section 2.1.6) (Gladstones, 1970).

The introduction and naturalisation of lupins in Australia is detailed in Gladstones (1994). It is believed that *L. polyphyllus*, a small seeded ornamental perennial now naturalized to Tasmania and highland Victoria, was the first lupin species introduced (Gladstones, 1994). The first annual Old World species introduced was *L. cosentinii* during the mid 19th century. It was cultivated for green manuring or the seed was harvested and ground into flour for use in bread-making in Western Australian (Gladstones, 1994). *L. cosentinii* is now naturalized on the coastal sand dunes and is commonly referred to as the Western Australian sandplain lupin (Gladstones, 1994). It has been grown in these areas as a self regenerating crop (annual pasture) for sheep and soil improvement (B Buirchell, pers. comm.). Gladstones (1994) also describes two lines of *L. angustifolius* which are naturalized to Western Australia; P20639, which resembles material collected in Portugal and is common from Perth to north of Gingin, and a soft seeded line from around Bunbury, which closely resembles the cultivar, New Zealand Blue (probably a soft-seeded selection from northern European varieties). The other cultivated species, *L. albus* and *L. luteus* have become semi-naturalized to the high rainfall zones of south west Western Australia, where they had been previously cultivated (Gladstones, 1994).

The species *L. angustifolius*, which is now widely cultivated throughout the Western Australian cereal belt and in many parts of South Australia, western Victoria and New South Wales, was introduced into Australia in the 1930's and 40's and, initially, was mainly grown for green manuring of orchards and vineyards (Gladstones, 1994).

The beneficial aspects of lupins in rotations on cereal farms ranges from providing alternate strategies for weed and pest control to improved soil nutrition and structure. For example, populations of grass weeds which build up during the cereal phase of the rotation can be reduced during the lupin phase of the rotation by the application of selective herbicides (Gill, 1994). Reeves, *et al.* (1984) concluded that the incidence of diseases such as take-all (*Gaumannomyces graminis*) and *Fusarium* spp. were significantly reduced in a wheat crop following a lupin crop. In addition, it has been suggested that lupins can increase soil nitrogen reserves, thus the wheat crop in a wheat - lupin rotation will have a much higher yield than the wheat in a continuous wheat rotation without the addition of inorganic N fertilizers (Rowland, *et al.*, 1988). Another nutritional benefit of lupins in rotation is from proteoid roots that some species (*L. albus*, *L. cosentinii*, *L. pilosus* and *L. atlanticus*) produce (Clements, *et al.*, 1993). Citric acid released from these roots enhances the mobilization of P, Fe and Mn (Gardner, *et al.*, 1981; Dinkelaker, *et*

*al.*, 1989; Bolland, 1995) and may also increase the availability of these nutrients to the following crop.

Australia is now the largest lupin producer in the world. The major species of lupin grown is *L. angustifolius* (approx. 95 % total production). There are smaller areas of *L. albus* (5 % total area) for stock feed and human consumption and more recently *L. luteus* has been released for its adaptation to highly acidic soils in Western Australia. In the past *L. cosentinii* had been grown on some of the sandy calcareous soils in Western Australia, but currently is only used as a volunteer pasture for stockfeed (W Cowling, pers. comm.). In total the area sown to lupins has increased from 5000 hectares in 1969 to 1215000 hectares in 1998 and the production has increased from 55000 tonnes in 1979 to 1380000 tonnes in 1998 (Fig. 2.1). The major increase in area has taken place in Western Australia, where acidic to neutral sandier soils are common and *L. angustifolius* can be grown widely. In Western Australia lupins are now the major pulse crop. In South Australia, Victoria and to a lesser extent New South Wales production is limited due to a large proportion of the soils in the cereal belt being calcareous. For example, in South Australia, approximately 70% of the arable cropping soils are calcareous, hence only 70000 to 90000 hectares are sown to lupins annually (producing 70000 - 90000 tonnes; Fig. 2.1), which is approximately half the level of production of field peas which are relatively well adapted to calcareous soils.

World production of lupins has risen from approximately 300,000 tonnes in 1980 to 1.5 million tonnes 1998 due to the large areas harvested and quantity of seed produced in Australia (Fig. 2.2a). Throughout the rest of the world lupin production has either declined or remained constant. For example the USSR (Russian Federation from 1991 to 1998) was the major producer of lupins until the late 1970's, often producing more 500,000 tonnes, but currently it only produces 12,000 tonnes. The total areas harvested peaked in the early 1960's when the USSR was the major producer, but steadily declined until the 1980's, when the areas sown in Australia rapidly increased (Fig. 2.2b). From these graphs it can be seen that yields (t/ha) have also increased, from approximately 0.5 - 0.7 t/ha to 1.0 - 1.2 t/ha, largely due to the breeding advances in Australia over the last 30 years.

**Figure 2.1** (a) Australian production of lupins from 1979 to 1998 and (b) total area harvested from 1969 to 1998 (ABARE, 1999).

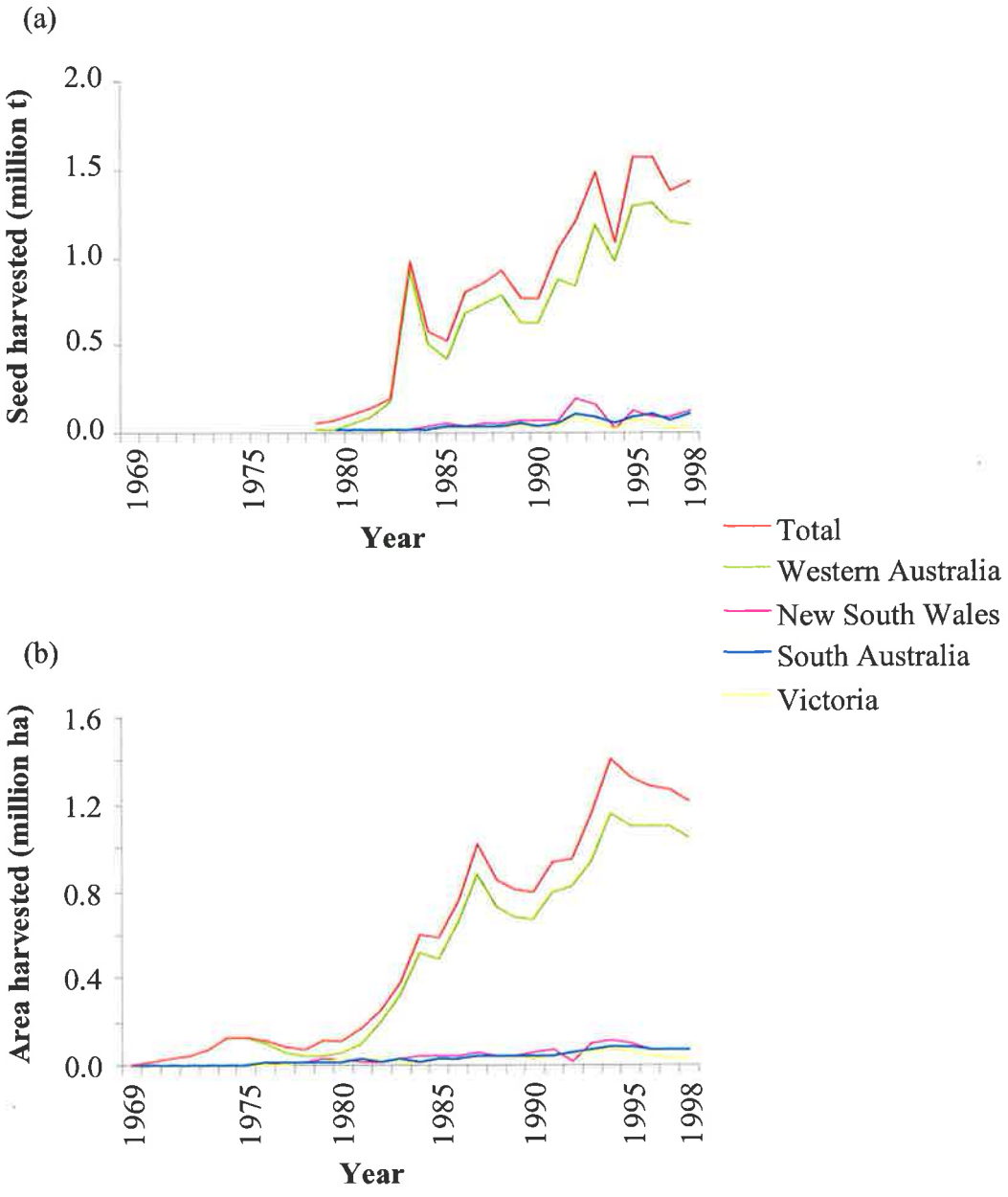
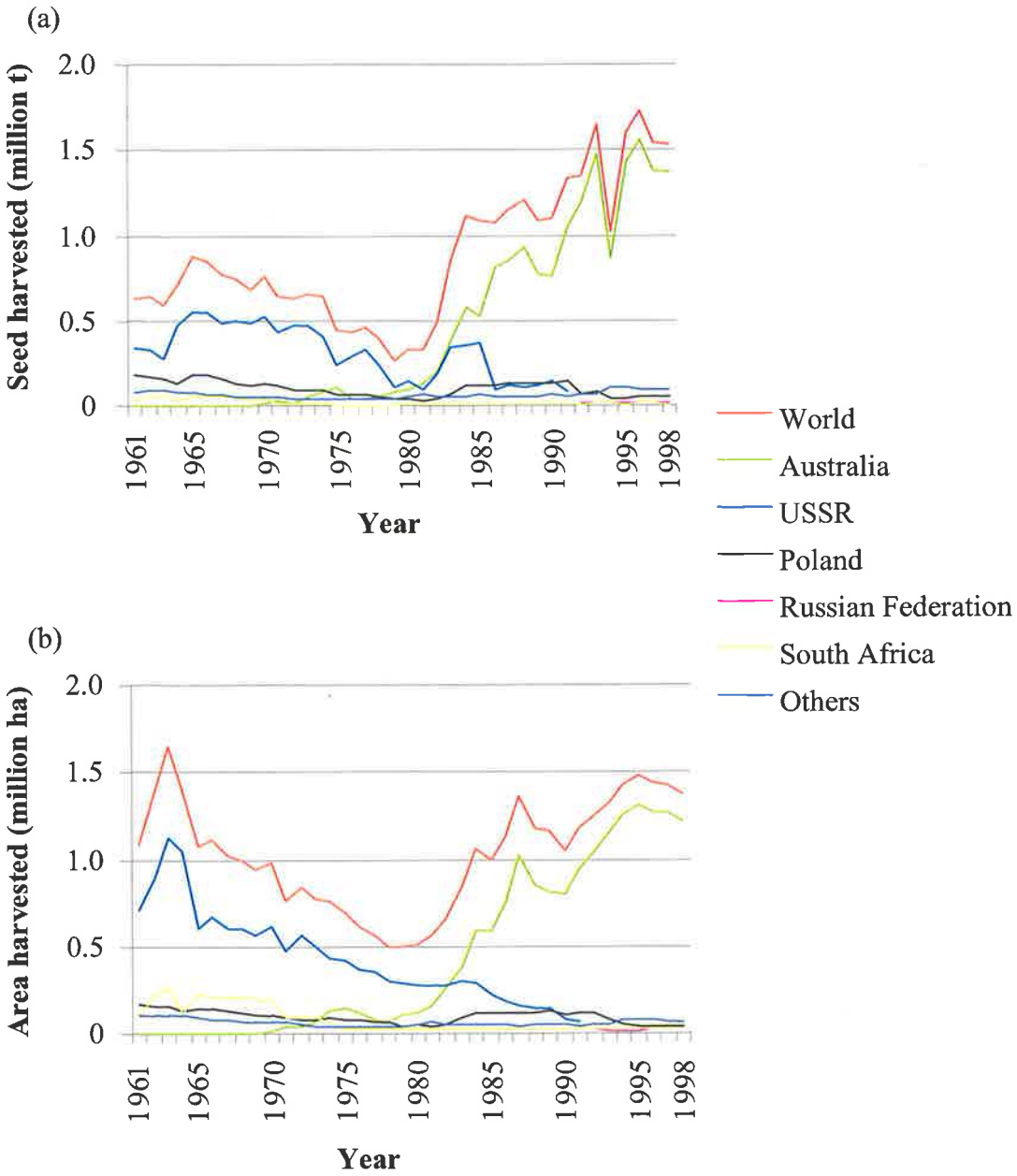


**Figure 2.2** (a) World production of lupins and (b) total area harvested from 1961 to 1998 (FAOSTAT database, 1999). Note: The USSR became the Russian Federation in 1991.



### 2.1.4 Diseases and pathogens of lupins

*Fungal*

Lupin species vary in their susceptibility to a number of fungal pathogens including phomopsis, brown leaf spot, pleiochaeta root rot, rhizoctonia and grey leaf spot and anthracnose (Table 2.4). The most important of these until recently was phomopsis stem blight (*Diapotle woodii* punith.) which can lead to stubble toxicity and lupinosis in sheep (Cowling, *et al.*, 1986). The outbreak of anthracnose (*Colletotrichum gleosporiodes*) in Western Australia and South Australia in 1996 caused major crop losses (grain yields reduced by up to 100% and in many cases affected crops were used as a green manure), especially in the highly susceptible *L. albus* varieties (Sweetingham, 1997). The major symptoms are bending and twisting of stems with a lesion in the crook of the bend (Sweetingham, 1997). Brown leaf spot and pleiochaeta root rot are both caused by the fungus *Pleiochaeta setosa* (Landers, 1991; Sweetingham, 1993). Brown leaf spot leads to irregular brown spots and leaf drop (Landers, 1991), whereas pleiochaeta root rot attacks the root system, stunting growth and killing the seedling (Sweetingham, 1993). Rhizoctonia (*Rhizoctonia solani*) occurs as several strains which cause quite different diseases in lupins (Sweetingham, *et al.*, 1993). All strains can cause serious damage to crops resulting in reduced yields. Grey leaf spot (*Stemphylium vesucarium*) is now not as significant, since resistance has been incorporated into commercial varieties (Landers, 1991).

*Viral*

There are two major viral pathogens of lupins, cucumber mosaic virus and bean yellow mosaic virus. Both are transmitted non-persistently by aphids and are seed-borne in some lupin species (Jones and Latham, 1996). Different species of lupins vary in tolerance levels to both pathogens, ranging from totally resistant to highly susceptible (Table 2.5). In *L. angustifolius*, cucumber mosaic virus causes symptoms of leaf curling, stunting of leaves and whole plant, epinasty, pale mottling of leaflets and occasionally death of plants, whereas infection by bean yellow mosaic virus results in blackening and bending over of the growing point, followed by systemic necrosis and death of the plant (Landers, 1991).

*Nematodes*

Under field conditions *L. angustifolius* crops are usually unaffected by *Pratylenchus* sp. (B Buirchell pers. comm.). V Vanstone (pers. comm.) has reported that *L. angustifolius* genotypes differ in resistance to *Pratylenchus* spp., ranging from moderately resistant to resistant. Another important nematode in the cereal belt of southern Australia is the cereal cyst nematode

**Table 2.4** The genetic variation and range of resistance to fungal pathogens in the agriculturally important species of lupins (Buirchell, pers. comm.; Cowling et al., 1986; Landers, 1991; Sweetingham, 1993; Sweetingham, et al., 1993; M Sweetingham, pers. comm.).

| | Fungal Pathogen | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Anthracnose | | Phomopsis | | Pleiochaeta root rot | | Brown spot | | Rhizoctonia | | Grey leaf spot | |
| Lupin species | Genotypic variation | Range of Resistance[1] | Genotypic variation | Range of resistance | Genotypic variation | Range of resistance | Genotypic variation | Range of resistance | Genotypic variation | Range of resistance | Genotypic variation | Range of resistance |
| *L. angustifolius* | present | R to S | present | R to S | present | MS to S | present | S to MS | unknown | S | present | R to S |
| *L. albus* | present | MR to VS | present | MR to S | present | MS to VS | present | MS to VS | unknown | S | | |
| *L. luteus* | present | MS to VS | present | MR to S | | R | | MR | unknown | S[2] | | |
| *L. cosentinii* | | | present | MR to S | | MR | | R | unknown | unknown | | |
| *L. pilosus* | | | unknown | MR | present | MS | unknown | R | unknown | unknown | unknown | unknown |
| *L. atlanticus* | | | unknown | MR | unknown | VS | unknown | R | unknown | unknown | unknown | unknown |

[1] VS - very susceptible; S - susceptible; MS - moderately susceptible; MR - moderately resistant; R - resistant.
[2] *L. luteus* is resistant to the Eradu patch *Rhizoctonia* sp., but not to hypocotyl rot or bare patch.

**Table 2.5** The genetic variation and range of resistance to viral pathogens in the agriculturally important species of lupins (B Buirchell, pers. comm.; Jones, 1993; Landers, 1991).

| | Viral Pathogen | | | |
|---|---|---|---|---|
| | Bean Yellow Mosaic Virus | | Cucumber Mosaic Virus | |
| Lupin species | Genotypic variation | Range of resistance | Genotypic variation | Range of resistance |
| *L. angustifolius* | present | MR to MS | none | S |
| *L. albus* | present | MR to MS | | R |
| *L. luteus* | present | S | present | R to S |
| *L. cosentinii* | unknown | S | unknown | R |
| *L. pilosus* | unknown | S | unknown | R |
| *L. atlanticus* | unknown | S | unknown | R |

(*Heterodera avenae*). Lupins have been found to be unaffected when grown on soils where this nematode was present (B Buirchell, pers. comm.). There have been no reports of nematodes affecting the growth and yield of rough-seeded lupins (B Buirchell, pers. comm.).

*Insects*

Young crops of *L. angustifolius* are susceptible to a number of insects with aphids being the major problem (Landers, 1991). Apart from the feeding damage, aphids are vectors of both viruses (Berlandier, *et al.*, 1997). Within *L. angustifolius* there is a genetic variation in susceptibility to aphids; generally varieties with higher concentrations of alkaloids in the leaves are less susceptible (Jones and Cowling, 1995; Cowling, 1998).

At flowering and pod filling, the most destructive insect is the native budworm (*Heliocoverpa punctigera*) (Landers, 1991). The insect is currently controlled by using insecticides and biological control, although current research is aimed at developing more resistant cultivars (Walden, 1994).

### 2.1.5 Seed composition and products of lupins

The most common use of lupin seed is in rations given to animals, although they can be used as a high protein substitute to soybean in human diets. The plant can also be used as forage for animals.

*Seed composition*

All lupin species which have been utilised in animal foods and for human consumption have high seed protein concentration which is comparable to that of soybeans (Table 2.6) (Hill, 1986). In terms of amino acids, lupins like other grain legumes are deficient in methionine and cysteine (Petterson and Mackintosh, 1994). Seeds of most lupin species also contain greater amounts of oil and the fibre content than many other grain legume species (Table 2.6) (Petterson and Mackintosh, 1994). The carbohydrate composition of lupin seed differs between the hulls and cotyledons Brillouet (1984). The hull generally contains structural polysaccharides (cellulose, hemicelluloses and pectins), whilst the cotyledons main carbohydrate reserves are non-structural polysaccharides (galactose, arabinose and uronic acid) (Brillouet, 1984). It has been established that the seed contains virtually no starch (Hill, 1986). The mineral composition of lupin seed varies between species (Hung, *et al.*, 1988). Generally nutrient contents are comparable to field pea, but lower than soybean (Petterson, 1998). Lupins contain many anti-nutritional factors

including alkaloids, oligosaccharides and tannins, but in modern cultivars they are only at a low concentration (Petterson and Mackintosh, 1994). Bitter varieties of *L. angustifolius* and *L. albus* lupins contain appreciable amounts of alkaloids (0.9-1.3%) compared with sweet varieties (0.002-0.058%), which make them unsuitable for consumption unless the alkaloids are removed (Williams and Harrison, 1983; W Cowling pers. comm.).

*Animal feed*

There have been many reviews on the nutritive value of lupins to animals (Barnveld and Hughes, 1994; Hough and Jacobs, 1994; Murray, 1994). With the exception of Ca, both *L. angustifolius* and *L. albus* provide an adequate source of dietary minerals for both sheep and cattle, although there may be Mn toxicity problems from feeding *L. albus* seeds of some crops (Table 2.7) (Hung, *et al.*, 1988). The major problem with using lupins in animal feed is their relatively low methionine and cysteine levels, but this can be overcome by supplementation.

**Table 2.6** Comparative protein, oil and fibre composition of *L. angustifolius*, *L. albus* and *Glycine max* (soybean). (from Hill, 1986; Clements and Cowling, 1991; Petterson and Mackintosh, 1994)

| Species | Protein concentration Whole Seed % | Oil % | Fibre % |
|---|---|---|---|
| *L. angustifolius* | 32-40 | 3.0-7.2 | 17.0 |
| *L. albus* | 36.6 | 6.4-11.5 | 14.1 |
| *Glycine max* | 35.0 | 16.3 | 10.2 |

**Table 2.7** A comparison of the mineral nutrient requirements of livestock with the mineral content of meal produced from seeds of *Lupinus albus* and *L. angustifolius*. (from Hung, *et al.*, 1988)

| | *L. albus* | *L. angustifolius* | Cattle | Sheep | Poultry | Pigs |
|---|---|---|---|---|---|---|
| P (g $kg^{-1}$) | 4.0 | 2.4 | 1.8-4.3 | 1.6-3.7 | 5 | 4 |
| S (g $kg^{-1}$) | 2.3 | 2.4 | 1 | 1.4-2.6 | - | - |
| K (g $kg^{-1}$) | 9.5 | 8.1 | 6-8 | 5 | 1.6 | 2.5 |
| Ca (g $kg^{-1}$) | 1.9 | 2.4 | 1.8-6.0 | 2.1-5.2 | 8 | 5 |
| Mg (g $kg^{-1}$) | 1.4 | 1.6 | 0.4-1.0 | 0.4-0.8 | 0.5 | 0.5 |
| Na (g $kg^{-1}$) | 0.5 | 6.5 | 1.0 | 0.4-1.0 | 1.2 | 0.9 |
| Cl (g $kg^{-1}$) | 1.2 | 0.6 | 0.7 | 1.0 | 8.0 | 1.0 |
| Cu (mg $kg^{-1}$) | 7 | 4 | 4 | 5 | 4 | 6 |
| Zn (mg $kg^{-1}$) | 38 | 35 | 10-30 | 35-50 | 30-40 | 45-50 |
| Fe (mg $kg^{-1}$) | 43 | 52 | 10 | 30-50 | 65-105 | 60-125 |
| Mo (mg $kg^{-1}$) | 2.3 | 3.1 | 20.5 | 20.5 | - | - |
| Mn (mg $kg^{-1}$) | 1516 | 61 | 1-10 | 20-40 | 55-64 | 40-60 |

*Human consumption*

In Australia lupins have been cleared for human consumption (Landers, 1991), although the only likely market is as a soybean substitute (Hung, *et al.*, 1988) or in health food products. Other possible uses of lupins include fresh sprouts, flour, dietary fibre for inclusion in bread (Landers, 1991) and in Asian food such as tofu and tempeh (Hung, *et al.*, 1988; Kyle, 1994). In some Mediterranean countries the seed of *L. albus* is deep-fried and served as a snack food (B Buirchell, pers. comm.)

### 2.1.6 Modern lupin breeding

The first active breeding of bitter *L. luteus*, *L. angustifolius* and *L. albus* for rapid growth and early maturity by private breeders in Germany began soon after the first world war. Breeding of lupins as a grain and forage crop began when von Sengbusch isolated the first alkaloid free (< 0.05%; sweet) plants of *L. luteus* and *L. angustifolius* in 1928-29 (Sengbusch, 1938). Alkaloid free lines of *L. albus* were also isolated during the 1930's from soft-seeded parents with indehiscent pods (Gladstones, 1970). 'Sweetness' in lupins is due to simple recessive genes, believed to have arisen from natural mutants in breeding populations (Hackbarth and Sengbusch, 1934). Due to the privacy and secret nature of the German work, independent research in Russia resulted in the discovery of another gene for low alkaloid production in *L. angustifolius* and some low alkaloid lines of *L. luteus* (Fedotov, 1932; Fedotov, 1934).

Later breeding in Germany on *L. angustifolius* and *L. luteus* concentrated on further selection for naturally occurring mutant genes for desirable characters (i.e. permeable seed coat and indehiscent pods) and the incorporation of these into alkaloid free lines (Sengbusch, 1938). The major breeding emphasis was on *L. luteus* which was adapted to the acidic sands of the Baltic coastal plain (Gladstones, 1970). In 1943, Weiko II (*L. luteus*), the first true crop variety of lupin (alkaloid free, soft-seeded, indehiscent pods), apart from the alkaloid free selections of *L. albus* which already possessed the attributes of permeable seed coat and indehiscent pods in the parent bitter lines, was released (Gladstones, 1970). Breeding of *L. albus* in Germany at this time was aimed at combining earlier maturity with alkaloid freedom and increasing oil content of the seed (Hackbarth, 1953; Gladstones, 1967). Sengbusch (1938) and Hackbarth, *et al.* (1935) also indicated that there was a need to breed for tolerance to free lime, disease resistance (especially in *L. albus*) and seed quality characteristics.

There was only limited breeding success with *L. angustifolius*, in which soft-seeded and lower alkaloid lines were produced (Sengbusch, 1938), but an indehiscent strain was later proven to be dehiscent (Gladstones, 1967). In the 1960's a fully indehiscent line was found in field trials in Australia (Gladstones, 1967). Two individual recessive genes were found to code for the indehiscent phenotype (Gladstones, 1967). It was also in these trials that indehiscent lines of *L. cosentinii* were found (Gladstones, 1967) (Section 2.1.6.1).

Overall, the breeding of lupins has generally been restricted by the low intercrossability between species, thus breeders have not been able to easily transfer domestication genes from one species to another. This has been compounded by the lack of knowledge of the interrelationships of the *Lupinus* spp. genomes and the origins of each species. Gupta, *et al.* (1996) found that of 30 different interspecific crosses attempted between six rough-seeded lupin species, only eight produced viable $F_1$ seed. Only two of these crosses were viable at the $F_2$ stage, hence gene transfer was only possible between *L. cosentinii*, *L. digitatus* and *L. atlanticus*. The authors concluded that, in terms of genomes, *L. atlanticus* is closer to *L. digitatus* than *L. cosentinii*, *L. pilosus* is closer to *L. atlanticus* than *L. cosentinii* and *L. princei* has an isolated genome. Since $F_1$ hybrids were obtained by Kazimierski (1961) and Pazy, *et al.* (1981) between *L. palaestinus* and *L. pilosus*, it appears that these genomes are also related.

### 2.1.6.1 Lupin breeding in Australia

The breeding of lupins in Australia has been extensively documented by Gladstones (1994) and is only summarised here. Most of the lupin breeding in Australia has been undertaken in Western Australia. The major species of interest has been the narrow-leafed lupins, *L. angustifolius*, because of its adaptation to infertile acidic sands which are prevalent throughout the Western Australian cropping zone. In 1989 a program was started at Wagga Wagga in New South Wales to breed for both *L. angustifolius* and *L. albus* lupins adapted to heavier soils and longer seasons with higher rainfall. This program now focuses on white lupins (*L. albus*).

*Lupinus angustifolius (narrow-leafed lupin)*

Breeding of *L. angustifolius* began in 1960 at the University of Western Australia (Gladstones, 1994), following preliminary genetic and agronomic studies conducted by Gladstones from 1954-57 (Gladstones, 1958). Initially the breeding was only concerned with the domestication of the species and resulted in the world's first true crop-type cultivars of this species Uniwhite (1967), Uniharvest (1971) and Unicrop (1973). Once domestication had been achieved the

principal aims of the breeding program were to improve yield, harvestability and other field characteristics, disease and insect resistance, and breadth of adaptation, and maintain protein content and other aspects of seed quality (Gladstones, 1982). A full list of the current selection criteria used in the Western Australian *L. angustifolius* breeding program has been given in Gladstones (1994). Throughout the breeding program only lines with domestication characters (low-alkaloids, permeable seeds, indehiscent pods, early flowering) have been retained (Gladstones, 1967; Gladstones and Hill, 1969; Gladstones, 1982).

The pedigree breeding method has been used for narrow-leafed lupins and it generally takes a minimum of 14 years from the initial cross to the release of a new cultivar (Gladstones, 1982; Cowling, 1993). More recently recurrent selection has been used to incorporate horizontal resistance to brown leaf spot (Cowling, *et al.*, 1997). The recent outbreak of anthracnose (1996) in Australia has lead to the adoption of a backcrossing program aimed at incorporating resistance in high yielding cultivars (W Cowling pers. comm.).

Until recently the most widely grown cultivars have been Gungurru and Merrit; Gungurru being the first variety with phomopsis resistance. In the last four years a number of cultivars have been released displaying improved yield and resistance to pests and diseases, such as brown spot (Myallie), aphids (Kalya) and anthracnose (Tanjil) (Cowling, 1998). A moderately restricted branching cultivar (Tallerack) has also been released to test whether this modification of the plant architecture is suitable for agriculture (Cowling, 1998). One of the major aims currently is to incorporate resistance for the major pests and diseases into one cultivar. The program has been developing varieties that are targeted to specific environments (W Cowling, pers. comm.). For example the cultivar, Belara, was released because of its improved adaptation to low rainfall areas.

In New South Wales the breeding of *L. angustifolius* has aimed at developing later flowering cultivars, compared with Western Australian releases, to allow for the longer seasons, and resistance to fungal diseases (D Luckett, pers. comm.). The first varietal release was Wonga which had good anthracnose and phomopsis resistance (Cowling, 1998). In breeding trials Wonga has showed better adaptation than the Western Australian material to the heavier soils common throughout Victoria, New South Wales and South Australia (Cowling, 1998).

*Lupinus cosentinii (Western Australian sand-plain lupin)*

The breeding of *L. cosentinii* which was undertaken from 1954 to 1980, followed similar objectives to the narrow-leafed program. Although showing potential for use in agriculture, the breeding program was suspended due to the following reasons (Gladstones, 1994):

1. resistance to phomopsis had not been identified within the species,
2. the species was sensitive to low temperatures,
3. the sweet genotypes were highly susceptible to aphids,
4. the seed would have had to be segregated from that of *L. angustifolius* for marketing reasons and
5. because of the adoption of phosphate fertilizers, *L. angustifolius* could be grown on many soils which had been assumed to be only suitable for *L. cosentinii*.

*Lupinus albus (white lupin)*

White lupins are adapted to fertile loamy soils with good drainage. On these soils in Western Australia industry interest in lupins was low because more profitable crops, such as field peas, could be adequately grown, hence the breeding in Western Australia was initially confined to evaluation of new genotypes from programs in other countries (Gladstones, 1994). The first direct crossing was undertaken in 1968 and continued on a small scale until 1983 and then from 1989 to 1994 (Gladstones, 1994), when the program was moved to Wagga Wagga in New South Wales (D Luckett, pers. comm.). The main aim of the initial program in Western Australia was to select pure breeding sweet lines as white lupins have a high outcrossing rate and much of the imported material was contaminated with bitter types (Gladstones, 1994). The first varieties released in Australia were selections from northern European material. These include Hamburg and Kiev Mutant, which are still recommended in many growing areas. The breeding program at Wagga Wagga has focussed on developing resistance to root rots and improving yields (D Luckett, pers. comm.). The recent outbreak of anthracnose, to which white lupins are highly susceptible, has severely restricted the industry, particularly in Western Australia. Resistance has been identified in landraces and wild types and is being incorporated into the breeding material (Buirchell, 1999).

As *L. albus* has superior seed protein and oil content than *L. angustifolius* (Table 2.6) for animal feed and is used for human consumption, the future of breeding is positive, particularly if anthracnose can be controlled. The first varieties from the New South Wales program will be

released in the next three years, but larger improvements will be seen in four to five years when anthracnose resistant and root rot resistant lines are released (D Luckett, pers. comm.).

*Lupinus luteus (yellow lupin)*

Initial expectations in the 1950's and 1960's across southern Australia for *L. luteus* were high as it was a fully developed crop plant adapted to sandy acid soils and the most widely grown of the original sweet lupins of northern Europe (Gladstones, 1994). However, it failed to live up to expectations as most genotypes were late flowering, intolerant to drought, susceptible to aphids and native budworm, transmit bean yellow mosaic virus through seeds and very poor yielding compared to the *L. angustifolius* cultivars available at that time (Gladstones, 1994). Breeding of *L. luteus* continued until the early 1970's without much success. The program has been restarted, since this species has the best adaptation and yield on extremely acidic soils (pH < 5.0) which occur mainly in Western Australia. The current lines have better aphid and lodging resistance, shatter resistant pods and have grain yields 45% greater than the current variety Wodjil (Buirchell, 1999).

*Lupinus pilosus and Lupinus atlanticus (rough-seeded lupins)*

The breeding of the rough-seeded lupins, *L. pilosus* and *L. atlanticus*, was first attempted in 1975 although many years earlier *L. pilosus* had been identified as being adapted to alkaline loams and clay loams (Gladstones, 1994). As both species were undomesticated initial work was targeted at introducing domestication genes via interspecific crossing or mutagenisis. Interspecific crosses were attempted between *L. pilosus*, *L. atlanticus* and *L. cosentinii*, as well as seeds of *L. pilosus* being treated with a mutagen. As there were no successful crosses or mutants, work on *L. pilosus* ceased until the late 1980's (Gladstones, 1994) when there was a renewed interest in developing a grain legume for calcareous or alkaline clay loams in South Australia (W Hawthorne, pers. comm.), Victoria, New South Wales and Western Australia (Buirchell and Cowling, 1989).

Initially it was found that *L. atlanticus* could be readily inter-crossed with *L. cosentinii* to produce an $F_1$ population that had good flower and pod set, but shrivelled and non-viable $F_1$ seeds (Gladstones, 1994). Concentrated attempts with *L. cosentinii*, resulted in successful interspecific crosses with a number of seed being generated and the population grown through to the $F_3$ (Roy and Gladstones, 1985). Roy and Gladstones (1988) also found that interspecific crossing was possible between other rough-seeded species, such as *L. digitatus*, although there were often problems with sterility of the $F_1$ and $F_2$ plants. A later mutagen program on *L.*

*atlanticus* produced a variety of mutations, including one sweet line and one with white flowers and seeds. These two mutants remained viable (Gladstones, 1994).

In 1990 a concentrated breeding effort on *L. pilosus* and *L. atlanticus* began and this has lead to the development of a fully domesticated line of *L. atlanticus* in 1997. In both species all domestication characters were available from mutagenisis, although all of the initial fully domesticated lines of *L. atlanticus* carry the gene for soft-seededness from a interspecific cross with *L. cosentinii*, which results in smaller seeds than those of the landraces and wild types (B Buirchell, pers. comm.). Currently *L. pilosus* has all the domestication characters in individual breeding lines, but these are yet to be combined into one breeding line (B Buirchell, pers. comm.). The release of the first *L. atlanticus* and *L. pilosus* cultivars is likely to occur over the next 5 to 10 years. As *L. atlanticus* and *L. pilosus* can be grown on a wide range of soils including calcareous soils, to which narrow-leafed lupins are intolerant, the potential areas which can be cropped in Australia is large (B Buirchell, pers. comm.).

## 2.2 Calcareous soils

Calcareous soils are commonly found throughout much of the wheat belt in South Australia, Victoria and New South Wales with smaller areas in Western Australia. Intolerant crop varieties grown on calcareous soils display symptoms resembling bicarbonate ($HCO_3^-$) induced Fe deficiency (Coulombe, *et al.*, 1984a). Other nutritional deficiencies and toxicities that are commonly associated with calcareous soils include B toxicity, and Mn, Zn, P and Cu deficiency (Reuter, *et al.*, 1973; Cartwright, *et al.*, 1984; Mengel and Geurtzen, 1986; Fairbanks, *et al.*, 1987; Russell, 1988; Rashid, *et al.*, 1990; Rimmer, *et al.*, 1993; Marschner, 1995; Cakmak, *et al.*, 1997). They are generally classified in the Great Soil Groups system as solonised brown soils, calcareous sands, grey-brown and red calcareous soils (Stace, *et al.*, 1968) and in the Australian Soil Classification Order as calcarosols (Isbell, *et al.*, 1997).

In the farming community calcareous soils are often termed as alkaline, limey, and high pH. Although these terms are partly correct, they can lead to confusion as calcareous soils are always alkaline, but some alkaline soils do not contain substantial amounts of calcium carbonate ($CaCO_3$). It is the $CaCO_3$ in the soil that leads to the $HCO_3^-$ induced chlorosis and it has been shown that narrow-leafed lupins will grow on alkaline soils without free $CaCO_3$, although they are intolerant to calcareous soils (J Brand, pers. obs.; W Hawthorne, pers. comm.).

Approximately 70-80% of the South Australian cereal cropping zone has calcareous soils (D Maschmedt, pers. comm.), 75-80% is alkaline (pH > 7.0) and 63% is sodic (Naidu, *et al.*, 1993). All three phenomena commonly occur together in the soil. Distribution of $CaCO_3$ throughout the soil profile can be highly variable, both horizontally and vertically. An example of this in the field are 'lime patches', where there are small outcrops of active $CaCO_3$ in the soil in a paddock. These are particularly notable in a crops of lupins as there are chlorotic symptoms with stunted growth and the yield loss in these areas can be up to 100% (Plate 2.1). Also in duplex soils, high concentrations of $CaCO_3$ generally occur in the subsoil which is also often sodic and may contain concentrations of B which are toxic to intolerant genotypes.

### 2.2.1 Origins and natural occurrence calcium carbonate

Calcite ($CaCO_3$) is the most common form of carbonate in soils, but aragonite, dolomite ($CaCO_3.MgCO_3$) and magnesite ($MgCO_3$) also occur (Russell, 1988). Aragonite readily weathers to calcite (Donner and Lynn, 1989).

Soil carbonates originate from several sources or combinations of sources, either directly in the form of carbonates or by a solution-precipitation mechanism (Donner and Lynn, 1989). The most common sources and mechanisms are (Donner and Lynn, 1989):

1. *Parent Material.* The most direct method is inheritance from the parent material. The principal requirement is insufficient leaching to remove carbonates from the profile.
2. *Dissolution of Ca-bearing minerals.* The common context is dissolution of more soluble minerals, such as gypsum or anorthite (Ca-silicate), within the immediate soil and precipitation of calcite.
3. *Mineralization of Plant Materials.* Plants recycle Ca to the surface. Ca released by decomposition of residues reacts with $CO_2$-charged $H_2O$ to form $CaCO_3$.
4. *Wind Deposition.* Carbonate minerals can be transported some distance, suspended in air, and deposited on the surface as dust or in rain. Subsequent relocation within the soil by solution and re-precipitation is possible.
5. *Rain.* Ca ions enter the soil in rainwater and subsequently combine with $HCO_3^-$ associated with $CO_2$-charged $H_2O$.
6. *Surface Water.* Natural surface $H_2O$ or irrigation $H_2O$ can contain $Ca^{2+}$ ions that react with $CO_2$-charged $H_2O$ to form carbonates.

**Plate 2.1** A crop of narrow-leafed lupins (*L. angustifolius*) showing symptoms of $HCO_3^-$ induced chlorosis on a patch of calcareous soil (yellowing in centre of photograph) in an otherwise non-calcareous paddock.

7. *Ground Water*. Ground water may move through carbonate containing soils or strata and bring either $Ca^{2+}$ or $HCO_3^-$ ions into the soil, where they combine to form $CaCO_3$ when the soil dries or the temperature rises.

In the semi-arid climates across the southern Australian cereal growing zone it has been suggested that most of the carbonate rich soils have been formed by wind deposition of loess from the continental shelf during glacial periods or from parent material (K G Weatherby, pers. comm.). As the rainfall is low, the carbonates have remained in the topsoil (Weatherby and Oades, 1975), whereas in higher rainfall climates the carbonate would have been leached out of the topsoil to the lower layers

### 2.2.2 Physical forms and chemistry of calcium carbonate

*Physical forms*

There are three general physical forms of calcium carbonate in soils:

1. sheet limestone (calcrete),
2. limestone rubble and
3. high proportions of powdery $CaCO_3$ (active $CaCO_3$) (Beare, *et al.*, 1960).

Sheet limestone is generally found below the topsoil and can be a physical impedance to root growth, preventing roots from getting to subsoil water. Limestone rubble can be found either throughout the topsoil or in the subsoil. Both of these forms have some physical impedance to root growth, but due to their small surface area, chemical effects on root growth via $HCO_3^-$ are minimal. Conversely, active $CaCO_3$ has a large surface area which reacts with water and $CO_2$ to produce $HCO_3^-$ (see below).

*Chemistry*

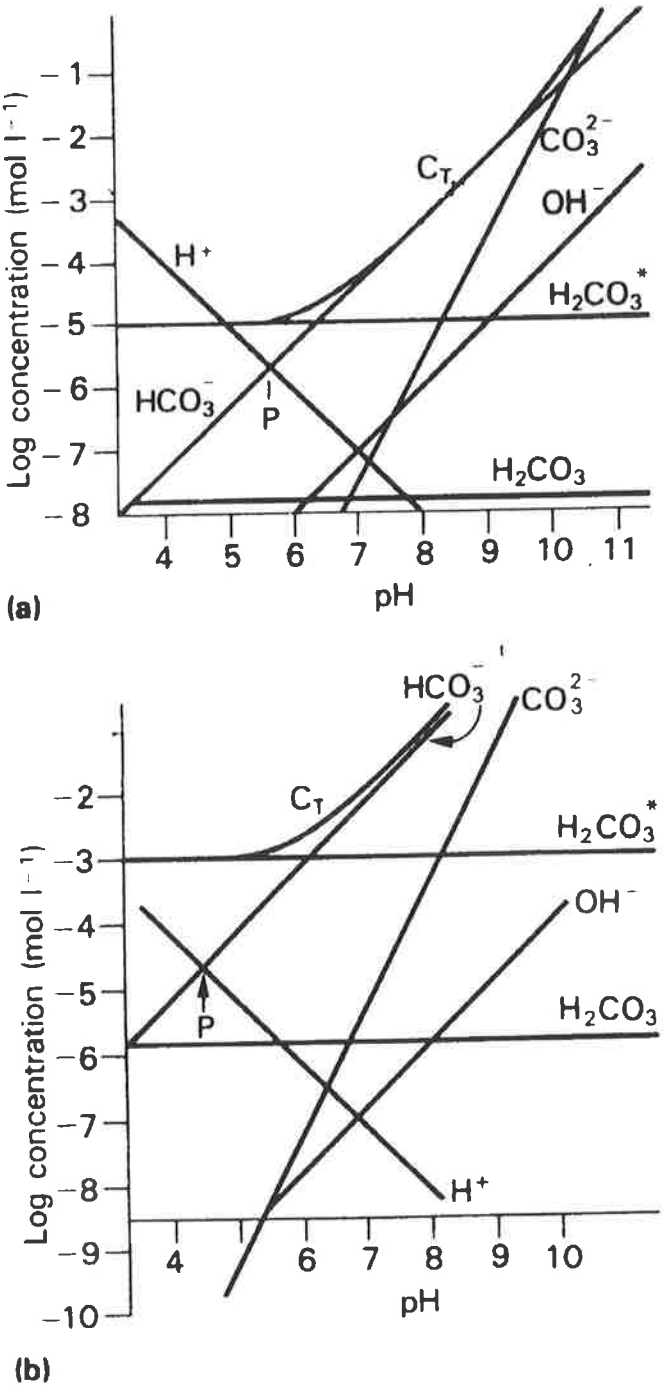
All carbonate minerals have moderate solubility in water (Donner and Lynn, 1989).

The following is an equilibrium reaction of carbonate in soil:

$CaCO_3(\text{calcite}) + H_2O + CO_2 \leftrightarrows Ca^{2+}_{(aq)} + 2HCO_3^-$ (Donner and Lynn, 1989).

The pH of calcareous soils is generally between 7 and 8.5, but depends on the equilibria between $CaCO_3$, $H_2O$ and $CO_2$ which affects the concentration of $HCO_3^-$ in the soil solution (Russell, 1988). Bicarbonate is the predominant carbonate species between pH 6 and 9 with concentrations increasing 10-fold for each pH unit rise, while $CO_3^{2-}$ is predominant above pH 10 (Fig. 2.3) (Russell, 1988).

**Figure 2.3** (From Russell, 1988) Carbonate species in pure water in equilibrium with (a) atmospheric $CO_2$. Partial pressure $P_{CO_2} = 10^{-3.5}$ atmosphere
(b) $P_{CO_2} = 10^{-1.5}$ atmosphere (equivalent to a soil system)
$[H_2CO_3^*] = [CO_2\ aq] + [H_2CO_3]$
$C_T = [H_2CO_3^*] + [HCO_3^-] + [CO_3^{2-}]$



concentrations increasing 10-fold for each pH unit rise, while $CO_3^{2-}$ is predominant above pH 10 (Fig. 2.3) (Russell, 1988).

$CaCO_3$ in the soil can act as a buffer, maintaining a high pH. However, its effectiveness as a buffer depends on the carbonate solubility and rate of dissolution, with the latter being increased with increased surface area (Russell, 1988). Thus, active $CaCO_3$ has a large chemical effect on the growth of intolerant genotypes as $HCO_3^-$, which has been shown to induce Fe deficiency symptoms (Coulombe, *et al.*, 1984a), is produced and its concentration maintained by the buffering of high levels of $CaCO_3$.

The soil moisture and partial pressure of $CO_2$ ($P_{CO_2}$), which is usually up to 100 times greater in the soil atmosphere compared with air, can also have a significant effect on the equilibrium. As soil moisture increases in a calcareous soil microbial respiration and $pCO_2$ increases whilst gas exchange decreases leading to an increase in the concentration of $HCO_3^-$ in soil, although the pH could decrease or remain constant (Russell, 1988).

Subsoils that are calcareous commonly have a pH greater than 8.5 as little $CO_2$ is being produced, the soil approximates to a closed system (Russell, 1988). Under these conditions when water, initially in equilibrium with the $CO_2$ in the atmosphere, percolates through the soil it has been recorded that the pH can rise to 9.9 (Turner, *et al.*, 1958). Also when the subsoil is sodic pH values have often been recorded above 10 which is due to the presence of excess Na ions in solution (Russell, 1988).

## 2.3 Plant growth on calcareous soils

The effects of $CaCO_3$ on plant growth have been extensively studied (Woolhouse, 1966a; Schinas and Rowell, 1977; Froehlich and Fehr, 1981; Mengel, *et al.*, 1984; Mengel and Geurtzen, 1986; Singh, *et al.*, 1986; Ao, *et al.*, 1987; Fairbanks, *et al.*, 1987; Treeby, *et al.*, 1989; White and Robson, 1989c; Jessop, *et al.*, 1990; White, 1990; White and Robson, 1990; Chand and Tomar, 1994; Tyler, 1994; Tang and Robson, 1995; Tang, *et al.*, 1995b; Cakmak, *et al.*, 1997). It is not the pH and $CaCO_3$ *per se* that affects the plant, other than physical restriction of root growth, rather it is the $HCO_3^-$ that is formed in the soil solution of calcareous soils (Section 2.2.2). In general, plant species can be classified into three main groups:

1. calcicoles - tolerant to calcareous soils and $HCO_3^-$,

2. calcifuges - intolerant to calcareous soils and $HCO_3^-$ (Woolhouse, 1966b) and
3. those that are tolerant of a wide range of pH (Russell, 1988).

Many reports indicate that current species of lupins used in agriculture are calcifuges (Schinas and Rowell, 1977; White and Robson, 1989b; Jessop, *et al.*, 1990; White, 1990), although it has been suggested that *L. albus* is tolerant to moderate levels of active $CaCO_3$ (Dinkelaker, *et al.*, 1989). In a study comparing various soils with a range of active $CaCO_3$ Schinas and Rowell (1977) found that the fresh weight of tops of *L. albus* were reduced at active $CaCO_3$ concentrations greater than 10 to 20%.

In South Australia it is currently recommended that narrow-leafed lupins not be grown on soils with greater than 4% $CaCO_3$ (Mayfield, *et al.*, 1996). Some reports have indicated that $CaCO_3$ as low as 2-3% may cause yield reductions (Egan, *et al.*, 1992-93).

In calcareous soils there are toxicities of $HCO_3^-$ and B, particularly in the subsoil and many nutrients (Fe, Mn, Zn, P and Cu) become less available. The most important of these for dicotyledonous plants is Fe. Also the subsoil is often sodic which can cause further limitations to plant growth. Active $CaCO_3$ in the soil can also result in reduced nodulation (Tang and Robson, 1995), root dry weight, shoot dry weight, branching and height (Jessop, *et al.*, 1990) in intolerant genotypes.

### 2.3.1 Nutrient toxicities and deficiencies associated with calcareous soils

*Bicarbonate*

Narrow-leafed lupins grown on calcareous soils show symptoms resembling Fe deficiency chlorosis (Jessop, *et al.*, 1990). In other crops which display similar symptoms on calcareous soils high (toxic) concentrations of $HCO_3^-$ in the soil solution has been implicated as the major cause of the chlorosis (Coulombe, *et al.*, 1984a; Mengel, *et al.*, 1984; Mengel and Geurtzen, 1986). In solution culture it has been shown that high $HCO_3^-$ concentrations induce symptoms resembling Fe deficiency in a range of pulse crops including soybeans (Coulombe, *et al.*, 1984b), chickpeas (Chaney, *et al.*, 1992b) and lupins (Tang, *et al.*, 1996a). It has been suggested that pH could also cause the symptoms (Tang and Robson, 1993), however Chaney, *et al.* (1992a) has discounted this by using a system whereby the solution was aerated with a air that has a higher proportion of $CO_2$; the pH of the solution could be maintained at 7 whilst the concentrations of $HCO_3^-$ was increased. This demonstrated that the symptoms were dependent on the $HCO_3^-$ concentration, not pH. Another indication that $HCO_3^-$ is the major factor in calcareous soils is

that Fe deficiency increases with flooding (Bloom and Inskeep, 1986; White and Robson, 1989b). Under flooded conditions the concentration of $CO_2$ in the soil increases resulting in higher concentrations of $HCO_3^-$ (Ao, *et al.*, 1987).

Bicarbonate induced Fe deficiency is generally only associated with non-graminaceous crops, such as soybeans, lupins, apples and grapevines, which display the Strategy I response to Fe deficiency (Marschner, 1995). In the graminaceous species, such as wheat, sorghum and maize, which display the Strategy II response to Fe deficiency, $HCO_3^-$ concentrations in soil solution are only poorly correlated with Fe chlorosis (Chaney, 1984).

High $HCO_3^-$ concentrations can affect the uptake, translocation and utilization of Fe in plants and based on these observations several reasons for $HCO_3^-$ induced Fe deficiency symptoms have been proposed (Marschner, 1995):

1. high $HCO_3^-$ concentrations in soil solution both raises and buffers the pH and thus further lowers the concentrations of soluble inorganic Fe.
2. in Strategy I plants, root responses to Fe deficiency (i.e. excretion of protons and release of reductants/chelators) are severely inhibited by the high pH. These impaired root responses include a reduction in the effectivity of the $H^+$-efflux pump by neutralization of the $H^+$, reducing the release of phenolics and Fe(III) reduction at the plasma membrane.
3. Fe transport to the shoots is possibly impaired through sequesteration of Fe in the vacuoles of the roots by organic acids. Organic acid synthesis has been found to increase under high $HCO_3^-$ concentrations.
4. the utilization of Fe in the leaves may be inhibited. It has been noted that Fe concentration remains similar or may increase in plants susceptible to $HCO_3^-$ induced Fe deficiency. A possible reason for this is that Fe is inactivated in the leaf of the plant (Kaur, *et al.*, 1984), although this could not be confirmed by Dockendorf and Höfner (1990). Alternatively, the high Fe contents in the leaves may be a consequence of a limitation on other factors required for leaf expansion, chloroplast development and chlorophyll formation (Marschner, 1995). For example, it has been suggested that $HCO_3^-$ inhibits shoot growth prior to the occurrence of Fe deficiency chlorosis (Shi, *et al.*, 1993).
5. high $HCO_3^-$ concentrations may also inhibit root growth, root respiration, root pressure-driven solute export into the xylem and the rate of cytokinin export (necessary for protein synthesis and chloroplast development) to the shoot (Marschner, 1995).

*Boron toxicity*

B toxicity, commonly associated with the clay 'B' horizon of duplex soils (Cartwright, *et al.*, 1986), was identified as limiting to growth of barley on which brown spots and marginal chlorosis had been observed. Since that time genetic variation for tolerance to B toxicity in many crops including wheat (Paull, *et al.*, 1988), barley (Jenkin, 1993), peas (Bagheri, *et al.*, 1994), and lupins (Brand, 1995) has been observed and a number of cultivars of wheat have been released for their tolerance to B, enhancing the grain yield.

*Sodicity*

In Australia approximately 25-30% of soils are sodic (exchangeable sodium capacity (ESP) greater than 6%)(Northcote and Skene, 1972; Rengasamy and Olsson, 1991; Chartres, 1995). Some calcareous soils have sodicity problems, particularly in the clay B horizon of duplex soils, and exhibit poor soil-water and soil-air relations. Sodicity can adversely affect root growth, restrict plant production and make the soil difficult to work when wet or dry (Rengasamy and Olsson, 1991). Also in sodic soils $HCO_3^-$ concentrations are likely to be higher than in non-sodic soils, as the $P_{CO_2}$ is increased due to poor aeration and pH is increased (Russell, 1988), further restricting growth of intolerant crops. The pH in sodic soils can reach as high as 10, at which level $CO_3^{2-}$ becomes the predominant ion and is likely to be more toxic than $HCO_3^-$ (Russell, 1988).

*Phosphorus deficiency*

P is often deficient in calcareous soils because of its low solubility, despite soil analysis indicating high concentrations (Tyler, 1992; Rahmatullah *et al.*, 1994). It becomes increasingly insoluble as pH increases and precipitates as calcium phosphates. High $HCO_3^-$ concentrations in soil solution may directly affect the uptake of phosphate, or there may be an indirect effect through Fe or Mn deficiencies (Russell, 1988). Bloom and Inskeep (1986) indicated that increased P supply also may intensify the chlorotic symptoms associated Fe deficiency and it has been found that the addition of P to calcareous soils may increase the probability of Zn deficiency (Safaya, *et al.*, 1977; Loneragan, *et al.*, 1979; Parker, *et al.*, 1992).

*Micronutrient deficiencies (Fe, Mn, Zn, Cu)*

The major micronutrient deficiencies associated with calcareous soils are Fe (Loeppert, 1986; Mengel and Geurtzen, 1986; Singh, *et al.*, 1986; Fairbanks, *et al.*, 1987; Plessner, *et al.*, 1992),

Mn (Reuter, *et al.*, 1973; Rashid, *et al.*, 1990) and Zn (Plessner, *et al.*, 1992; Cakmak, *et al.*, 1997). At high pH in calcareous soils the solubility of Fe and Zn is decreased, thus the concentrations of these nutrients available for plant uptake tend to be low (Marschner, 1995). Increasing levels of $CaCO_3$ reduce the solubility of Mn due to the absorption of Mn on $CaCO_3$ and possibly the precipitation of Mn calcite (Jauregui and Reisenauer, 1982). Another micronutrient that has been observed to be often deficient on calcareous soils is Cu (Kausar, *et al.*, 1976; Gutser, 1990). Similar to the other micronutrients its solubility, thus availability to plant uptake, decreases with increased pH.

### 2.3.2 Adaptive mechanisms for tolerance

*Bicarbonate*

The mechanisms of tolerance to $HCO_3^-$ induced chlorosis are probably related to an ability to overcome limitations on absorption of Fe and other nutrients by the roots and translocation to the shoots. It has been shown that chlorosis tolerant cultivars of soybeans have a significantly greater excretion of protons resulting in a pH drop of up to 2 units (Chaney and Bell, 1987). In other $HCO_3^-$ resistant non-graminae plants, rhizodermal transfer cells in young roots with dense long root hairs have been found to form (Chaney and Bell, 1987). From these areas there is likely to be highly localised acidification allowing the root hairs to take up Fe, before the protons are consumed by $HCO_3^-$ or other reactions with soil (Chaney and Bell, 1987). Genotypes that are able to exclude $HCO_3^-$ from the roots are also likely to show less chlorosis as the $HCO_3^-$ is unlikely to affect the translocation of Fe or other nutrient within the plant. There is a possibility that tolerant lines are able to excrete higher levels of Fe chelators (Marschner, 1995). For example, sunflower plants grown in Fe deficient solutions exuded larger quantities of Fe chelates than plants grown in Fe sufficient solutions (Marschner, *et al.*, 1982).

*Boron toxicity*

In a range of species, all genotypes showing tolerance to B toxicity, were able to maintain lower concentrations of B in the shoots and grain than sensitive genotypes (Paull, *et al.*, 1988). Thus, the mechanism of tolerance is related to the ability of the plant to either exclude or remove B from the root system or sequester B inside the root (Paull, *et al.*, 1988).

*Sodicity*

The tolerance mechanism of plants to sodicity is not well understood, although it is probably related to an ability to overcome the physical and chemical (high Na) limitations in a sodic soil.

In sodic soils anoxic conditions prevail and it has been found that in plants that can tolerate low oxygen or waterlogging, the concentration of alcohol dehydrogenase increases (Buchor and Kuhlemeier, 1993). In white clover it has been shown that genotypes with higher alcohol dehydrogenase activities during flooding have greater flooding tolerance (Chan and Burton, 1992). Also in rice it has been found that tolerant lines are able to maintain high K concentrations in leaves, while preventing Na from accumulating (Sharma, 1986; Gorham, *et al.*, 1993). Other authors have also indicated that the Na:K ratio is lower in tolerant cultivars (Joshi, *et al.*, 1979).

*Phosphorus deficiency*

There are a number of mechanisms that plants can use to overcome P deficiency. Hyper-excretion of protons in the rhizosphere may enable the root to enhance efficiency of P uptake (Hedly, *et al.*, 1982). Other mechanisms include enhancement of root elongation, root hair formation and increased extracellular phosphatase activity (Dinkelaker, *et al.*, 1989). Infection of roots by vesicular-arbuscular mycorrhizae may be indirectly influenced by an increased exudate release by roots in response to P deficiency (Dinkelaker, *et al.*, 1989; Kothari, *et al.*, 1991). The vesicular-arbuscular mycorrhizae have been shown to enhance the uptake of P (Kothari, *et al.*, 1991). Also, under P deficiency some plant species, such as *L. albus*, produce proteoid roots which are sections of dense bottle-brush-like cluster of short (5-10 mm) rootlets covered with a dense mat of root hairs (Dinkelaker, *et al.*, 1989). In these proteoid root zones citrate and protons are released which aid the uptake of P and micronutrients (Gardner, *et al.*, 1982a; Gardner, *et al.*, 1983a; Gardner, *et al.*, 1983b; Dinkelaker, *et al.*, 1989).

*Micronutrient deficiency (Fe, Mn, Zn, Cu)*

The tolerance of plants to micronutrient deficiencies is related to the efficiency of uptake, utilisation and internal requirement of the nutrient. For example, (Graham, 1988) proposed five possible mechanisms for Mn efficiency:

1. superior internal utilisation or lower functional Mn requirement.
2. improved internal redistribution.
3. faster specific rate of absorption from the soil solution at low Mn concentrations.
4. more extensive root geometry
5. greater root excretion of the substances into the rhizosphere to mobilise insoluble Mn:
   a) $H^+$
   b) reducing substances

c) Mn binding ligands

d) microbial stimulants.

It is likely that these mechanisms will also apply for the other micronutrients (Treeby, *et al.*, 1989; Cakmak, *et al.*, 1996).

Vesicular-arbuscular mycorrhizae, as discussed above, have been shown to aid in the uptake of other micronutrients, especially Zn, as well as P (Dinkelaker, *et al.*, 1989; Kothari, *et al.*, 1991).

### 2.3.3 Breeding for tolerance to calcareous soils

Breeding for tolerance to calcareous soils involves overcoming the major limiting factors which were discussed above.

*Bicarbonate*

The tolerance to $HCO_3^-$ induced chlorosis has been extensively studied in soybeans (Coulombe, *et al.*, 1984a) and chickpeas (Chaney, *et al.*, 1992b) and to a lesser extent in grapevines (Mengel, *et al.*, 1984), apples (Ao, *et al.*, 1987), peaches (Shi, *et al.*, 1993), peanuts (Tang, *et al.*, 1991) and lupins (Tang, *et al.*, 1996a). The major breeding efforts have been in soybeans (Rodriguez de Cianzio, *et al.*, 1979; Froehlich and Fehr, 1981; Byron and Lambert, 1983; Coulombe, *et al.*, 1984a; Coulombe, *et al.*, 1984b; Jessen, *et al.*, 1986; Fairbanks, *et al.*, 1987) and chickpeas (Singh, *et al.*, 1986; Hamze, *et al.*, 1987; Saxena, *et al.*, 1990; Chaney, *et al.*, 1992b) in which soil and solution screening methods have been developed and a range of tolerance, in terms of chlorosis symptoms, from intolerant (chlorotic) to tolerant (healthy) has been found (Froehlich and Fehr, 1981; Byron and Lambert, 1983; Coulombe, *et al.*, 1984b; Jessen, *et al.*, 1986; Chaney, *et al.*, 1992a; Chaney, *et al.*, 1992b). The solution method was most desirable for breeding programs as it was more efficient and reliable than the soil methods (Chaney, *et al.*, 1992a). In soybeans it has been identified that there is one major gene, recessive for susceptibility, and many other minor genes controlling tolerance (R Chaney, pers. comm.). The minor genes were incorporated via recurrent selection to develop highly tolerant cultivars (R Chaney, pers. comm.).

*Boron toxicity*

There have been three main screening methods (soil, solution and filter paper) developed to identify genotypes that are tolerant to high levels of soil B (Paull, *et al.*, 1988; Chantachume, *et al.*, 1995; Campbell, *et al.*, 1998). The methods correlate well (Campbell, *et al.*, 1998) and show

a wide range of tolerance in wheat and barley ranging from intolerant to tolerant. B tolerance in wheat is controlled by four major genes (Paull, *et al.*, 1991).

*Sodicity*

The most common method for identifying genotypes tolerant to sodic conditions is to grow them in soil with high exchangeable Na and then measure growth and the Na:K ratio (Joshi, *et al.*, 1979; Sharma, 1986; Gorham, *et al.*, 1993). Tolerant genotypes generally display better growth and have a lower Na:K ratio (Joshi, *et al.*, 1979). A range of variation for tolerance to sodicity has been found in a number of crops including chickpea (Kumar, *et al.*, 1983), rice (Sharma, 1986), and wheat (Joshi, *et al.*, 1979). The number of genes involved in tolerance has not been identified.

*Micronutrient efficiencies*

There has been some breeding of crops for micronutrient efficiency, but most of the work so far has been aimed at understanding genetics and developing molecular markers, particularly for Mn and Zn efficiency (Khabaz-Saberi, 1999). Screening methods have generally involved growing plants either in deficient soils under controlled conditions and measuring growth and nutrient concentrations or in the field and measuring grain yield and nutrient concentrations (Marcar and Graham, 1987; Bansal, *et al.*, 1992; Graham, *et al.*, 1992; Huang, *et al.*, 1994; Grewal, *et al.*, 1997). A range of genetic variation for Mn and/or Zn has been found in wheat (Graham, 1988; Graham, *et al.*, 1992; Cakmak, *et al.*, 1997), barley (Reuter, *et al.*, 1973; Huang, *et al.*, 1994) and canola (Grewal, *et al.*, 1997). There appears to be one major gene for Cu efficiency in cereals, for Mn there may be a single major gene (per genome in wheat) controlling efficiency, and for Zn there are probably three or four major genes with several minor genes giving additional efficiency (Khabaz-Saberi, 1999; R Graham pers. comm.).

### 2.3.4 Tolerance of lupins to calcareous soils

The tolerance of lupins, particularly, to calcareous soils has not been studied extensively across a range of species (White and Robson, 1989a; Jessop, *et al.*, 1990). A majority of the work has focussed on the tolerance to alkaline soils *per se* and not calcareous soils (Mengel and Geurtzen, 1986; White, 1990; Cowling and Clements, 1993; Mock and Gibson, 1993; Tang, *et al.*, 1993; Tang, 1995; Tang and Robson, 1995; Tang, *et al.*, 1995b). For alkaline soil tolerance, a soil and solution screening trial at pH 7 showed that within *L. angustifolius* there was a range of tolerance in terms of chlorotic symptoms, but no lines were completely tolerant (Tang and Thomson,

1996; Tang and Robson, 1998). However the soil used in this trial was only slightly calcareous (1% $CaCO_3$) and results are unlikely to be indicative of responses on a majority of calcareous soils.

As discussed previously most lupin species appear to be intolerant of calcareous soils. The only species of importance in agriculture to show some tolerance are *L. albus*, *L. pilosus* and *L. atlanticus* (Dinkelaker, *et al.*, 1989; Egan, *et al.*, 1992-93; B Buirchell per comm.). The current cultivars of *L. angustifolius* are highly intolerant, often resulting in total yield loss in the farming situation (J Brand pers. obs., M Wilksch, pers. comm., W Hawthorne, pers. comm.).

White lupins have shown intermediate tolerance to calcareous soils with moderate levels of $CaCO_3$, but poor yields in the farming situation make them an uneconomic alternative to other legumes such as field peas. Both *L. pilosus* and *L. atlanticus* appear to be the species best adapted to grow on calcareous soils, as landraces and wild types have shown few or no symptoms of chlorosis in field trials and yields equivalent to or better than *L. angustifolius* when grown on acidic soils (Egan, *et al.*, 1992-93; B Buirchell, pers. comm.). Landraces and wild types of *L. pilosus* have been commonly collected from calcareous soils, while it appears that *L. atlanticus* has come from alkaline soils that are not necessarily calcareous (Clements and Cowling, 1990).

## 2.4 Conclusion

The tolerance of *L. pilosus* and *L. atlanticus* to calcareous soils has not been established experimentally, although generally it has been concluded that they show better tolerance than any other lupin species. It is necessary not only to identify the tolerance of these species, but also to investigate the range of intraspecific genotypic variation as a prelude to a breeding program to develop varieties for these soil types. Previous research indicates that the major limitation to growth of some pulses in calcareous soils is $HCO_3^-$ (Section 2.3.1). Therefore in lupins, particularly *L. pilosus* and *L. atlanticus*, it is desirable to understand how $HCO_3^-$ effects plant growth. If genetic variation for tolerance to calcareous soils is present in these species, then the mechanisms of tolerance need to be defined and number of genes controlling tolerance identified.

# CHAPTER 3

## Adaptation of *Lupinus angustifolius* L. and *L. pilosus* Murr. to calcareous soils of South Australia

## 3.1 Introduction

Current varieties of narrow-leafed lupin (*Lupinus angustifolius* L.) are poorly adapted to calcareous soils which are prevalent throughout much of the South Australian and Victorian cereal cropping zones, as well as smaller areas in New South Wales and Western Australia (D Maschmedt, pers comm.). The soils throughout these areas are commonly duplex having shallow sandy or loamy A horizons and a clay B horizon (D Maschmedt, pers. comm.). They generally contain $CaCO_3$ through the whole profile and often the B horizon is also sodic and contains concentrations of B which are toxic to B intolerant genotypes (Cartwright, *et al.*, 1986; Paull, *et al.*, 1988; Bagheri, *et al.*, 1994; Isbell, *et al.*, 1997).

The most common grain legume crop grown in these areas, field peas (*Pisum sativum* L.), has major limitations of a sprawling growth habit conducive to high levels of leaf and stem disease, and a weak stem base leaving the soil exposed to erosion after harvest. Lupins have the potential to fill this niche, if species with adaptation to calcareous soils can be identified, as they are adapted to low rainfall, having strong erect stems for ease of harvest and prevention of erosion, whilst still providing a pest and disease break which can result in substantial yield responses in the subsequent cereal crops.

Of the current domesticated species of lupins, both *L. angustifolius* and *L. luteus* are limited to non-calcareous, acid to neutral soils, displaying chlorotic symptoms and in some cases total yield loss when grown on calcareous soils. No tolerance has been identified in these two species to calcareous soils. Both *L. albus* and *L. cosentinii* show some adaptation, but grain yields have been low compared with peas (J P Egan, pers. comm.). Although there appears to be some genetic variation in *L. albus*, no fully tolerant lines have been identified, and in *L. cosentinii* no variation has been recorded. Hence to find a suitable lupin it has been necessary to look for an undomesticated species tolerant to calcareous soils. This has lead to the identification of two rough-seeded species, *L. pilosus* and *L. atlanticus* (Buirchell and Cowling, 1989).

In field trials at calcareous sites both *L. pilosus* and *L. atlanticus* were identified as having better adaptation to calcareous soils than the narrow-leafed lupins (Egan and Hawthorne, 1994). The grain yields of a collection of landraces of these species ranged between 0.2 and 0.7 t/ha greater than the cultivar Merrit (*L. angustifolius*) (Egan and Hawthorne, 1994). Tang, *et al.* (1993) also showed that on a soil with 0.6-3% $CaCO_3$, *L. pilosus* showed a lower degree of chlorotic symptoms.

In this study, fourteen soils were collected from across the South Australian cereal zone. Single genotypes from *L. pilosus* and *L. angustifolius* were grown on these soils and their growth compared to identify the factors governing their adaptation and to identify the potential for *L. pilosus* in southern Australian agriculture.

## 3.2 Materials and methods

*Genetic material*

The two species used in this experiment were *L. angustifolius* cv. Gungurru and *L. pilosus* P20954. Gungurru is a commonly grown cultivar throughout South Australia and P20954 is landrace naturalized to many areas of Western Australia (Table 6.1). In a field trial at Minnipa during 1993 the grain yield of P20954 was 1.35 t/ha compared with Merrit (a sister line of Gungurru with similar yields) at 0.75 t/ha (Egan, *et al.*, 1992-93).

*Soil and pot preparation*

Fourteen soils (approximately 30 kg), representative of the range of topsoils (0-10 cm) and subsoils (10-20 cm) found throughout the region, were collected from across the South Australian cereal belt (Mid North, Eyre Peninsula and Murray Mallee) during August 1996 (Table 3.1). In particular, most soils were collected from areas where the narrow-leafed lupins cannot be grown economically or cultivation limited due to the high $CaCO_3$ content. The soils CTY and NTY were collected 5 m apart in the same paddock and have similar physical and chemical characteristics, except for $CaCO_3$ content. The UC modified potting mix was used as the control (see below). The field soils and a potting mix were air-dried for three to four days in soil drying ovens at approximately 40°C. All soils were analysed for pH, electrical conductivity, sodium absorption ratio, total $CaCO_3$, colour, texture, field capacity and wilting point (see 'soil analyses' below; Table 3.1).

The air-dried soils were placed in plastic containers (35 cm x 39 cm x 32 cm) and watered to the moisture contents indicated in Table 3.1 with water purified by reverse osmosis (RO). The moisture contents had been determined via a visual assessment accounting for the texture and moisture holding capacity. Generally the sandier soils were watered to between 10 and 15% moisture content, whilst the soils with more clay were watered to 15-20%. The soils were mixed twice weekly for three weeks to ensure an even distribution of moisture. The containers were reweighed and RO water added to weight as required.

Twenty five centimetre lengths of ten centimetre diametre poly vinyl chloride (PVC) pipe lined with plastic were used as pots. The plastic lining was 19 cm diameter layflat tubing cut into 30 cm lengths. One end was heat sealed to form the bottom and the extra length allowed for folding over at the top of the PVC pipe. The moist soils were transferred to the pots and gently compacted by tapping on a hard surface. Pots were allowed to stand for two weeks and watered to weight before seeding and at weekly intervals for the duration of the experiment.

University of California (UC) modified potting mix

The UC soil was prepared as follows: 350 L of coarse washed sand was steam sterilised at 100°C for 30 minutes, before being mixed with 250 L of Euroturf® peatmoss. After cooling for 10 minutes 450 g calcium hydroxide, 400 g calcium carbonate and 500 g Nitrophoska (N-P-K, 15-4-12) were mixed in for 20 seconds, resulting in a pH of 6.8. The soil was passed through a 10 mm sieve to remove stones and agglomerations of peat.

*Soil analyses*

Before all analyses, soils were air-dried at 50°C for 48 hours and passed through a 2 mm sieve.

pH, electrical conductivity and sodium absorption ratio

In this experiment both $pH_w$ (1:5, soil: $H_2O$) and $pH_s$ (1:5, soil:0.1 M $CaCl_2$)of soils were recorded, whilst for following chapters pH of soils only refers to measurements of $pH_w$.

To determine $pH_w$, electrical conductivity (EC) and sodium absorption ratio (SAR): 8 g of air-dried soil was weighed into a 100 ml tube and 40 ml of RO water added. All three measurements were recorded from one sample. For $pH_s$, 40 ml of 0.1 M $CaCl_2$ was used rather than RO water. Samples were mixed on an end over end shaker for 60 minutes and allowed to settle for a further 60 minutes. The pH and EC of the solution was determined 1 cm above the settled soil layer using an Activon® pH meter and a Radiometer® conductivity meter, respectively.

**Table 3.1** Soil collection site, texture, $pH_w$, $pH_s$, field capacity, wilting point, $CaCO_3$ content, sodium absorption ratio (SAR) and electrical conductivity (EC) of soils used in the study. Code for pot names: C, calcareous; N, non-calcareous; T, topsoil; S, subsoil; the last letter of the pot name refers to the district.

| Pot name | District | Topsoil / Subsoil | Texture (%) Sand | Silt | Clay | $pH_w$ | $pH_s$ | Field capacity (%) | Wilting point (%) | $CaCO_3$ content (%) | SAR | EC (dS/cm) | Moisture content (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | UC potting mix | | 99 | 0 | 1 | 5.8 | 4.4 | 8.1 | 5.4 | 0.0 | 2.85 | 321 | 18 |
| CTK | Kimba | T | 82 | 5 | 13 | 8.5 | 7.4 | 20.5 | 8.8 | 1.0 | 1.27 | 57 | 17 |
| CSK | Kimba | S | 62 | 11 | 27 | 8.7 | 7.8 | 39.6 | 15.7 | 6.6 | 3.00 | 314 | 21 |
| CSK2 | Kimba | S | 69 | 4 | 27 | 9.6 | 7.8 | 38.4 | 21.8 | 6.3 | 24.56 | 441 | 20 |
| CTC | Cungena | T | 14 | 4 | 82 | 8.7 | 7.5 | 17.9 | 7.9 | 46.7 | 0.93 | 377 | 16 |
| CTL | Lameroo | T | 84 | 3 | 14 | 8.7 | 7.5 | 16.5 | 6.8 | 1.8 | 0.83 | 335 | 15 |
| CTT | Tarlee | T | 77 | 8 | 15 | 8.4 | 7.3 | 21.8 | 9.3 | 5.0 | 1.15 | 316 | 13 |
| CTT2 | Tumby Bay | T | 66 | 13 | 21 | 7.8 | 6.6 | 38.3 | 24.4 | 0.3 | 3.43 | 107 | 20 |
| CTY | Yeelanna | T | 69 | 8 | 23 | 8.5 | 7.4 | 33.1 | 15.5 | 5.0 | 1.30 | 157 | 20 |
| NTY | Yeelanna | T | 74 | 15 | 10 | 8.0 | 6.8 | 33.6 | 16.5 | 0.0 | 1.61 | 106 | 16 |
| NTU | Ungarra | T | 97 | 0 | 3 | 6.4 | 5.3 | 3.8 | 3.3 | 0.0 | 1.14 | 112 | 13 |
| NSU | Ungarra | S | 97 | 0 | 3 | 6.4 | 5.1 | 3.6 | 2.4 | 0.0 | 1.88 | 266 | 11 |
| NTW | Wharminda | T | 95 | 1 | 4 | 7.0 | 5.7 | 8.6 | 4.1 | 0.0 | 1.50 | 308 | 12 |
| NSW | Wharminda | S | 85 | 5 | 10 | 7.8 | 6.5 | 12.6 | 6.5 | 0.0 | 2.11 | 343 | 13 |
| NTU2 | Ungarra | T | 92 | 3 | 5 | 6.0 | 4.5 | 9.5 | 2.7 | 0.0 | 4.83 | 524 | 11 |

To determine SAR: the soils were allowed to stand for 48 hours and the supernatant filtered through a Whatmann® 42 filter paper. The solution was analysed for nutrient concentrations by inductively coupled-plasma absorption emission spectrometry (ICP-AES) (Zarcinas, *et al.*, 1987). The SAR was calculated as follows:

$$SAR = \frac{[Na]}{([Ca]+[Mg])^{½}}$$

Total $CaCO_3$ (Black, 1965)

Between 0.25 and 5 g of air-dried soil (0.25 if highly calcareous, 5g if non-calcareous) was weighed into a glass flask. A 25 ml flat bottom plastic tube containing acidified $FeCl_2$ solution was carefully placed in the bottom (preventing spillage of the solution) of the flask held in a Griffen® flask shaker and connected to the calcimeter so that the seal was airtight (for construction of the calcimeter see Black (1965)). The flask was shaken (spilling the $FeCl_2$ solution) at moderate speed for two minutes or until the reaction had been completed. The production of $CO_2$ from the reaction of carbonates in the soil with acid was measured and the $CaCO_3$ content of the sample calculated.

On the basis of 50 mg $CaCO_3$ releasing approximately 10 ml $CO_2$ the formula below was used.

$$\%CaCO_3 = \frac{5 \text{ x (estimated volume of } CO_2 \text{ from the soil) x } 10^{-1}}{\text{weight of soil}}$$

Organic carbon (Allison, 1965)

One gram of air-dried soil was weighed into a 500 ml conical flask and 10 ml of 1 *M* potassium dichromate ($K_2Cr_2O_7$) solution followed by 20 ml of concentrated sulphuric acid ($H_2SO_4$) were added. The solution was shaken and allowed to digest for 30 minutes. Ten ml of concentrated orthophosphoric acid, 0.5 ml of o-phenanthroline solution and approximately 200 ml of RO water were added. After the flasks had cooled, the excess chromic acid was titrated by adding 0.5 *M* ferrous sulfate ($FeSO_4$) solution from a burette until the solution turned from green to red. The organic carbon was calculated, given that each ml of 1 *M* $K_2Cr_2O_7$ equals 3 mg of carbon.

Field capacity and wilting point

Sintered glass funnels and ceramic pressure plates were used to determine field capacity (FC; $\psi_m$ = -10 Kpa) and wilting point (WP; $\psi_m$ = -1500 Kpa). The air-dried soils were poured into 3 cm diametre plastic PVC tubing placed on the ceramic plates. The soils were saturated with RO

water, mixed to allow even surface contact with the ceramic plate and covered to prevent moisture loss via evaporation. The samples were allowed to drain for 48 hours to determine field capacity and 7 days for wilting point, before being removed and moist weights recorded. The moist soil was placed in a plastic container and oven dried at 105$^{O}$C for 48 hours and the dry weight recorded. Field capacity and wilting point was calculated as follows:

$$\text{FC (\%) or WP (\%)} = \frac{\text{(g moist soil - g dry soil)}}{\text{(g dry soil)}} \times 100$$

Texture (Sheldrick and Wang, 1993)

To determine the proportions of sand (> 0.02 mm), silt (0.02 - 0.002 mm) and clay (< 0.002 mm), 40 g of air-dried soil was weighed into a 1 L plastic bottle and 150 ml RO water, 50 ml Calgon® solution and 3 ml of 1.6 M NaOH added. The bottle was shaken overnight on a end over end shaker. The contents of the bottle were transferred into a 1 L measuring cylinder and the volume adjusted to 1 L with RO water. The solution was mixed for 30 seconds by inversion and the time of commencement of sedimentation recorded. After 5 minutes ($R_1$) and 5 hours ($R_2$) a hydrometer reading was taken. To calculate sand silt and clay content the following formula was used:

$$\text{\% clay} \quad \frac{(R_2 - \text{standard})}{\text{(soil added(40 g))}} \times 100$$

standard refers to the hydrometer reading in the solution as described above without soil.

$$\text{\% silt} = \frac{(R_1 - R_2)}{40} \times 100$$

% sand = 100 - (%clay + %silt).

Colour

The colour of air-dried soils was determined using a Munsell Soil Colour Chart, (1992 rev. ed. Pub.: Newburgh, N.Y. : Macbeth Division of Kollmorgen Instruments Corp.)

*Plant growth and experimental design*

Prior to sowing seeds of P20954 were scarified with a scalpel by cutting the seed coat through to the endosperm opposite the hilum, without damaging the cotyledons, to remove the effects of an impermeable seedcoat. Seeds of both genotypes were imbibed on moistened filter paper for 48 hours at 20°C. Three seeds, one genotype was used per pot, were sown at 1 cm depth in the soil and inoculated with 2 mls of a concentrated suspension of Nitrogerm 100 group G inoculant

(*Bradyrhizobium* sp. *Lupinus*; WU425 - Bio-Care Technology Pty. Ltd.). Pots were watered to weight (Table 3.1) with RO water. Seven days after sowing plants were thinned to two per pot, after four weeks to one per pot and this was grown for a further three weeks.

The experiment, arranged as a randomised complete block design with three replicates of fifteen soils and two species, was conducted in an evaporatively cooled glasshouse. Minimum and maximum temperatures ranged from 15$^{O}$C at night to 30$^{O}$C during the day.

*Measurements*

Chlorosis score of the youngest emerging leaf (YEL) (Table 3.2; Plate 3.1) and number of leaves were recorded weekly from 14 to 49 days after sowing (DAS). At 49 DAS the youngest emerging leaf (YEL) and the whole shoots were harvested separately, washed in 0.1 *M* hydrochloric acid (HCl) for 30 seconds and rinsed in RO water for 60 seconds to remove surface contamination, and dried in an oven at 80$^{o}$C for 48 hours. Dry weights were recorded and the samples were analysed for nutrient concentrations by ICP-AES analysis. Critical concentrations of nutrients in the YEL for narrow-leafed lupins are summarised in Table 3.3. The YEL dry weight was added to the whole shoot dry weight to give a total dry weight.

**Table 3.2** Visual chlorosis scoring system for lupins.

| Score | Description |
|---|---|
| 0 | healthy, i.e. no chlorosis |
| 1 | slight yellowing of youngest leaves |
| 2 | moderate chlorosis, more distinct yellowing |
| 3 | moderate to highly chlorotic, YEL mostly yellow - slight stunting |
| 4 | highly chlorotic, leaves almost totally yellow and stunted |
| 5 | dead |

**Table 3.3** Critical concentrations of nutrients in the YEL for narrow-leafed lupins (*L. angustifolius*)(Reuter and Robinson, 1997).

| Fe | Mn | B | Cu | Zn | Ca | Mg | Na | K | P | S | Al |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 44 | 40 | 20 | 1.0 | 15 | 10000 | 2000 | 100 | 25000 | 2200 | 2400 | na |

*Statistical analyses*

To test for significant differences, a two-way analysis of variance (ANOVA) between species and soil (Genstat 5; Lawes Agricultural Trust, Rothamsted, U.K.) was used for all harvest measurements (total dry weight and nutrients) and measurements of leaves and chlorosis score 49 DAS. The weekly measurement of chlorosis score and number of leaves was analysed by a three-way ANOVA between soil, lupin species and DAS. Individual means were compared by

**Plate 3.1** Chlorosis scoring system for lupins.

least squares differences (LSD). To identify the major soil factors and nutrient concentrations affecting chlorosis scores and dry weights of Gungurru and P20954 a multiple linear regression analysis using backward elimination was calculated. Results of the CTC and NTU2 soils were removed from the analyses as they apparently showed herbicide and Zn toxicity, respectively.

## 3.3 Results

*Correlations between soil properties*

Table 3.4 shows the correlations between various soil measurements. Particularly notable are the positive relationships between total $CaCO_3$ and $pH_w$, $pH_s$ and clay. As there is a very high correlation between $pH_w$ and $pH_s$, only $pH_w$ has been presented in later results and discussion.

**Table 3.4** Correlation coefficients (r) between sand, silt and clay contents, $pH_w$ and $pH_s$, total $CaCO_3$, SAR and EC.

| | Sand | Silt | Clay | $pH_w$ | $pH_s$ | Total $CaCO_3$ | SAR |
|---|---|---|---|---|---|---|---|
| **$pH_w$** | -0.81** | 0.48 | 0.86** | | | | |
| **$pH_s$** | -0.83** | 0.55* | 0.86** | 0.98** | | | |
| **Total $CaCO_3$** | -0.69** | 0.27 | 0.82** | 0.73** | 0.71** | | |
| **SAR** | -0.32 | -0.06 | 0.48 | 0.46 | 0.30 | 0.48 | |
| **EC** | 0.04 | -0.35 | 0.14 | 0.18 | 0.07 | 0.40 | 0.49 |

* $P < 0.05$, ** $P < 0.01$.

*Plant growth*

Soil type had little effect on the emergence of either species of lupin, although plants in the soils with a higher clay content (CTT2, CSK, CTY, CSK2) emerged slightly more slowly.

There was a significant three-way interaction ($P < 0.01$) between soil, lupin species and DAS for both chlorosis score and number of leaves (Fig. 3.1 and 3.2). The first recording of chlorosis, 14 DAS, was on the youngest leaves of both species grown on the calcareous soils (CTC, CSK2, CSK, CTK, CTT2, CTY, CTL). Gungurru showed more severe symptoms and stunted growth than P20954. In CTC and NTU2 the growth of both Gungurru and P20954 appeared abnormal. The CTC plants were severely stunted, leaves were curled and in Gungurru fully chlorotic suggesting a herbicide toxicity. T Patterson (pers. comm.) indicated that sulfonylurea herbicides, which are highly toxic to lupins and are residual in calcareous and alkaline soils for many years, had been used in the past 10 years (Plate 3.2). The Zn concentration was extremely high in chlorotic and stunted plants grown on NTU2 (Table 3.10) so the results from these two soils have been excluded from further analysis.

**Plate 3.2** Suspected symptoms of sulfonylurea herbicide toxicity on *L. pilosus* P20954 grown on the Cungena calcareous topsoil.

**Figure 3.1** Changes in the chlorosis score of P20954 and Gungurru on the calcareous soils from 14 to 42 days after sowing. Bar indicates LSD ($P < 0.05$).

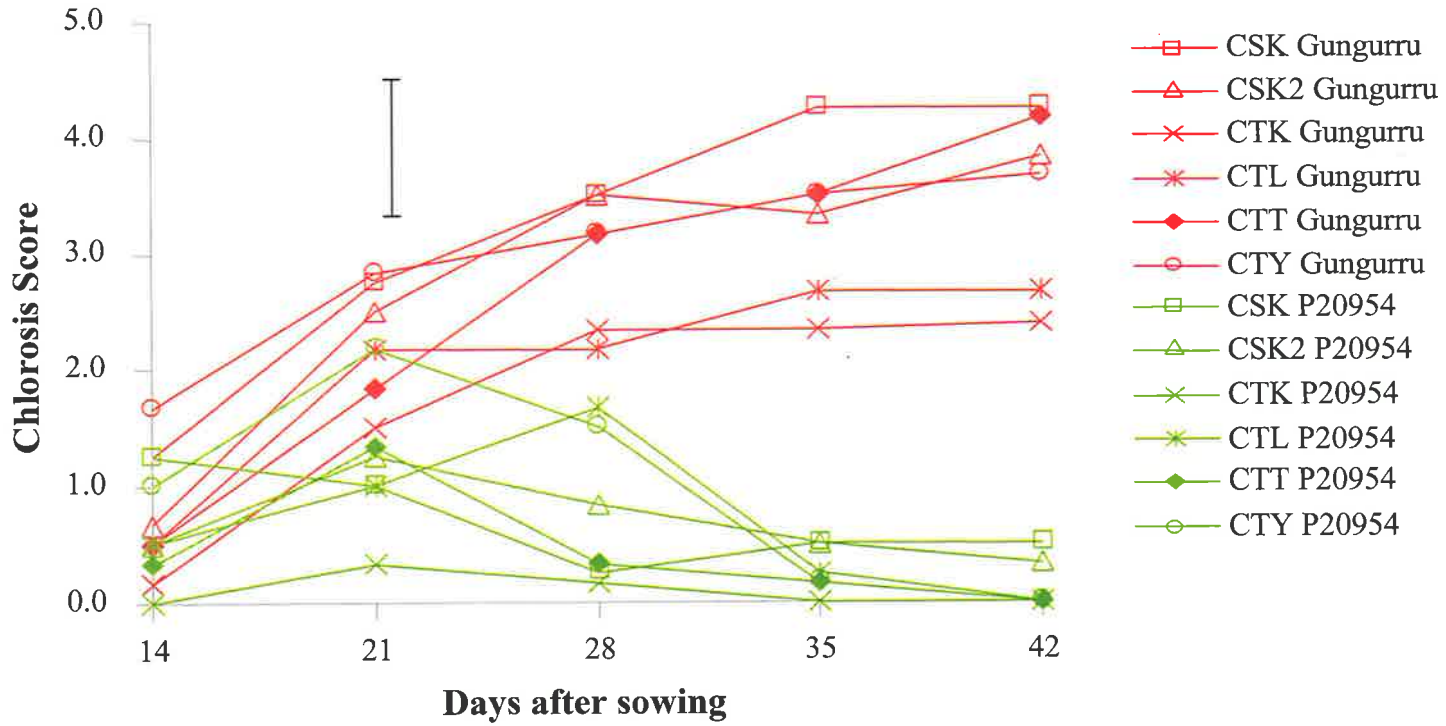
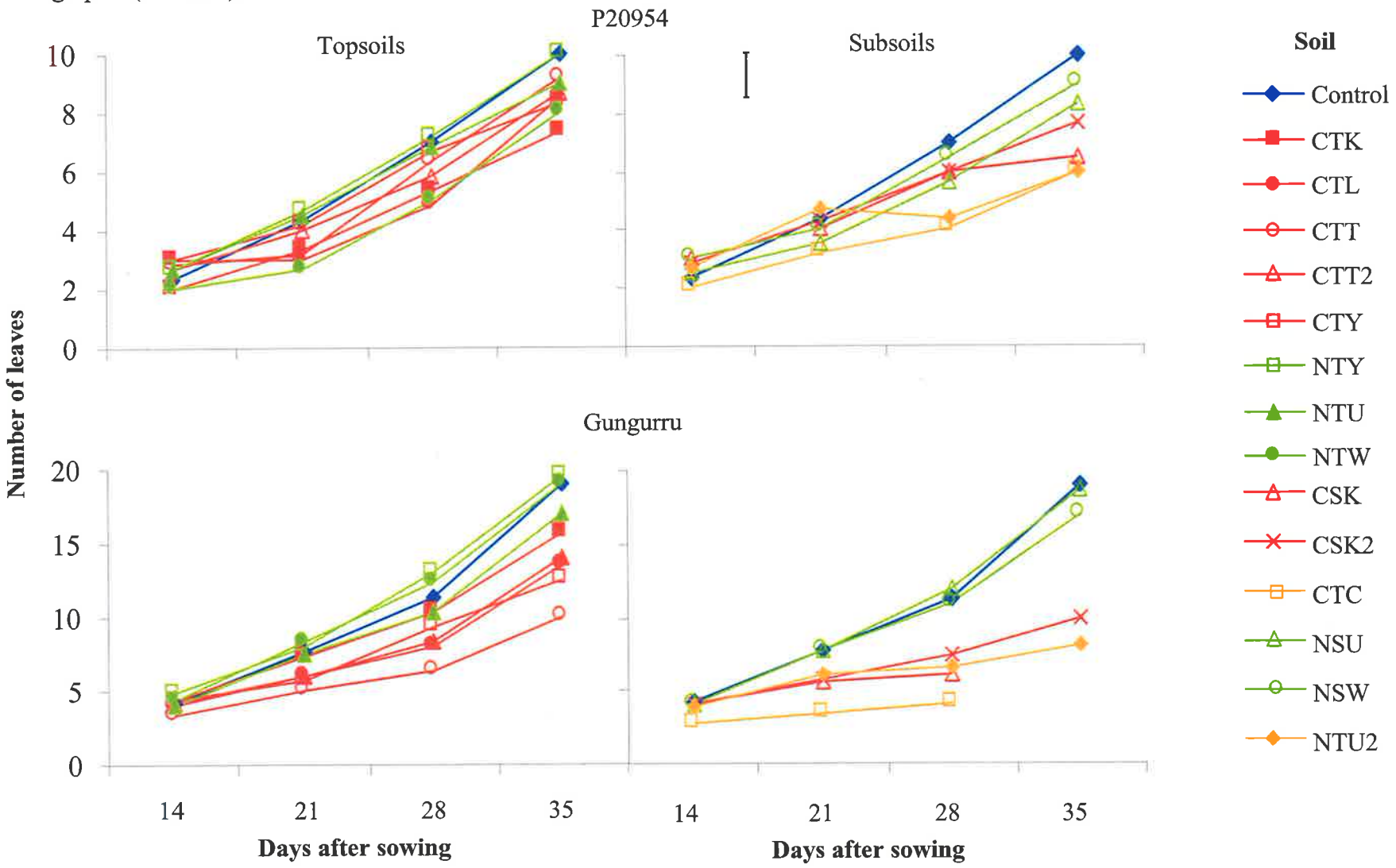


**Figure 3.2** Changes in number of leaves of P20954 and Gungurru on the calcareous and non-calcareous topsoils and subsoils from 14 to 35 days after sowing. Toxic topsoils (CTC and NTU2) are included with the subsoils. Bar indicates LSD for all graphs ($P < 0.05$).



The chlorosis scores of both species continued to increase until 21 DAS on all calcareous soils. By 28 DAS P20954 had begun to recover on all soils, except CTL until later weeks (i.e. the chlorosis score decreased; the youngest leaves became green), whereas the chlorosis scores for Gungurru still increased (Fig. 3.1). By 35 DAS chlorotic symptoms on the YEL were moderate to severe for Gungurru, but minor for P20954 (Fig. 3.1). On the calcareous soils abscission of the older leaves of Gungurru occurred from 14 DAS onwards. At 49 DAS (harvest), Gungurru displayed significantly more severe chlorotic symptoms than P20954 in all the calcareous soils, including CTL (Table 3.5). It appeared that the Gungurru plants would not have survived to produce seed in any of these calcareous soils.

The number of leaves for Gungurru on the calcareous soils was generally less than that on the non-calcareous soils, whereas for P20954 there was little difference between the calcareous and non-calcareous soils (Fig. 3.2).

At the final harvest, there was a significant interaction ($P < 0.01$) between species and soil type for both chlorosis score and dry weight (Table 3.5). The effect of soil type on leaf number was significant ($P < 0.01$) for both species (Table 3.5). The number of leaves of the plants on the calcareous soils was reduced by 35-73% for Gungurru and 14-31% for P20954 compared to the control. The highest leaf number was recorded on NTY, showing a similar trend to shoot dry weight.

Shoot dry weight of Gungurru on all the calcareous topsoils was < 25% of that on the control soil, whereas on these soils the shoot dry weight of P20954 remained > 50% of that in the control soil (Table 3.5). Plants on NTY showed the greatest growth of field soils for both species, being significantly above that on the control soil for P20954 and not significantly different for Gungurru (Table 3.5). The growth of both genotypes on the two calcareous clay subsoils, CSK2 (also sodic) and CSK, was poor, although P20954 showed less reduction in growth than Gungurru relative to the control (72% reduction *cf.* 95%). The growth of P20954 on the other two subsoils, NSU and NSW, was similar to the growth on the control soil and the non-calcareous topsoils, but the growth of Gungurru was markedly depressed. On the two soils collected about 5 m apart, there was a 93% and 45% biomass reduction on the calcareous soil (CTY) for Gungurru and P20954, respectively, compared with that on the non-calcareous soil (NTY).

**Table 3.5** Shoot dry weight, chlorosis score and leaf number of *L. angustifolius* (Gungurru) and *L. pilosus* (P20954) grown for 49 days.

| | Shoot dry weight | | Relative shoot weight | | Chlorosis score | | Leaf number | |
|---|---|---|---|---|---|---|---|---|
| **Soil** | **Gungurru** | **P20954** | **Gungurru** | **P20954** | **Gungurru** | **P20954** | **Gungurru** | **P20954** |
| Control | 2.85 | 3.81 | 100 | 100 | 0.0 | 0.0 | 22.6 | 16.0 |
| CTK | 0.38 | 2.14 | 13 | 56 | 2.0 | 0.0 | 14.0 | 11.3 |
| CSK | 0.14 | 1.04 | 5 | 27 | 4.5 | 0.5 | 6.0 | 8.5 |
| CSK2 | 0.16 | 1.07 | 5 | 28 | 2.3 | 0.2 | 10.0 | 9.0 |
| CTL | 0.57 | 2.02 | 20 | 53 | 2.7 | 1.3 | 14.6 | 11.3 |
| CTT | 0.22 | 3.23 | 8 | 85 | 4.3 | 0.0 | 8.6 | 13.3 |
| CTT2 | 0.46 | 1.93 | 16 | 51 | 0.3 | 0.0 | 18.6 | 13.3 |
| CTY | 0.17 | 2.73 | 6 | 72 | 2.2 | 0.0 | 12.6 | 13.7 |
| NTY | 2.42 | 4.83 | 85 | 127 | 0.0 | 0.0 | 24.0 | 17.3 |
| NTU | 1.46 | 2.41 | 51 | 63 | 0.0 | 0.0 | 19.4 | 14.7 |
| NSU | 1.40 | 3.13 | 49 | 82 | 0.0 | 0.0 | 21.4 | 14.0 |
| NSW | 0.92 | 3.06 | 32 | 80 | 0.0 | 0.0 | 21.4 | 15.0 |
| NTW | 1.70 | 2.81 | 60 | 74 | 0.0 | 0.0 | 20.6 | 12.7 |
| LSD[1] ($P < 0.05$) | 0.89 | | | | 1.7 | | 6.5 | 3.9 |

[1] Interaction effect significant ($P < 0.01$) for shoot dry weight and chlorosis score. Effect of soil type on leaf number was significant ($P < 0.01$).

For Gungurru, chlorosis score at all weeks correlated positively with clay content, pH and $CaCO_3$ content; shoot dry weight and leaf number were negatively correlated, with clay content, pH and $CaCO_3$ content (Table 3.6). For P20954 shoot dry weight was negatively correlated with clay content, and $CaCO_3$ content and leaf number was negatively correlated with clay content, pH, and $CaCO_3$. No soil parameter was correlated with chlorosis score at final harvest for P20954 (Table 3.6) although in weeks 2, 3, 4, and 6 chlorosis score was positively correlated with $CaCO_3$, clay content, and pH (Table 3.7). The dry weight of Gungurru was negatively correlated with B, Mg and K in the YEL and the chlorosis score positively correlated with B and K (Table 3.8). There were no significant correlations for the chlorosis score at any week or for dry weight of P20954 with any of the YEL nutrient concentrations.

The multiple linear regression analysis indicated that the major soil factor affecting chlorosis score of Gungurru at harvest was $CaCO_3$ ($P < 0.01$) accounting for 73% of the variance. Sodium absorption ratio was also significant ($P < 0.05$) due to the high SAR value associated with CSK2. There were no individual or combined soil factors which were significant in the multiple linear regression for the chlorosis score of P20954 at harvest although, in weeks 2 and 3 $CaCO_3$ was significant ($P < 0.01$) accounting for 72% of the variance. The dry weights of both genotypes were significantly affected by a number of soil factors. In Gungurru clay content ($P <$

0.01) combined with electrical conductivity ($P < 0.05$) and field capacity ($P < 0.01$) accounted for 89% of the variance, whilst for P20954, clay content ($P < 0.01$), sodium absorption ratio ($P < 0.01$), sand ($P < 0.01$), and pH ($P < 0.05$) combined accounted for 91% of the variance. Nutritional results indicated the probability of B toxicity in Gungurru on CSK and CSK2 but not in P20954 (Table 3.10), so the multiple linear regression was recalculated for the dry weight and chlorosis score of Gungurru omitting these two soils from the analysis. For chlorosis score $CaCO_3$ ($P < 0.01$) accounted for 73% of the variance and was the only significant factor, whereas for dry weight, clay content ($P < 0.01$), wilting point ($P < 0.01$), and pH ($P < 0.01$) together accounted for 96% of the variance.

Shoot dry weight, chlorosis score and leaf number were all significantly correlated for Gungurru, whereas for P20954 only shoot dry weight and leaf number were correlated (Table 3.9).

**Table 3.6** Correlation coefficients (r) between soil characteristics and shoot dry weight, chlorosis score and leaf number of *L. angustifolius* cv. Gungurru and *L. pilosus* P20954 at the final harvest (49 DAS).

| | Shoot dry weight | | Chlorosis score | | Leaf number | |
|---|---|---|---|---|---|---|
| | **Gungurru** | **P20954** | **Gungurru** | **P20954** | **Gungurru** | **P20954** |
| **Clay** | -0.79** | -0.63* | 0.69** | 0.25 | -0.78** | -0.67* |
| **$pH_w$** | -0.77** | -0.51 | 0.70** | 0.37 | -0.73** | -0.64* |
| **$CaCO_3$ content** | -0.70** | -0.56* | 0.87** | 0.22 | -0.92** | -0.70** |
| **Field capacity** | -0.52 | -0.36 | 0.50 | 0.09 | -0.54 | -0.41 |
| **Wilting point** | -0.43 | -0.34 | 0.28 | -0.03 | -0.37 | -0.31 |
| **SAR** | -0.26 | -0.46 | 0.14 | -0.02 | -0.32 | -0.48 |
| **EC** | -0.10 | -0.24 | 0.29 | 0.34 | -0.27 | -0.38 |

* $P < 0.05$, ** $P < 0.01$.

**Table 3.7** Correlation coefficients (r) between chlorosis score and $CaCO_3$ content, pH and clay content for *L. angustifolius* cv. Gungurru and *L. pilosus* P20954 from 14 to 42 DAS.

| | Days after sowing | | | | |
|---|---|---|---|---|---|
| | **14** | **21** | **28** | **35** | **42** |
| | | | *$CaCO_3$ content* | | |
| **Gungurru** | 0.82** | 0.91** | 0.93** | 0.93** | 0.96** |
| **P20954** | 0.86** | 0.86** | 0.51 | 0.90** | 0.73** |
| | | | *$pH_w$* | | |
| **Gungurru** | 0.58* | 0.79** | 0.81** | 0.78** | 0.77** |
| **P20954** | 0.58* | 0.68** | 0.58* | 0.70** | 0.48 |
| | | | *Clay* | | |
| **Gungurru** | 0.71** | 0.79** | 0.80** | 0.77** | 0.77** |
| **P20954** | 0.75** | 0.71** | 0.49 | 0.78** | 0.65* |

* $P < 0.05$, ** $P < 0.01$.

**Table 3.8** Correlation coefficients (r) between nutrient concentrations in the youngest emerging leaf and whole shoots and shoot dry weight, chlorosis score and leaf number of *L. angustifolius* cv. Gungurru and *L. pilosus* P20954 at the final harvest.

| | Dry weight | | Chlorosis score | | Leaf number | |
|---|---|---|---|---|---|---|
| **Nutrient** | **Gungurru** | **P20954** | **Gungurru** | **P20954** | **Gungurru** | **P20954** |
| | | | *Youngest emerging leaf* | | | |
| Fe | 0.11 | -0.32 | -0.43 | -0.42 | 0.33 | -0.09 |
| Mn | 0.27 | -0.44 | -0.34 | 0.18 | 0.26 | -0.49 |
| B | -0.60* | -0.54 | 0.65** | 0.20 | -0.78** | -0.67** |
| Cu | -0.13 | 0.12 | -0.31 | -0.31 | 0.22 | 0.29 |
| Zn | 0.19 | -0.15 | -0.33 | 0.06 | 0.33 | -0.12 |
| Ca | -0.46 | -0.51 | 0.27 | 0.30 | -0.22 | -0.56* |
| Mg | -0.55* | -0.54 | 0.42 | 0.52 | -0.51* | -0.62* |
| Na | -0.34 | -0.35 | 0.37 | -0.02 | -0.52* | -0.33 |
| K | -0.54* | 0.08 | 0.84** | 0.41 | -0.78** | -0.08 |
| P | 0.30 | 0.47 | 0.08 | 0.05 | 0.09 | 0.57* |
| S | 0.41 | 0.34 | -0.35 | -0.05 | 0.32 | 0.43 |
| Al | -0.33 | -0.53 | 0.54 | 0.17 | -0.58* | -0.68** |
| | | | *Whole shoot* | | | |
| Fe | -0.52* | -0.38 | 0.40 | -0.06 | -0.41 | -0.40 |
| Mn | 0.74** | -0.34 | -0.54* | 0.03 | 0.55* | -0.37 |
| B | -0.49 | -0.58* | 0.49 | 0.10 | -0.64** | -0.65** |
| Cu | 0.05 | -0.06 | -0.41 | -0.18 | 0.36 | 0.14 |
| Zn | 0.72** | 0.44 | -0.39 | -0.23 | 0.47 | 0.47 |
| Ca | -0.15 | -0.01 | 0.41 | 0.07 | -0.23 | -0.08 |
| Mg | -0.07 | -0.25 | 0.40 | 0.66** | -0.25 | -0.31 |
| Na | -0.31 | -0.47 | 0.24 | 0.00 | -0.41 | -0.40 |
| K | -0.37 | 0.22 | 0.48 | 0.37 | -0.37 | 0.04 |
| P | 0.75** | 0.44 | -0.38 | -0.10 | 0.45 | 0.54* |
| S | -0.43 | 0.31 | 0.49 | -0.02 | -0.57* | 0.33 |
| Al | -0.56* | -0.34 | 0.85** | 0.18 | -0.82** | -0.44 |

* $P < 0.05$, ** $P < 0.01$.

**Table 3.9** Correlation coefficients (r) between shoot dry weight, chlorosis score and leaf number of *L. angustifolius* cv. Gungurru and *L. pilosus* P20954 at the final harvest.

| | | Shoot dry weight | | Chlorosis score | |
|---|---|---|---|---|---|
| | | **Gungurru** | **P20954** | **Gungurru** | **P20954** |
| **Chlorosis score** | **Gungurru** | -0.72** | | | |
| | **P20954** | | -0.39 | | |
| **Leaf number** | **Gungurru** | 0.83** | | -0.95** | |
| | **P20954** | | 0.91** | | -0.46 |

* $P < 0.05$, ** $P < 0.01$.

*Nutrient concentrations*

The statistical analysis of nutrient concentrations indicated a significant interaction between species and soil type ($P < 0.05$) for Mn, B, Cu, Zn, Ca, Mg, K and P in the YEL and Mn, Zn, Ca and S in the whole shoot (Table 3.10 and 3.11). The concentrations of all nutrients measured in the YEL of Gungurru except Mn on the calcareous soils and K an the non-calcareous soils, were above the critical levels (Table 3.3; Reuter and Robinson, 1997). The critical nutrient concentrations for *L. pilosus* are unknown, hence deficiencies of particular nutrients could not be accurately determined. The concentrations of the nutrients in the plant differed between whole shoots and the YEL; in general the YEL measurements seemed more indicative of the chlorotic symptoms than the whole shoot (Table 3.10 and 3.11).

Although not significant, the Fe concentrations in the YELs of Gungurru grown on the calcareous soils were generally less than those on the non-calcareous soils (Table 3.10). For P20954, there was no difference between the non-calcareous and calcareous soils and generally it had more Fe in the YEL compared with Gungurru on the calcareous soils. The Mn concentrations showed a similar trend to Fe, although for P20954 there may be a slight decrease in concentrations on the calcareous soils (Table 3.10). The Mn concentrations in plants of Gungurru on the calcareous soils were generally less than the critical concentrations (Table 3.10 and 3.3). In the whole shoot samples the Fe and Mn concentrations generally showed similar trends to that in the YEL (Table 3.11).

B concentrations in both the YEL and whole shoot of Gungurru and P20954 were in the adequate range, except for in the YELs of Gungurru grown on CSK and CSK2 in which B levels were elevated significantly above all other soils indicating B toxicity (Table 3.10 and 3.11).

The Cu and Zn concentrations in the YEL were slightly less on the calcareous soils than the non-calcareous soils for both genotypes (Table 3.10). P20954 had higher concentrations than Gungurru on all soils. In the whole shoots Cu concentrations showed no significant trends, whereas for Zn trends were similar to the YEL (Table 3.11). On NTU2 there appeared to be Zn toxicity as concentrations were more than eight times that in any other soil.

The concentrations of Ca, Na and K were all greater in the YEL of both Gungurru and P20954 on the calcareous soils with similar trends in the whole shoots (Table 3.10 and 3.11). It was particularly notable that concentrations of Na in plants on the calcareous sodic subsoil (CSK2)

**Table 3.10** Nutrient concentrations (mg $kg^{-1}$) in the youngest emerging leaves of Gungurru and P20954 after seven weeks growth in calcareous and non- calcareous soils. n.s. - not significant.

| Soil | Genotype | Fe | Mn | B | Cu | Zn | Ca | Mg | Na | K | P | S | Al |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | Gungurru | 116 | 66 | 49 | 0.2 | 61 | 15802 | 4039 | 1417 | 22863 | 6034 | 4253 | 24 |
| CTK | Gungurru | 72 | 39 | 94 | 1.2 | 60 | 30089 | 6053 | 495 | 22378 | 2635 | 3354 | 12 |
| CSK | Gungurru | 121 | 70 | 233 | 4.0 | 59 | 25465 | 8609 | 1980 | 27735 | 2151 | 2599 | 129 |
| CSK2 | Gungurru | 81 | 10 | 231 | 6.5 | 56 | 13988 | 7595 | 6334 | 33962 | 2934 | 3000 | 78 |
| CTC | Gungurru | Dead | Dead | Dead | Dead | Dead | Dead | Dead | Dead | Dead | Dead | Dead | Dead |
| CTL | Gungurru | 50 | 16 | 87 | 2.7 | 63 | 25302 | 6812 | 907 | 33853 | 4907 | 2920 | 53 |
| CTT | Gungurru | 45 | 14 | 71 | 2.9 | 73 | 27523 | 4569 | 1255 | 37180 | 7108 | 3236 | 50 |
| CTT2 | Gungurru | 123 | 28 | 61 | 12.2 | 54 | 28318 | 4905 | 578 | 19148 | 2887 | 3036 | 30 |
| CTY | Gungurru | 212 | 117 | 110 | 5.8 | 67 | 33202 | 5746 | 1043 | 24437 | 4287 | 4090 | 10 |
| NTY | Gungurru | 112 | 47 | 43 | 8.7 | 58 | 23666 | 4474 | 264 | 18584 | 4044 | 3241 | 24 |
| NTU | Gungurru | 121 | 106 | 58 | 5.0 | 76 | 21047 | 4722 | 549 | 15784 | 4628 | 4266 | 23 |
| NSU | Gungurru | 134 | 104 | 68 | 6.8 | 105 | 22536 | 5645 | 440 | 15364 | 3852 | 3454 | 22 |
| NTW | Gungurru | 131 | 63 | 58 | 2.7 | 83 | 25508 | 6494 | 417 | 14598 | 3478 | 2869 | 68 |
| NSW | Gungurru | 197 | 48 | 61 | 6.9 | 80 | 24190 | 7417 | 428 | 15849 | 3285 | 2936 | 40 |
| NTU2 | Gungurru | 48 | 115 | 64 | 4.3 | 2175 | 21296 | 4921 | 4843 | 13842 | 2308 | 3725 | 12 |
| Control | P20954 | 112 | 52 | 39 | 1.4 | 87 | 6903 | 3452 | 4693 | 20503 | 5173 | 5100 | 58 |
| CTK | P20954 | 139 | 75 | 57 | 8.7 | 83 | 13255 | 3239 | 2213 | 22057 | 2587 | 2282 | 97 |
| CSK | P20954 | 131 | 334 | 59 | 9.8 | 102 | 22242 | 6048 | 3387 | 18064 | 1392 | 2031 | 75 |
| CSK2 | P20954 | 172 | 22 | 80 | 6.4 | 70 | 5769 | 3142 | 8897 | 19562 | 2866 | 2309 | 113 |
| CTC | P20954 | 31 | 13 | 43 | 5.7 | 41 | 24490 | 4217 | 7752 | 30067 | 2171 | 2979 | 45 |
| CTL | P20954 | 105 | 23 | 56 | 4.9 | 83 | 9915 | 4103 | 3848 | 26415 | 4569 | 2881 | 71 |
| CTT | P20954 | 122 | 26 | 75 | 6.1 | 72 | 7813 | 3200 | 6487 | 19345 | 2906 | 2315 | 79 |
| CTT2 | P20954 | 180 | 35 | 43 | 18.7 | 76 | 8132 | 3158 | 4337 | 15753 | 3068 | 2205 | 43 |
| CTY | P20954 | 152 | 49 | 38 | 13.8 | 77 | 14953 | 3487 | 2494 | 26773 | 3207 | 2631 | 65 |
| NTY | P20954 | 126 | 31 | 38 | 16.6 | 70 | 5328 | 2496 | 2116 | 20562 | 3658 | 2312 | 35 |
| NTU | P20954 | 143 | 75 | 51 | 10.4 | 93 | 6964 | 3201 | 5012 | 16438 | 4896 | 3440 | 71 |
| NSU | P20954 | 134 | 63 | 51 | 12.6 | 100 | 5917 | 2726 | 4557 | 16273 | 3798 | 2418 | 38 |
| NTW | P20954 | 136 | 38 | 49 | 4.4 | 104 | 6192 | 3533 | 3458 | 23838 | 4453 | 2920 | 55 |
| NSW | P20954 | 185 | 37 | 48 | 13.6 | 93 | 7844 | 3569 | 3354 | 19788 | 3139 | 2476 | 69 |
| NTU2 | P20954 | 61 | 52 | 70 | 5.1 | 810 | 4242 | 2037 | 4845 | 22613 | 2769 | 2028 | 93 |
|  | LSD | n.s. | 39 | 35 | 3.6 | 145 | 6237 | 1409 | n.s. | 6839 | 1660 | n.s. | n.s. |

**Table 3.11** Nutrient concentrations (mg $kg^{-1}$) in the whole shoots of Gungurru and P20954 after seven weeks growth in calcareous and non-calcareous soils. n.s. - not significant.

| Soil | Genotype | Fe | Mn | B | Cu | Zn | Ca | Mg | Na | K | P | S | Al |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | Gungurru | 59 | 114 | 23 | 1.6 | 228 | 28494 | 5721 | 5377 | 25169 | 6560 | 6117 | 82 |
| CTK | Gungurru | 68 | 23 | 53 | 1.9 | 25 | 28417 | 3386 | 2773 | 35582 | 1084 | 3461 | 113 |
| CSK | Gungurru | 128 | 29 | 89 | 3.3 | 31 | 14858 | 5186 | 5275 | 27330 | 1138 | 5633 | 209 |
| CSK2 | Gungurru | 75 | 9 | 143 | 3.4 | 29 | 7736 | 4010 | 27053 | 21439 | 1525 | 6924 | 141 |
| CTC | Gungurru | Dead | Dead | Dead | Dead | Dead | Dead | Dead | Dead | Dead | Dead | Dead | Dead |
| CTL | Gungurru | 68 | 18 | 47 | 1.9 | 53 | 48330 | 8387 | 2434 | 40804 | 2114 | 6003 | 107 |
| CTT | Gungurru | 95 | 17 | 28 | 2.1 | 53 | 54401 | 5618 | 4000 | 28797 | 1890 | 4999 | 180 |
| CTT2 | Gungurru | 112 | 18 | 21 | 7.5 | 28 | 22855 | 3329 | 4135 | 24026 | 1176 | 5602 | 95 |
| CTY | Gungurru | 95 | 30 | 36 | 3.5 | 35 | 25187 | 4275 | 3908 | 41662 | 1491 | 7499 | 88 |
| NTY | Gungurru | 72 | 32 | 22 | 6.3 | 39 | 19059 | 3276 | 1002 | 27614 | 2130 | 2728 | 68 |
| NTU | Gungurru | 48 | 88 | 29 | 3.6 | 74 | 28539 | 4879 | 2796 | 15934 | 3540 | 4150 | 43 |
| NSU | Gungurru | 61 | 75 | 34 | 4.7 | 85 | 16524 | 3931 | 3245 | 15784 | 1922 | 3065 | 43 |
| NTW | Gungurru | 86 | 39 | 30 | 2.3 | 63 | 13960 | 4106 | 2097 | 23381 | 2083 | 2752 | 125 |
| NSW | Gungurru | 113 | 31 | 27 | 4.5 | 60 | 14790 | 4452 | 2039 | 24437 | 1427 | 3199 | 79 |
| NTU2 | Gungurru | 157 | 68 | 45 | 3.5 | 2223 | 11208 | 3299 | 10352 | 21538 | 1594 | 7057 | 291 |
| Control | P20954 | 41 | 121 | 25 | 1.7 | 357 | 17877 | 6302 | 8859 | 28455 | 4899 | 8205 | 73 |
| CTK | P20954 | 116 | 161 | 46 | 4.3 | 87 | 47380 | 4857 | 3820 | 35264 | 960 | 1720 | 156 |
| CSK | P20954 | 96 | 318 | 47 | 6.2 | 69 | 29570 | 5986 | 5029 | 28105 | 653 | 1578 | 133 |
| CSK2 | P20954 | 187 | 51 | 141 | 4.8 | 36 | 7861 | 4355 | 31192 | 12623 | 805 | 2099 | 262 |
| CTC | P20954 | 48 | 10 | 24 | 5.3 | 27 | 18696 | 4319 | 5302 | 26840 | 1922 | 4046 | 90 |
| CTL | P20954 | 103 | 31 | 32 | 4.1 | 73 | 34107 | 6689 | 6635 | 40560 | 1927 | 2873 | 165 |
| CTT | P20954 | 101 | 32 | 21 | 3.7 | 104 | 49308 | 4727 | 6101 | 26396 | 1431 | 2071 | 133 |
| CTT2 | P20954 | 121 | 57 | 20 | 10.3 | 60 | 28340 | 3903 | 10734 | 18286 | 1255 | 1754 | 89 |
| CTY | P20954 | 165 | 93 | 20 | 7.3 | 68 | 50064 | 4902 | 3295 | 39314 | 1307 | 1659 | 244 |
| NTY | P20954 | 110 | 74 | 21 | 8.1 | 62 | 20802 | 3620 | 3105 | 33636 | 1586 | 1740 | 157 |
| NTU | P20954 | 79 | 115 | 28 | 6.8 | 141 | 20030 | 4629 | 10366 | 16388 | 3330 | 2970 | 119 |
| NSU | P20954 | 105 | 110 | 34 | 6.6 | 161 | 25493 | 4085 | 9956 | 14011 | 1757 | 2541 | 96 |
| NTW | P20954 | 116 | 57 | 28 | 3.7 | 131 | 18312 | 5126 | 4384 | 34716 | 2138 | 2711 | 128 |
| NSW | P20954 | 129 | 71 | 22 | 5.7 | 117 | 23003 | 4852 | 4839 | 22525 | 1117 | 1482 | 92 |
| NTU2 | P20954 | 108 | 52 | 40 | 4.7 | 1678 | 5686 | 1991 | 14206 | 10583 | 1286 | 2941 | 215 |
| | LSD | n.s. | 26 | n.s. | n.s. | 111 | 12310 | n.s. | n.s. | n.s. | n.s. | 2280 | n.s. |

**Table 3.12** Correlation coefficients between nutrient concentrations in the YEL of Gungurru and P20954 and the soil measurements.

| | Fe | Mn | B | Cu | Zn | Ca | Mg | Na | K | P | S | Al |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | *Gungurru* | | | | | | |
| **Clay** | -0.04 | -0.34 | 0.77** | 0.30 | -0.59* | 0.24 | 0.52 | 0.57* | 0.60* | -0.39 | -0.44 | 0.48 |
| **$pH_w$** | -0.25 | -0.55* | 0.65* | 0.13 | -0.51 | 0.26 | 0.56* | 0.52 | 0.67* | -0.31 | -0.56 | 0.40 |
| **$CaCO_3$ content** | -0.15 | -0.17 | 0.84** | -0.11 | -0.34 | 0.06 | 0.49 | 0.67* | 0.76** | -0.06 | -0.24 | 0.59* |
| **Field capacity** | -0.03 | -0.35 | 0.60* | 0.45 | -0.72** | 0.22 | 0.29 | 0.45 | 0.44 | -0.39 | -0.40 | 0.36 |
| **Wilting point** | 0.00 | -0.37 | 0.49 | 0.60* | -0.71** | 0.09 | 0.15 | 0.48 | 0.32 | -0.39 | -0.31 | 0.22 |
| **SAR** | -0.18 | -0.38 | 0.66* | 0.18 | -0.31 | -0.61* | 0.37 | 0.96** | 0.39 | -0.28 | -0.20 | 0.35 |
| **EC** | -0.13 | -0.32 | 0.43 | -0.27 | 0.14 | -0.54 | 0.50 | 0.58* | 0.46 | 0.15 | -0.37 | 0.61* |
| | | | | | | *P20954* | | | | | | |
| **Clay** | 0.36 | 0.34 | 0.40 | 0.23 | -0.42 | 0.57* | 0.44 | 0.20 | 0.10 | -0.77** | -0.60* | 0.46 |
| **$pH_w$** | 0.23 | 0.10 | 0.55* | 0.09 | -0.51 | 0.40 | 0.27 | 0.16 | 0.33 | -0.69** | -0.69** | 0.50 |
| **$CaCO_3$ content** | 0.03 | 0.43 | 0.61* | -0.17 | -0.26 | 0.57* | 0.51 | 0.40 | 0.15 | -0.65* | -0.38 | 0.50 |
| **Field capacity** | 0.31 | 0.28 | 0.17 | 0.39 | -0.55* | 0.44 | 0.27 | 0.05 | 0.02 | -0.71** | -0.56* | 0.21 |
| **Wilting point** | 0.47 | 0.10 | 0.12 | 0.46 | -0.62* | 0.22 | 0.07 | 0.18 | -0.11 | -0.57* | -0.47 | 0.14 |
| **SAR** | 0.40 | -0.11 | 0.59* | -0.17 | -0.36 | -0.19 | -0.08 | 0.75** | -0.14 | -0.22 | -0.14 | 0.59* |
| **EC** | -0.05 | 0.04 | 0.51 | -0.58* | 0.18 | -0.09 | 0.32 | 0.59* | 0.10 | -0.01 | 0.12 | 0.33 |

* $P < 0.05$, ** $P < 0.01$.

were significantly elevated compared with other soils. Mg, P, S and Al all showed no trends between calcareous and non-calcareous soils (Table 3.10). Comparing the genotypes, Ca, K and P concentrations were generally greater in Gungurru than P20954, whilst Na concentrations were greater in P20954.

The correlation of nutrient concentrations in the YEL with soil measurements indicated that both B and K were positively correlated with clay, pH and $CaCO_3$ content for Gungurru (Table 3.12). For P20954, P concentrations were negatively correlated with all three measurements, whilst Ca and B correlated with $CaCO_3$ content, B and S correlated with pH and Ca and S correlated with clay. For both genotypes, SAR correlated positively with Na concentrations in the YEL.

## 3.4 Discussion

The results from this experiment indicated that P20954 has better adaptation to a range of soil types than Gungurru. Particularly, P20954 grew adequately on calcareous soils where current varieties of narrow-leafed lupin cannot be grown economically, in agreement with the results of previous studies (White, 1990; Egan *et. al.*, 1992-93).

Previous work on calcareous soils under field conditions (J P Egan, pers comm.) and with solution culture at high pH / $HCO_3^-$ (Tang and Thomson, 1996) has demonstrated that *L. pilosus* yields higher and shows less chlorotic symptoms than *L. angustifolius*. These observations were verified in this experiment in which P20954 was able to recover from the chlorotic symptoms early in the experiment whereas on Gungurru the chlorosis persisted throughout. This is contrary to the findings of Tang *et al.* (1993) who found that the chlorotic symptoms on *L. angustifolius* genotypes grown on an alkaline soil in the field decreased after approximately six to eight weeks. In that experiment the soil contained 0.6 – 3% $CaCO_3$ which is generally toxic to the growth of *L. angustifolius* (Tang *et al.*, 1993). Soil moisture differences between the two studies might be the reason for the failure of Gungurru to recover as had been previously observed. In the present study, soil moisture content was kept constant whereas in the study of Tang *et al.* (1993) soil moisture fluctuated and the topsoil would have dried. Under dryer conditions the concentration of $HCO_3^-$ in the soil could have been reduced as the partial pressure of $CO_2$ decreases in drier soils (Russell, 1988), hence plants may have been able to recover.

High soil clay content, pH and $CaCO_3$ levels, correlated with reduced growth and increased chlorosis score of Gungurru, while high soil clay content and $CaCO_3$ levels correlated only with reduced growth of P20954. These soil factors were correlated with each other, hence it is difficult to determine from this the causative factor. The multiple linear regression analysis suggests that, in terms of chlorotic symptoms, Gungurru was more affected by increased levels of $CaCO_3$ than P20954. The growth of Gungurru was linked to $CaCO_3$ levels, although it was also significantly affected by a number of other soil factors particularly pH and clay content similar to findings from previous studies (White and Robson, 1989b; Atwell, 1991a; Atwell, 1991b). The growth and chlorosis score at harvest of P20954 was not affected by $CaCO_3$, pH or clay content *per se,* rather it was related to a combination of soil factors.

Soil $CaCO_3$ appeared to be a major factor affecting lupin growth at the field site where CTY (5% $CaCO_3$) was collected. Narrow-leafed lupins growing at this site showed chlorotic symptoms on the youngest leaves and stunted growth. There was a distinct boundary between the calcareous patch (CTY) and the rest of the paddock, i.e. lupins were highly chlorotic only centimetres from those which were without symptoms (NTY) (Chapter 2; Plate 2.1). It must be noted that NTY showed the highest growth rate of the field soils for both species in the current study although it had a relatively high pH (7·9) which has been reported to reduce the growth rate of narrow-leafed lupins such as Gungurru (Tang, *et al.*, 1995b).

The line P20954 also performed much better on the subsoils compared with Gungurru indicating that this genotype would be able to utilise subsoil moisture and nutrition to achieve high grain yields. The nutritional results also indicate that P20954 is more B tolerant than Gungurru. This is important from a South Australian perspective as many soils contain toxic levels of B, especially in the subsoil, hence crops need to be tolerant in order to achieve maximum yields (Bagheri, *et al.*, 1994).

In the presence of high soil $CaCO_3$ levels and high pH there are high $HCO_3^-$ concentrations which are toxic to the growth of plants (Boxma, 1972; Coulombe, *et al.*, 1984a). Bicarbonate has been used successfully to screen soybeans for tolerance to Fe deficiency. (Coulombe, *et al.*, 1984b; Chaney, *et al.*, 1992a). The mechanisms by which $HCO_3^-$ acts are varied (Marschner, 1995), but it commonly induces Fe deficiency in solution culture at rates above 5-10 mM (Chaney, *et al.*, 1992a; Chaney, *et al.*, 1992b; Shi, *et al.*, 1993). In the soil at a pH of 8·5 (common throughout many calcareous soils) the $HCO_3^-$ level can range theoretically up to 50

mM (Russell, 1988)(Chapter 2, Fig. 2.3). Hence the effects of $HCO_3^-$ observed in solution culture are within the range of $HCO_3^-$ levels that could occur in calcareous soils. In the soil, active $CaCO_3$ acts as a buffer maintaining $HCO_3^-$ concentrations in the soil solution.

The recovery from symptoms resembling Fe chlorosis observed in P20954 grown on the calcareous soils could be due to several alternative mechanisms.

1. a proton pump may be activated, leading to acidification of the rhizosphere and a reduction in the concentrations of $HCO_3^-$, which could allow the plant to take up more nutrients as the pH and buffering effects are decreased.
2. $HCO_3^-$ may be excluded from the roots analogous to that observed in B tolerant genotypes of wheat (Paull, *et al.*, 1992).
3. nitrogen fixation being initiated increases proton excretion due to excess uptake of cations over anions acidifying the rhizosphere.
4. proteoid root formation. *L. pilosus* is able to form proteoid roots which are stimulated by increased levels of $HCO_3^-$ (Tang and Thomson, 1996). Gardner, *et al.* (1981) found that the pH was decreased in the rhizosphere zone of the proteoid roots, which was likely to increase the availability of nutrients in alkaline soils. Dinkelaker, *et al.* (1989), who grew white lupins (*L. albus*) in a soil with 20% $CaCO_3$, found that the pH was 4.8 in the zones of proteoid roots compared with the surrounding soil pH 7.5. In these zones, citric acid was released in response to P deficiency and may have aided in the uptake of other nutrients such as Fe and Mn (Dinkelaker, *et al.*, 1989).
5. greater ability to mobilise, translocate and utilise the Fe and other nutrients present within the plant.

As discussed in Chapter 2 (Section 2.1.2) root morphology is also different between the species. A taproot dominant root system (Gungurru) appears suited to deep sandy soils where penetration of the taproot allows extraction of water from deep within the profile (Clements, *et al.*, 1993). If penetration of the taproot is restricted, such as on a finer textured soils, the lower density of secondary and tertiary laterals may limit the efficient uptake of soil water and nutrients (Clements, *et al.*, 1993). Thus the greater proportion primary, secondary and tertiary lateral roots in P20954 may give it an adaptive advantage on clay or clay loam soils.

Nutritional analyses indicated that the poor growth of lupins on calcareous soils was not due to the deficiency of any other nutrients. Although the Fe concentrations in plants grown on

calcareous soils were not indicative of Fe chlorosis, it has been suggested that Fe may be precipitated in the apoplasm of leaves and not be physiologically available (Kaur, *et al.*, 1984; Mengel and Geurtzen, 1986). As the nutritional concentrations were measured at the end of the experiment, not when symptoms were most severe on P20954, the Fe levels may have increased as the symptoms decreased.

# CHAPTER 4

## Development of a soil screening method to identify lupins tolerant to calcareous soils and investigation of possible mechanisms of tolerance

## 4.1 Introduction

As shown in previous research (Plessner, *et al.*, 1992; Egan, *et al.*, 1992-93) and chapters 2 and 3, both *L. pilosus* and *L. atlanticus* have potential as an alternative pulse crop on calcareous soils, but there has been no research identifying intraspecific variation for expression of adaptation to these soils. However, it is expected that a range of tolerance to calcareous soils exists in *L. pilosus* as landraces and wild types have been collected throughout the circum-mediterranean on both calcareous and non-calcareous soils (Table 2.2 and 6.1). In *L. atlanticus* the range of variation is likely to be less as all landraces and wild types have been collected from the Atlas mountains in Morocco (Table 2.2 and 6.1).

The most common method of identifying intraspecific variation for adaptation to soil characteristics is through the development of screening protocols which replicate the reactions observed in field trials. For example, genotypic variation was noted in soybean grown on calcareous soils, with some cultivars showing less $HCO_3^-$-induced Fe deficiency chlorosis than others (Rodriguez de Cianzio, *et al.*, 1979). Both soil and solution based screening methods were developed to replicate these observations and these enabled efficient and accurate identification of tolerant genotypes (Byron and Lambert, 1983; Coulombe, *et al.*, 1984b; Jessen, *et al.*, 1986; Chaney, *et al.*, 1992a). In undomesticated species, such as *L. pilosus* and *L. atlanticus*, formulation of screening methods is more difficult as they have not been tested on a range of soil types. To overcome the lack of field data either large scale multi-site replicated field experiments or glasshouse based controlled screening experiments must first be conducted on a calcareous soil. Due to the small numbers of seed available and hand sowing of scarified seed in field experiments being extremely laborious, a soil based screening system was developed to reflect the field situation. Once a range of tolerance had been identified, a few genotypes differing in response could then be tested under field conditions to confirm or refute the results from the screening method.

To be useful in commercial breeding programs, screening methods must be efficient and accurate. Methods should ideally be conducted under controlled conditions, thus being easily repeatable. In a soil system, while the atmospheric (temperature, water supply) environment can be controlled through growth either in a glasshouse or a growth room, the soil environment is difficult to control as there are many abiotic and biotic factors that may influence the response. Previous research has indicated that the major factors influencing the ranking of genotypes are the $CaCO_3$ content of the soil and the soil moisture, whilst the addition of Fe either as a foliar spray or through soil fertilization can help overcome limitations imposed by a calcareous soil (Fairbanks, *et al.*, 1987; Longnecker, 1988; Jessop, *et al.*, 1990). These factors are discussed further in the introductions to the relevant experiments in this chapter.

In a screening procedure, measurements to identify tolerance should be taken during the first few weeks of growth, are simple to record and non-destructive, but reflective of the yield losses due to intolerance. In soil systems, simple measurements include the extent of chlorosis, shoot weight, plant height and rate of leaf production. For example, in soybean and chickpea a visual chlorosis score has been used to identify tolerant genotypes (Rodriguez de Cianzio, *et al.*, 1979; Chaney, *et al.*, 1992b). Measurements of growth are often not significant until later in growth after the initial visual symptoms have been expressed and are often destructive.

The major aims of experiments in this chapter were to define a soil screening method which enables the identification of intraspecific variation for tolerance to calcareous soils and to investigate the mechanisms of tolerance in *L. pilosus*.

# 4.2 The effect of increasing $CaCO_3$ concentrations in the soil on the growth of four lupin species and field peas (Experiment 1)

## 4.2.1 Introduction

Previous research has indicated that the addition of $CaCO_3$ to the soil increased chlorotic symptoms in intolerant genotypes (Anderson, 1983; White and Robson, 1989b). Jessop, *et al.* (1990) mixed two soils, high and low in $CaCO_3$ content, at different ratios and found that height and shoot and root dry weight of narrow-leafed lupin decreased as the proportion of the soil with a higher $CaCO_3$ content increased. Adding 3 g $kg^{-1}$ of $CaCO_3$ to an acidic sand increased the pH of the soil and decreased the fresh weight and concentration of P, Mn, Cu and Zn in the youngest leaf of *L. angustifolius* cv. Yandee (White and Robson, 1989b). Agronomic studies on the effect of liming on acidic soils in the field have indicated a negative effect on lupin growth proportional to the $CaCO_3$ added (Mayfield, *et al.*, 1996). Thus, the addition of $CaCO_3$ to a non-calcareous soil is a possible method of identifying differences in tolerance to calcareous soil between lupin genotypes.

Paull, *et al.* (1988) in studies developing screening methods for B toxicity, first focussed on determining a suitable level of B in the soil (by addition of boric acid to a soil with low B) for selecting tolerant genotypes. Similarly, the concentration of $CaCO_3$ which provides the greatest differentiation between tolerant and intolerant genotypes should be determined. The critical concentration of active $CaCO_3$ in the soil under field situations, above which *L. angustifolius* shows chlorosis, is approximately 2-3% (Mayfield, *et al.*, 1996). The critical level for the other lupin species is unknown, although it is likely to be higher for *L. albus*, *L. pilosus* and *L. atlanticus* (Buirchell and Cowling, 1989; C Hyughe, pers. comm).

To determine the optimal concentration of $CaCO_3$ in the soil for screening tolerant genotypes, there are three methods by which different $CaCO_3$ contents can be created:

1. the addition of increasing quantities of $CaCO_3$ to a non-calcareous soil.
2. the addition of increasing quantities of an acid to a highly calcareous soil (> 50% $CaCO_3$). The acid proportionally reacts with the $CaCO_3$ and different contents can be achieved.
3. mixing of a calcareous and a non-calcareous soil at different ratios.

The simplest and most efficient method is the addition of $CaCO_3$, being easy to calculate the quantities needed compared with the other methods. After adding $CaCO_3$, the soil must be

equilibrate whilst moist for a period of time, allowing the soil solution to react with $CaCO_3$ and $CO_2$, producing $HCO_3^-$ (Section 2.2.2). As it is difficult to determine the amount of acid needed to neutralize the $CaCO_3$ and the chemical and physical properties of the soil are likely to be adversely affected by the acid this method was not investigated here. Similarly, mixing soils is possible, however it would be necessary to use two soils with similar physical and structural characteristics, but differing in $CaCO_3$ contents, making this a less desirable option.

As discussed previously both *L. pilosus* and *L. atlanticus* show greater tolerance than *L. angustifolius* to calcareous soils, but are undomesticated. Alternatively, C Huyghe (pers. comm.) and B Jørnsgård (pers. comm.) has indicated that there is some tolerance in *L. albus* under field conditions, but the species has not been screened in controlled systems. If suitable genotypes were found in *L. albus*, the genes controlling tolerance could be rapidly incorporated into released cultivars via backcrossing.

In this experiment four species of lupin and a field pea cultivar were compared across a range of $CaCO_3$ concentrations, induced by the addition of $CaCO_3$ to a non-calcareous soil, to determine suitable concentrations for screening.

### 4.2.2 Materials and methods

*Genetic material*

Genotypes were selected to represent the range of lupin species of interest in agriculture and to observe intraspecific variation in *L. albus*. The species used were *L. albus* (three genotypes: P27486, P27797 and Hamburg), *L. pilosus* (P20954), *L. atlanticus* (P22927), *L. angustifolius* (Gungurru) and *Pisum sativum* (Alma) (Table 6.1). The line P27486 had been classified as tolerant of Fe chlorosis, P27797 was collected on a calcareous soil in Egypt, while Hamburg was known to be intolerant (C Huyghe pers. comm.). Both Gungurru and Alma were used as reference lines, Gungurru is highly sensitive to active $CaCO_3$ in the soil, while Alma has been widely grown throughout South Australia and is tolerant of active $CaCO_3$.

*Soil and pot preparation*

The experimental soil was a loamy clay collected from the CSIRO Glenthorne Research Farm, O'Halloran Hill, South Australia (Paull, *et al.*, 1988)(Table 4.1). The UC modified potting mix was used as the control (Section 3.2; Table 3.1). A bulk sample of each soil was air-dried, sieved

through a five millimetre screen and added to twenty centimetre diametre plastic lined pots (4.5 litres) to prevent leaching of nutrients. The weight of each pot was recorded.

Calcium carbonate was added to the surface of the Glenthorne soil at rates of 0, 2, 5, 10 and 20%. It was thoroughly mixed with the soil by removing the plastic bag and shaking for three minutes, after which the bag was placed back in the pot. The pots were watered to 70% of field capacity with RO water and remixed with a trowel. No nutrients were added to either soil. Pots were allowed to equilibrate for four weeks, being remixed and watered at regular intervals. After this period a soil sample was taken and $CaCO_3$ content and pH were determined (Table 4.2; Section 3.2.2). Another set of samples were taken after harvest for pH determination (Table 4.2).

**Table 4.1** Total $CaCO_3$, pH, moisture content at field capacity and estimated $HCO_3^-$ concentration (Russell, 1988) of the Glenthorne (with no added $CaCO_3$) and Wangary soils. The Wangary soil was used in experiments 2a, 2b and 2c.

| Soil | $CaCO_3$ (%) | pH | Field capacity (%) | $HCO_3^-$ concentration (mM) |
|---|---|---|---|---|
| Glenthorne | 0 | 6.7 | 36 | Negligible |
| Wangary | 50 | 8.2 | 36 | 20 |

**Table 4.2** Calculated $CaCO_3$ content (%$CaCO_3$; Section 3.2), and pH of soil before and after plant growth in the five $CaCO_3$ treatments in the Glenthorne soil compared with the control (UC soil).

| Treatment | %$CaCO_3$ | pH | |
|---|---|---|---|
| | | before | after |
| 0% $CaCO_3$ | 0.0 | 6.59 | 6.67 |
| 2% $CaCO_3$ | 1.8 | 7.40 | 7.36 |
| 5% $CaCO_3$ | 4.6 | 7.44 | 7.36 |
| 10% $CaCO_3$ | 9.1 | 7.40 | 7.40 |
| 20% $CaCO_3$ | 18.7 | 7.51 | 7.46 |
| Control | 0.0 | 5.82 | 5.85 |

*Plant growth and experimental design*

Prior to sowing, seeds were surface sterilized by washing in 70% ethanol, then soaking in 1.4% hypochlorite for five minutes and rinsing in water. After sterilizing, seeds of *L. pilosus* and *L. atlanticus* genotypes were scarified (Section 3.2.4). Seeds of all species and genotypes were imbibed and sown with group G inoculant as described in Section 3.2.4. Two seeds of every

genotype were sown in each pot. Seven days after sowing plants were thinned to one plant per genotype, giving a total of seven plants per pot. Plants were grown under glasshouse conditions (15 - 30°C) and pots watered with RO water to weight every two days.

The pots were arranged as a randomized complete block with six $CaCO_3$ levels and four replicates.

*Measurements*

The duration of the experiment and measurements recorded, indicating days after sowing, are summarised in Table 4.3. Details of the methods of measurements are described in Section 4.3.

*Statistical analyses*

To test for significant differences a two-way (ANOVA) between genotypes and $CaCO_3$ content was used for chlorosis score at 21, 28, 35 and 42 DAS and shoot dry weight and concentration of nutrients in the YEL 42 DAS. Chlorosis score was also analysed by a three-way ANOVA between genotypes, $CaCO_3$ content and DAS. Individual means were compared by LSD. For correlation coefficients the relative shoot dry weight (percentage of each treatment relative to the 0% $CaCO_3$ treatment in the Glenthorne soil) was calculated.

The control (UC potting mix) treatment was omitted from the ANOVA for chlorosis score and correlation coefficients, as no chlorosis was observed in this soil and the concentrations of P, S, and Zn in the YEL of plants grown in this soil were much higher than in any of the treatments in the Glenthorne soil.

### 4.2.3 Results

*Soil structure, $CaCO_3$ content and pH*

The Glenthorne soil without amendments of $CaCO_3$ has a crumbly structure (Paull, *et al.*, 1988). The addition of $CaCO_3$ to the soil resulted in a change of soil structure, becoming more prone to waterlogging, especially at higher $CaCO_3$ contents. The calculated $CaCO_3$ contents from the calcimetre readings were highly correlated with amounts of $CaCO_3$ added ($r = 0.99$), but pH did not increase proportionally (Table 4.2). The pH remained similar in all the $CaCO_3$ treatments before and after the experiment (Table 4.2).

**Table 4.3** Duration of the each of the four experiments and days after sowing when measurements of chlorosis score, chlorophyll meter readings, chlorophyll and active Fe concentrations, shoot dry weight, number of leaves and nutrient concentrations were recorded. n.d. - not determined.

| Experiment | Length of experiment (days) | Chlorosis score | Chlorphyll meter readings | Chlorophyll concentration | Active Fe | Shoot dry weight | Number of leaves | Nutrient concentrations |
|---|---|---|---|---|---|---|---|---|
| 1. $CaCO_3$ concentrations | 42 | 21, 28, 35, 42 | n.d. | n.d. | n.d. | 42 | n.d. | 42 |
| 2a. Soil moisture | 35 | 14, 21, 28, 35 | 14, 21, 28, 35 | 14, 21, 28, 35 | 14, 21, 28, 35 | 14, 21, 28, 35 | 14, 21, 28, 35 | 14, 21, 28, 35 |
| 2b. Nutrient addition | 84 | 21, 42, 56, 84 | 21, 42, 56, 84 | 21, 42, 56, 84 | 21, 42, 56, 84 | 21, 42, 56, 84 | n.d. | 21, 42, 56, 84 |
| 2c. $N_2$ fixation | 28 | 14, 21, 28 | 28 | 28 | 28 | 28 | n.d. | 28 |

*Chlorosis score and shoot dry weight*

Statistical analyses indicated that the three-way interaction between $CaCO_3$ content, genotype and DAS for chlorosis score was not significant, but the two-way interaction between $CaCO_3$ content and genotype was significant ($P < 0.05$) at each week of the experiment. For shoot dry weight at harvest (42 DAS), the main effects of soil treatment ($P < 0.01$) and genotype ($P < 0.01$) were significant.

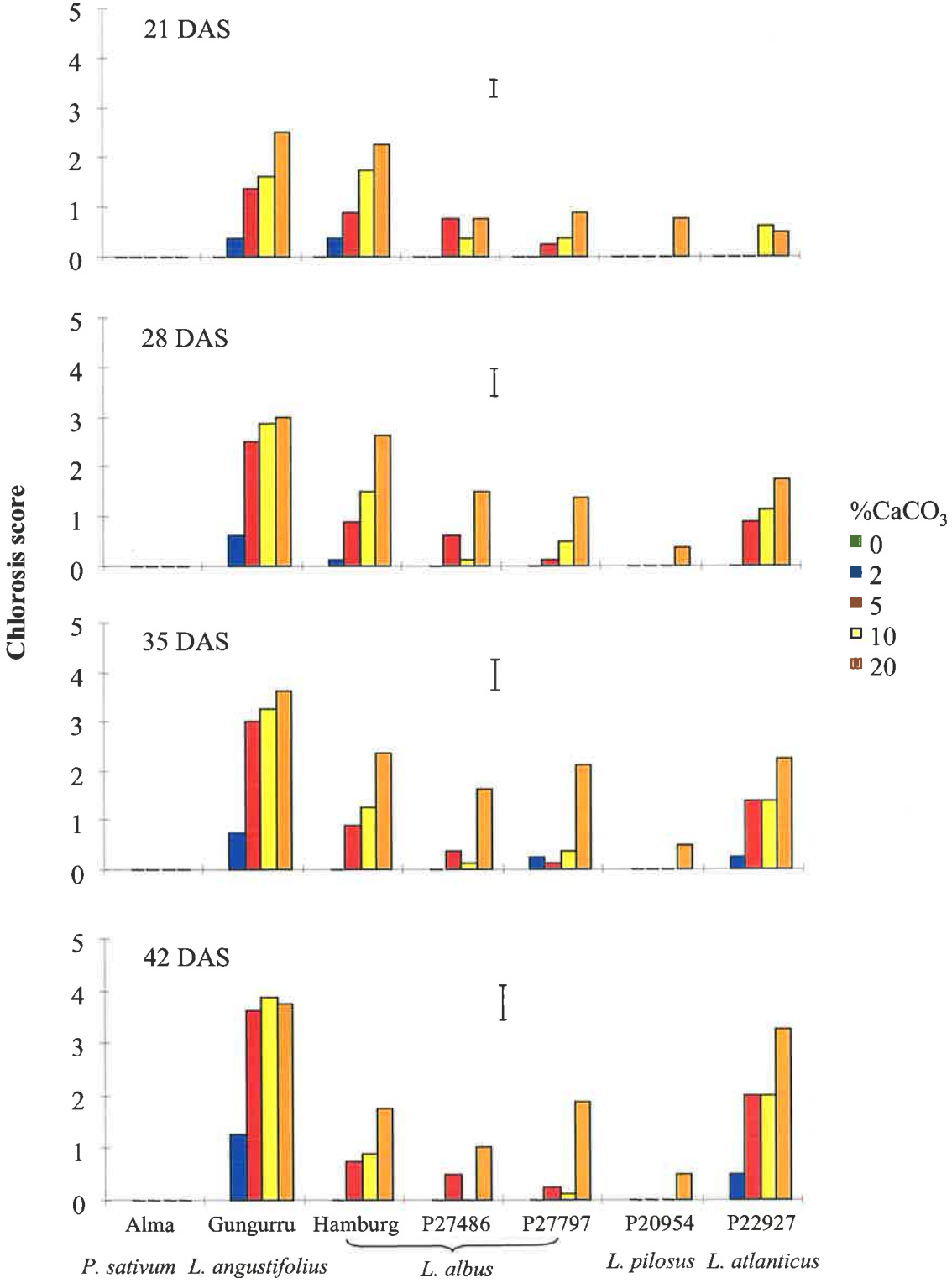
The addition of $CaCO_3$ to the Glenthorne soil had no effect on the rate of emergence of genotypes, however they all emerged slightly faster in the control.

The first symptoms of chlorosis, resembling Fe deficiency, were observed 14 DAS in the YEL of Gungurru and Hamburg grown on the soils with added $CaCO_3$. No chlorosis was noted on any genotype grown in the control or 0% $CaCO_3$ throughout the experiment. Plants of both *P. sativum* cv. Alma and *L. pilosus* P20954, grown in all $CaCO_3$ treatments, except 20% for P20954, showed no chlorosis on the YEL at any time during the experiment (Fig. 4.1). Conversely, plants of *L. angustifolius* cv. Gungurru showed severe symptoms in all treatments with added $CaCO_3$. The chlorosis score in all $CaCO_3$ treatments generally increased from 21 to 42 DAS so that at 42 DAS chlorotic symptoms were severe in 5%, 10% and 20% $CaCO_3$, and moderate in 2% $CaCO_3$ (Fig. 4.1). The chlorosis score of *L. atlanticus*, P22927, also increased significantly from 21 to 42 DAS. At 21 DAS there were only minor symptoms in 10% and 20% $CaCO_3$, but by 42 DAS, chlorosis symptoms were severe in 20% $CaCO_3$, moderate in 5% and 10% $CaCO_3$ and slight in 2% $CaCO_3$ (Fig. 4.1).

The three *L. albus* genotypes differing in tolerance to Fe chlorosis were compared to confirm intraspecific variation. Hamburg, similar to Gungurru, showed more severe chlorotic symptoms in all $CaCO_3$ treatments 21 DAS than P27486 and P27797 (Fig. 4.1). At 28 DAS, the chlorotic symptoms had increased in plants of P27486 and P27797 grown in 20% $CaCO_3$, but not in Hamburg. From 28 to 42 DAS, the chlorosis score generally decreased for Hamburg and remained constant for P27486 and P27797. At 42 DAS the three genotypes had similar chlorosis at 20% $CaCO_3$, but Hamburg suffered more chlorosis than the other two in $CaCO_3$ levels less than or equal to 10% (Fig. 4.1).

The shoot dry weights of plants of Alma and P20954, similar to chlorosis score, were least affected by the addition of $CaCO_3$. Generally shoot dry weight was 80 to 90% of that observed in

**Figure 4.1** The effect of increasing the $CaCO_3$ content in the Glenthorne soil on visual chlorosis score in the YEL of four lupin species and a pea (*P. sativum*) cultivar from 21 to 42 DAS. Control treatment (UC modified mix) not shown as no chlorotic symptoms were observed. Bars indicate LSD ($P < 0.05$).



0% $CaCO_3$ (Fig. 4.2). Both genotypes grew poorly on the control compared with all other treatments. For Gungurru, there was a 30% reduction in shoot dry weight of plants grown in 2% $CaCO_3$, 50% reduction in 5% $CaCO_3$ and 60% reduction in 10% and 20% $CaCO_3$. The relative shoot dry weight of Gungurru was greatest in the control, significantly higher than all other genotypes. For P22927 there was 40 to 60% reductions in all $CaCO_3$ treatments and the control compared with 0% $CaCO_3$ (Fig. 4.2).

The three *L. albus* genotypes generally showed the poorest growth on the control (30-40% of the 0% $CaCO_3$ treatment; Fig. 4.2). Unlike chlorosis, plants of all three genotypes showed a similar decrease (30% to 50%) in shoot dry weight as the $CaCO_3$ content increased (Fig. 4.2).

Shoot dry weight of all species at harvest correlated with chlorosis score during the experiment, particularly measurements taken at 28, 35 and 42 DAS (Table 4.4).

*Nutrient concentrations*

There was a significant ($P < 0.05$) interaction between $CaCO_3$ content and genotype for concentrations of all nutrients, except for Fe and K, for which only the main effects of $CaCO_3$ content and genotype were significant ($P < 0.05$).

The concentrations of Fe, Mn, Cu, B, Zn and P in the YELs of all genotypes were generally decreased and Ca, Mg, Na and S increased by the addition of $CaCO_3$ to the Glenthorne soil (Fig. 4.3). K concentrations showed no response. In the control soil concentrations of Mn, Cu, and Ca in the YELs of all genotypes were generally decreased and Zn, P and S were increased compared with concentrations in the amended and unamended Glenthorne soil (Fig. 4.3). The concentrations of Fe, B, Mg, Na and K were generally similar to that observed on the Glenthorne soil. The concentrations of all nutrients in all treatments except Mn in all species except *L. albus* on the amended and unamended Glenthorne soil were above critical levels (Table 3.3) defined for *L. angustifolius* (Fig. 4.3) (Reuter and Robinson, 1997).

When comparing among genotypes, P22927 generally had the highest Fe concentrations in the YEL and Alma and Gungurru the lowest (Fig. 4.3). The concentrations of Mn were extremely high in all *L. albus* genotypes and generally, P27486 and P27797 were able to maintain higher concentrations at the increased $CaCO_3$ contents compared with Hamburg. For B the *L. albus* genotypes (Hamburg, P27486, P27797) and Gungurru had significantly higher concentrations

**Figure 4.2** The effect of increasing the $CaCO_3$ content in the Glenthorne soil compared with the UC modified potting mix (control) on the shoot dry weight of plants of four lupin species and a pea cultivar at harvest (42 DAS) and the relative shoot dry weight (percentage of 0% $CaCO_3$). The two-way interaction between genotype and soil treatment was not significant. Bars indicate standard error.

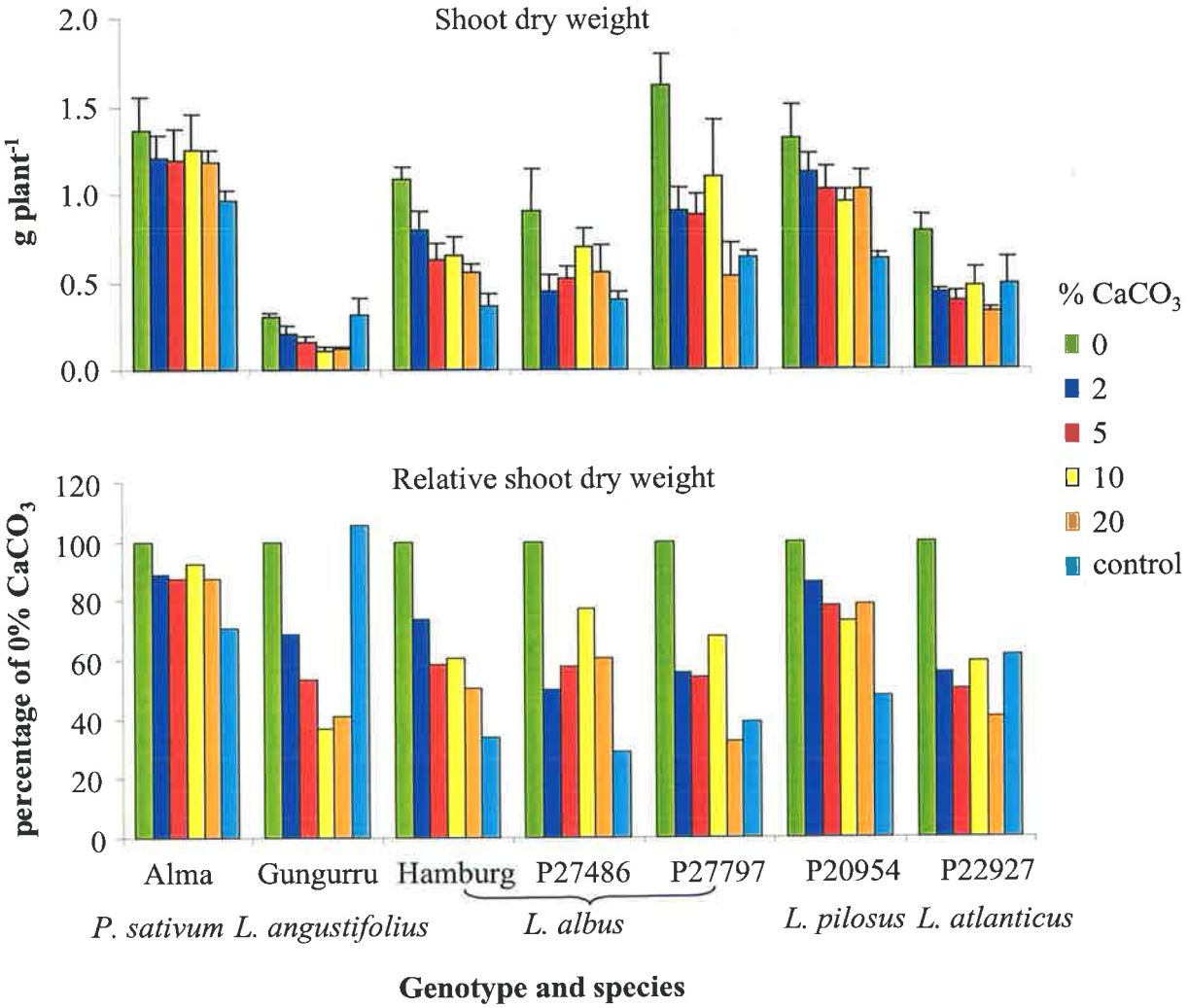


**Figure 4.3** The effect of increasing the $CaCO_3$ content in the Glenthorne soil compared with the UC modified potting mix (control) for four lupins species and a pea cultivar at harvest (42 DAS). Bars represent LSD ($P < 0.05$) for comparison of the $\%CaCO_3$ treatments (Control treatment was omitted from the analysis). n.s. - not significant.

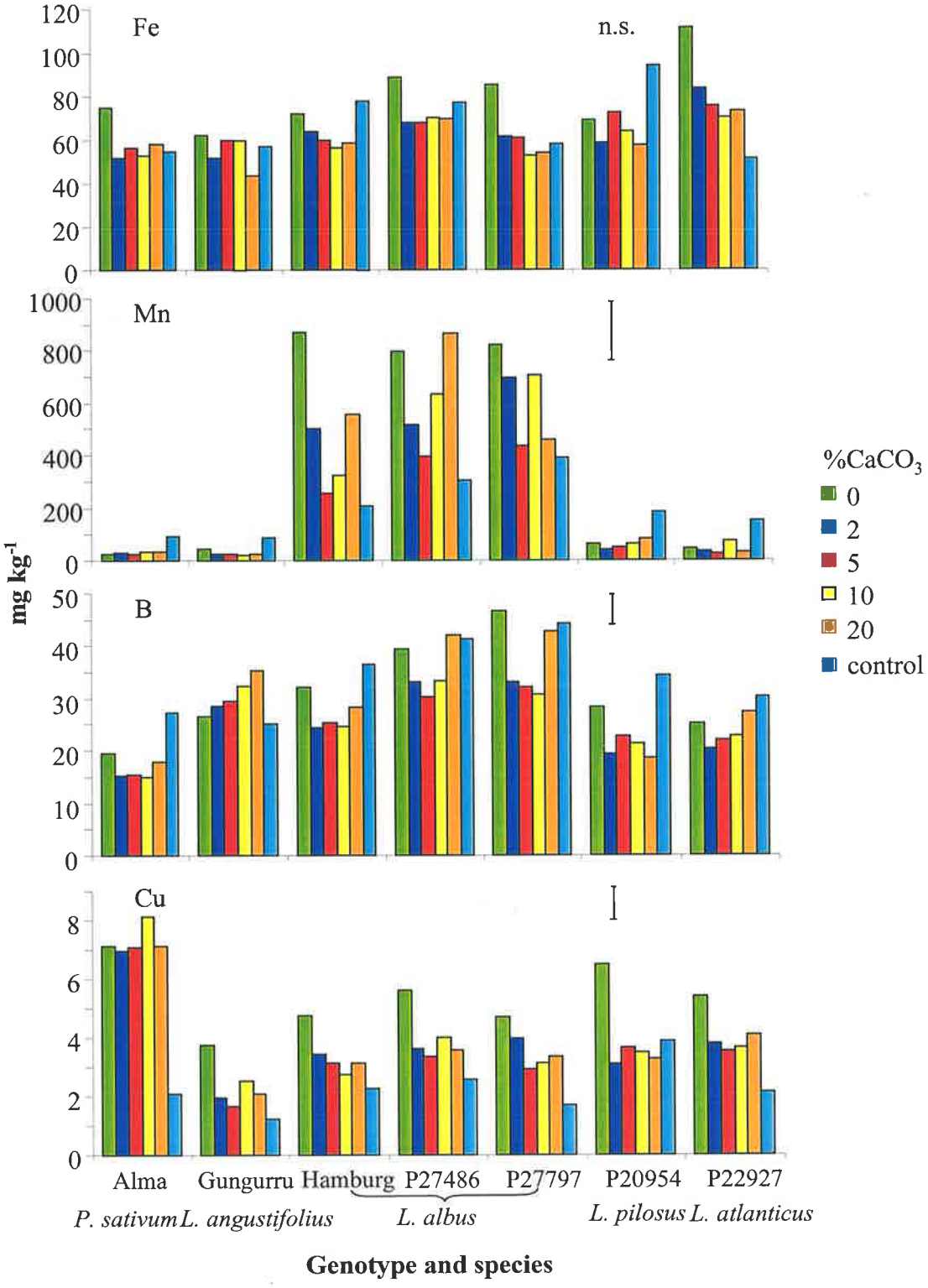


**Figure 4.3** *Continued* - The effect of increasing the $CaCO_3$ content in the Glenthorne soil compared with the UC modified potting mix (control) for four lupins species and a pea cultivar at harvest (42 DAS). Bars represent LSD ($P < 0.05$) for comparison of the $\%CaCO_3$ treatments (Control treatment was omitted from the analysis). n.s. - not significant.

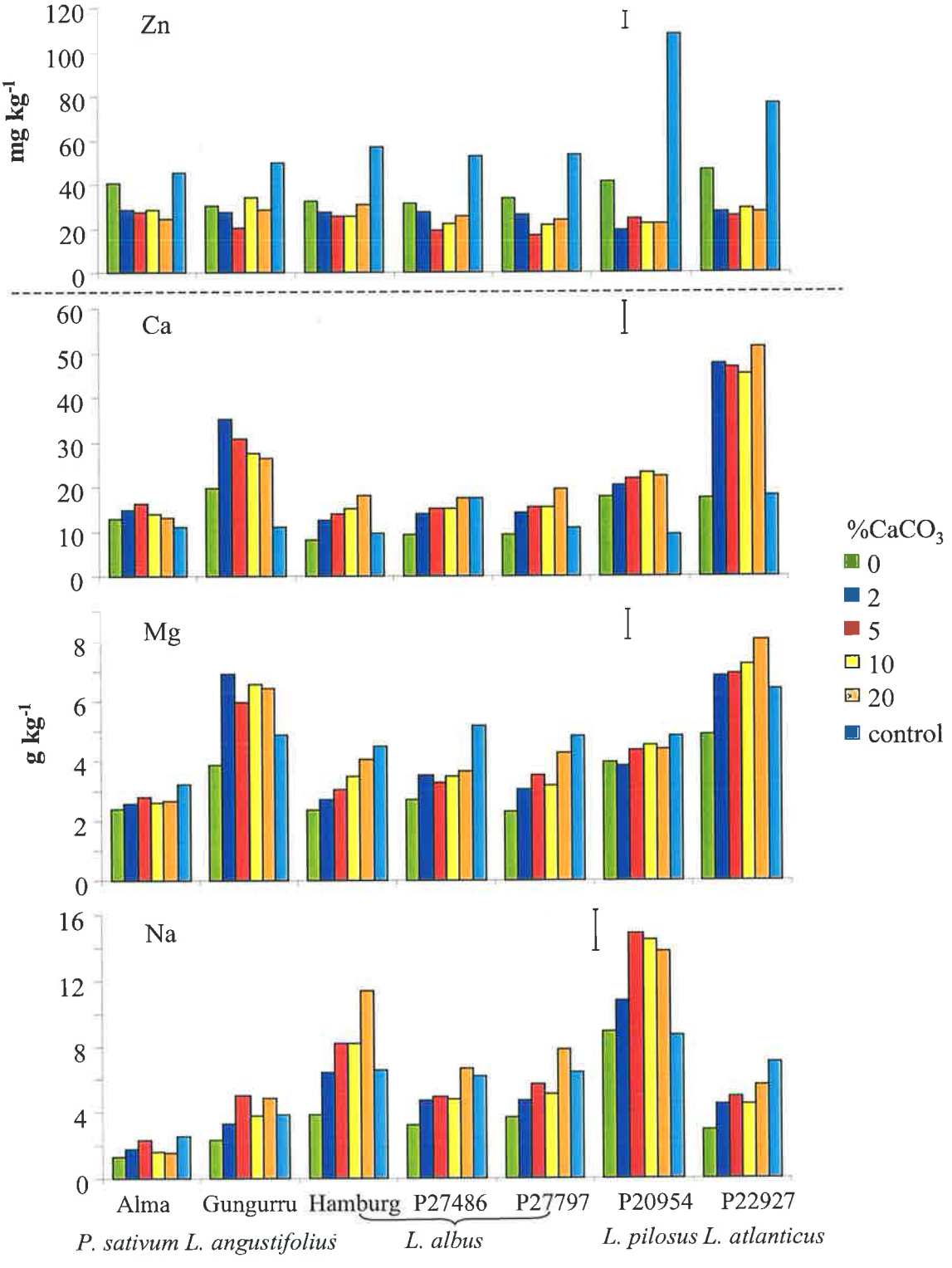


**Figure 4.3** *Continued* - The effect of increasing the $CaCO_3$ content in the Glenthorne soil compared with the UC modified potting mix (control) for four lupins species and a pea cultivar at harvest (42 DAS). Bars represent LSD ($P < 0.05$) for comparison of the $\%CaCO_3$ treatments (Control treatment was omitted from the analysis). n.s. - not significant.

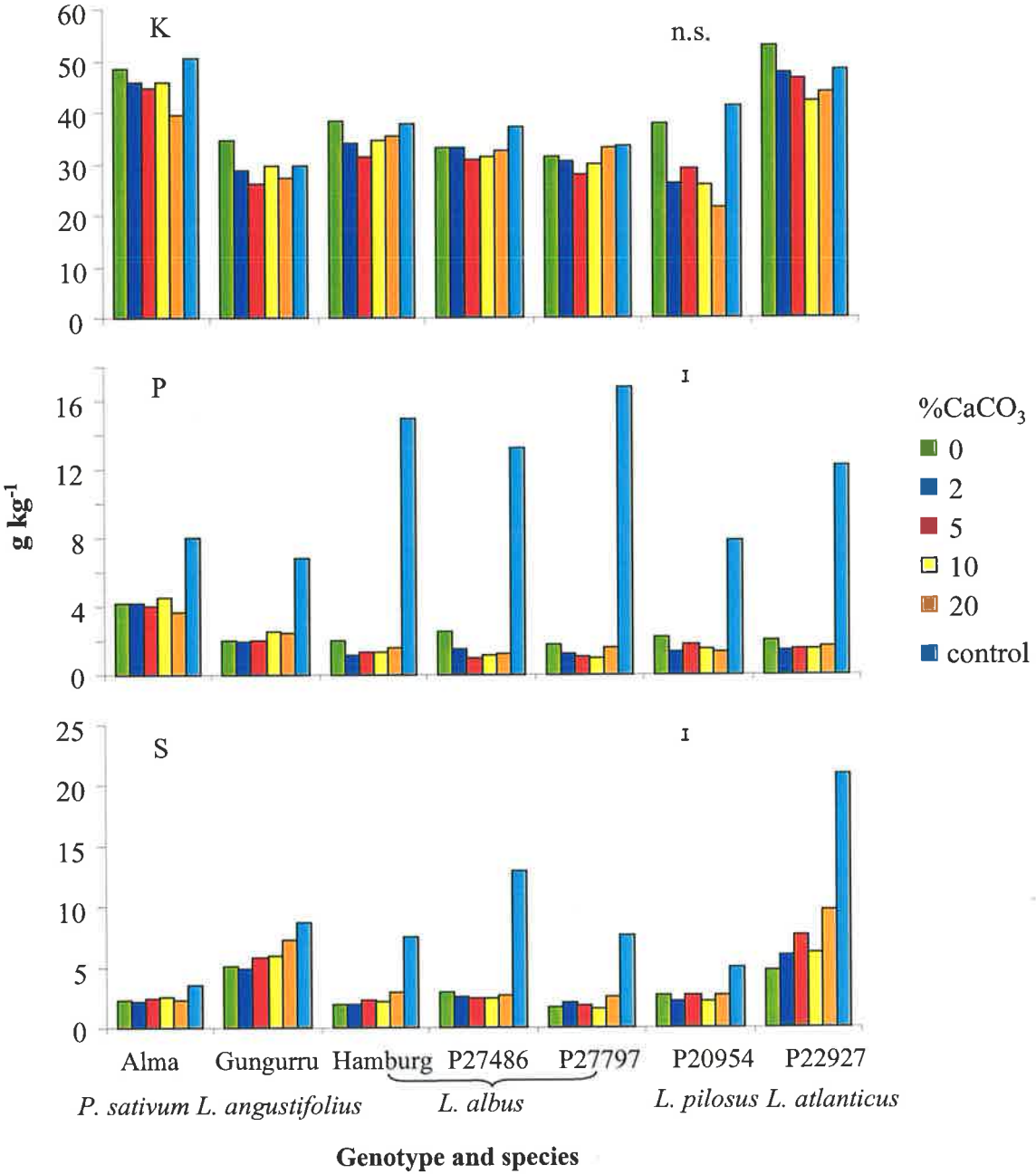


**Table 4.4** Correlation coefficients (r) between relative shoot dry weight (SDW %), chlorosis score at 21, 28, 35 and 42 DAS and nutrient concentrations in the YEL (Fe, Mn, B, Cu, Zn, Ca, Mg, Na, K, P and S) of the four species of lupin species and a pea cultivar grown in the Glenthorne soil at 0, 2, 5, 10 and 20% $CaCO_3$. Data from the control treatment (UC modified mix) was omitted from the correlation.

| | SDW % | CS 21 DAS | CS 28 DAS | CS 35 DAS | CS 42 DAS | Fe | Mn | B | Cu | Zn | Ca | Mg | Na | K | P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **CS 21 DAS** | -0.56** | | | | | | | | | | | | | | |
| **CS 28 DAS** | -0.68** | 0.92** | | | | | | | | | | | | | |
| **CS 35 DAS** | -0.70** | 0.85** | 0.98** | | | | | | | | | | | | |
| **CS 42 DAS** | -0.73** | 0.73** | 0.92** | 0.97** | | | | | | | | | | | |
| **Fe** | 0.09 | -0.37* | -0.28 | -0.26 | -0.19 | | | | | | | | | | |
| **Mn** | 0.18 | 0.01 | -0.09 | -0.13 | -0.26 | 0.13 | | | | | | | | | |
| **B** | -0.21 | 0.27 | 0.30 | 0.32 | 0.25 | 0.18 | 0.71** | | | | | | | | |
| **Cu** | 0.69** | -0.57** | -0.55** | -0.53** | -0.51** | 0.19 | -0.12 | -0.39* | | | | | | | |
| **Zn** | -0.04 | 0.14 | 0.18 | 0.17 | 0.19 | 0.44** | -0.15 | 0.08 | 0.24 | | | | | | |
| **Ca** | -0.59** | 0.12 | 0.36* | 0.44** | 0.59** | 0.08 | -0.52** | -0.21 | -0.34* | -0.02 | | | | | |
| **Mg** | -0.72** | 0.32 | 0.53** | 0.60** | 0.73** | 0.08 | -0.50** | -0.04 | -0.50** | 0.12 | 0.94** | | | | |
| **Na** | 0.03 | 0.19 | 0.11 | 0.08 | 0.01 | -0.14 | -0.02 | -0.05 | -0.43** | -0.30 | 0.05 | 0.09 | | | |
| **K** | 0.15 | -0.36* | -0.22 | -0.19 | -0.12 | 0.43** | -0.27 | -0.40* | 0.66** | 0.35* | 0.20 | 0.05 | -0.54** | | |
| **P** | 0.36* | -0.19 | -0.14 | -0.12 | -0.09 | -0.11 | -0.42* | -0.45** | 0.78** | 0.35* | -0.18 | -0.24 | -0.51** | 0.52** | |
| **S** | -0.70** | 0.27 | 0.52** | 0.60** | 0.73** | 0.16 | -0.49** | -0.04 | -0.28 | 0.22 | 0.86** | 0.90** | -0.18 | 0.26 | -0.02 |

* $P < 0.05$, ** $P < 0.01$

than other genotypes (Fig. 4.3). Cu concentrations were significantly higher in plants of Alma and slightly lower in Gungurru grown in all $CaCO_3$ treatments and the unamended Glenthorne soil, than all other genotypes (Fig. 4.3). However, in the control P20954 had the highest concentrations (Fig. 4.3). The concentrations of Zn were similar across all genotypes in all treatments, except P20954 and P22927 in the control, in which they had increased levels (Fig. 4.3).

Ca and Mg concentrations were extremely high in both Gungurru and P22927 when $CaCO_3$ was added to the Glenthorne soil compared with other genotypes (Fig. 4.3). The concentrations of Na in plants of P20954 and Hamburg were high and Alma was low with added $CaCO_3$ (Fig. 4.3). For K, Alma and P22927 generally had the highest concentrations in all treatments (Fig. 4.3). The concentrations of P in Alma and S in Gungurru and P22927 were higher than other genotypes in the amended and unamended Glenthorne soil (Fig 4.3). However in the control the concentrations of P in *L. albus* genotypes and S in P22927 and P27486 were higher than other genotypes.

Correlation coefficients indicate that shoot dry weight was positively related to Cu concentrations and negatively related to Ca, Mg and S concentrations (Table 4.4). The chlorosis score at 28, 35 and 42 DAS was also negatively related to Cu concentrations and positively related to Ca, Mg and S concentrations. It was also notable that Ca, Mg and S concentrations were positively related.

# 4.3 The effect of soil moisture, nutrition and inoculation on the growth and chlorosis of a tolerant, moderately tolerant and moderately intolerant genotype of *L. pilosus*, in a calcareous soil (Experiment 2)

## 4.3.1 Introduction

The previous experiment suggested that the addition of $CaCO_3$ to soil was an inappropriate method of screening for tolerance to calcareous soils due to limitations associated with the loss of soil structure and the confounding effects of water logging (Section 4.2.3). In addition, *L. albus* was poorly adapted to soil with added $CaCO_3$ compared with *L. pilosus* and showed little phenotypic variation. Thus, in the following experiments, a field soil with a high $CaCO_3$ content was used. A preliminary experiment was conducted with this soil to compare the growth of a collection of landraces and wild types of *L. pilosus*, collected from different countries and soil types. Results showed that there was a broad range of tolerance from tolerant to moderately intolerant. Three genotypes of *L. pilosus* were selected to cover this range of tolerance (P23370 - tolerant, P20954 - moderately tolerant, P22932 - moderately intolerant) and compared in the following experiments.

To develop a screening method in a calcareous soil it is important to test whether there are any confounding effects of soil moisture, nutrition, rhizobial inoculation or Fe fertilization on the performance of genotypes that differ in tolerance to calcareous soil.

In *L. angustifolius* grown on calcareous soils, chlorotic symptoms resembling Fe deficiency generally increase in severity as the soil moisture content increases. Previous research has indicated that this observation is also true for other intolerant crops such as soybeans (Inskeep and Bloom, 1986; Fairbanks, *et al.*, 1987) and apples (Ao, *et al.*, 1987). In calcareous soils $HCO_3^-$ has been shown as the major factor inducing chlorosis in susceptible cultivars of several crops (Coulombe, *et al.*, 1984a) and has been used in solution culture systems to screen for tolerance to Fe chlorosis (Chaney, *et al.*, 1992a; Chaney, *et al.*, 1992b). The moisture content in conjunction with other factors such as active $CaCO_3$ content, pH and $pCO_2$ affects the concentration of $HCO_3^-$ in the soil solution, thus the severity of the symptoms. As the soil moisture increases in a calcareous soil, microbial respiration and $pCO_2$ increases, whilst gas exchange decreases leading to an increase in the concentration of $HCO_3^-$ (Russell, 1988). Thus,

for the development of a soil screening method that enables selection of intolerant and tolerant genotypes, a suitable moisture content needs to be determined.

Symptoms of $HCO_3^-$ induced Fe deficiency of intolerant genotypes may be alleviated by the addition of Fe fertilizers such as Fe chelate (FeEDDHA) to the soil or as a foliar spray (Chen and Barak, 1982; Moraghan, 1985; Longnecker, 1988; Plessner, *et al.*, 1992). Both methods have been only partially successful as often it is the low availability rather than low total amount that prevents uptake of Fe by plant roots in calcareous soils (Longnecker, 1988; Plessner, *et al.*, 1992). Also it has been hypothesized that $HCO_3^-$ directly inhibits the uptake of Fe (Coulombe, *et al.*, 1984a; Römheld, 1986; Chaney, *et al.*, 1992a), rather than inactivating the Fe in leaves as has been previously suggested (Kaur, *et al.*, 1984), thus the addition of Fe does not overcome the $HCO_3^-$ effect.

Lupins, being a leguminous crop, rely on the formation of nodules and fixing $N_2$ to obtain an adequate supply of N. Previous research has shown that nodulation in narrow-leafed lupins is significantly reduced in an alkaline soil and in solution culture at high pH (Tang, *et al.*, 1993). It has also been shown that the inoculant used on lupins (*Bradyrhizobium* sp. *Lupinus*; WU425) has poor tolerance to calcareous soils and does not persist in the soil for long periods (Jessop, *et al.*, 1990). Hence it is important to avoid the confounding effect of poor nodulation or poor nitrogen fixation when identifying tolerance to calcareous soils.

In this section the effects of soil moisture (*Experiment 2a*), soil nutrition and the addition of Fe (*Experiment 2b*), and inoculation and nitrate addition (*Experiment 2c*) on the growth and chlorosis of three *L. pilosus* genotypes differing in tolerance to a calcareous soil were investigated.

### 4.3.2 Materials and methods

*Genetic material*

The three genotypes of *L. pilosus* grown in comparison in these experiments were P23370 - tolerant, P20954 - moderately tolerant and P22932 - moderately intolerant of calcareous soils based on preliminary screening (Section 4.3.1). Details of collection data and source of seed are contained in Table 6.1.

*Soil and pot preparation*

In all experiments, a bulk sample of the experimental soil was air-dried and mixed before adding to the required number of pots. The pots used were 20 cm diametre (4.5 L) and lined with plastic to prevent leaching of nutrients.

Effect of soil moisture (Expt. 2a)

The experimental soils were a calcareous sandy loam collected from a field site at Wangary on the Eyre Peninsula of South Australia (Wilhelm, *et al.*, 1988) and the Glenthorne soil included as the control (Table 4.1). The experiment consisted of three moisture levels in the Wangary soil compared with the Glenthorne soil and three genotypes in three replicates arranged as a randomized complete block.

The Wangary soil was weighed into 27 pots and basal nutrients added in solution to the soil surface of each pot (Table 4.5). After allowing to dry, the soil was mixed and watered to the required moisture contents (80%, 100% and 120% of field capacity). The Glenthorne soil had no $CaCO_3$ and all three genotypes of *L. pilosus* showed good growth on this soil (Experiment 1). It was weighed into nine pots and watered up to 70% of field capacity. All pots were allowed to equilibrate for two weeks before sowing. Pots were watered to weight every two days during the experiment.

Effect of nutrient addition (Expt. 2b)

The experimental soil was the Wangary calcareous sandy loam (Table 4.1). The experiment consisted of four nutrient treatments and three genotypes in three replicates. The nutrient treatments were:

1. Control - no added nutrients or Fe,
2. Added nutrients without foliar or soil applied Fe,
3. Added nutrients with soil applied Fe (5 mg $kg^{-1}$ FeEDDHA; 'iron ethylenediamine-di(o-hydroxyphenyl acetic acid)', Novartis),
4. Added nutrients with foliar applied Fe (0.5% $FeSO_4$ with 0.03% nonyl phenol ethylene oxide as a wetting agent sprayed onto leaves weekly from seven days after sowing).

Basal nutrients were added in solution to the soil surface of each pot (Table 4.5), except for the control treatment, in which the equivalent volume of RO water was added. The soil was re-dried, mixed and watered to 90% of field capacity (determined from experiment 2a). Soils were

allowed to stand for two weeks before the experiment was commenced, during which pots were watered to weight every two days.

Effect of $N_2$ fixation (Expt. 2c)

The experimental soil was the Wangary calcareous sandy loam (Table 4.1). It was added 10 pots and five replicates of two treatments were applied:

1. Inoculated without supply of chemical N,
2. Supply of $NH_4NO_3$ without inoculation.

Basal nutrients were added in solution to the soil surface of each pot (Table 4.5); in the +N treatment, $NH_4NO_3$ solution (100 mg $kg^{-1}$) was also added. The soil was re-dried, mixed and watered to 90% of field capacity. Soils were allowed to stand for two weeks before the experiment was commenced, during which, pots were watered to weight every two days.

**Table 4.5** Concentrations of nutrients added the surface of the Wangary soil in experiments 2a, 2b and 2c.

| Nutrient | Concentration (mg $kg^{-1}$ dry soil) |
|---|---|
| $MgSO_4.4H_2O$ | 50 |
| $CaCl_2.2H_2O$ | 125 |
| $KH_2PO_4$ | 250 |
| $K_2SO_4$ | 400 |
| $H_3BO_3$ | 2 |
| $Na_2MoO_4.2H_2O$ | 0.5 |
| $CoSO_4.7H_2O$ | 1 |
| $CuSO_4.5H_2O$ | 3.5 |
| $MnSO_4.H_2O$ | 25 |
| $ZnSO_4.H_2O$ | 12.5 |

*Plant growth*

In all experiments, prior to sowing, seeds were surface sterilized (Section 4.2.2). After sterilizing, seeds were scarified and imbibed on moistened filter paper for 48 hours. The seed were sown and inoculated as described in Section 3.2, except the +N treatment in experiment 2c where no inoculant was added. In experiment 2a and 2b, ten seeds of one genotype were sown in each pot. In experiment 2c, two seeds of each of the three genotypes were sown per pot. Seven days after sowing plants were thinned to eight plants per pot in experiment 2a and 2b. Plants were grown under glasshouse conditions (15 - 30°C) and pots watered to the required moisture content with RO water every two days.

*Measurements*

The duration of each experiment and the number of days after sowing at which measurements were recorded are summarised in Table 4.3. For experiments 2a and 2b there were four harvests, 14, 21, 28 and 35 DAS, and 21, 42, 56 and 84 DAS, respectively, whilst for experiment 2c there was only a final harvest. The four harvests in experiments 2a and 2b were included to observe nutritional changes in the leaves relating to changes in chlorosis over time, such as in the first experiment when *L. pilosus* P20954 recovered from chlorosis. At each individual harvest in these experiments two plants per pot were removed and the YEL and whole shoots separated.

In experiment 2a, chlorophyll meter readings, number of leaves, total shoot dry weight and chlorophyll concentrations of the plants grown on the calcareous soil relative to those on the control soil (Glenthorne) were used for the correlation analysis.

Chlorosis score

Chlorosis score (Table 3.2) of the YEL was recorded for each individual plant in the pot. The average of the plants of the same genotypes in each pot was used for the analyses of variance.

Chlorophyll meter

The chlorophyll meter (SPAD-502, Minolta Corp.) was used to determine leaf greenness in the YEL and provide a non-destructive estimation of chlorophyll concentration (Campbell, *et al.*, 1990). It also provided a non-subjective measurement which can be related to chlorosis score. Each individual reading for a plant was an average of four replicate readings on the YEL. The average of the plants of the same genotypes in each pot was used for the analyses of variance.

Chlorophyll concentration

Chlorophyll concentration was determined using a method modified from Hiscox and Israelstam (1979). Three 0.5 mm discs were taken with a cork borer from the YEL of each replicate plant in a pot at harvests and combined. The discs were weighed and placed in a 10 ml tube. Five ml of dimethyl sulfoxide (DMSO) was immediately added and allowed to incubate, covered, at 65$^{O}$C overnight until the tissue was clear. The extract was cooled to room temperature. A 3 ml subsample was pipetted into a cuvette and the optical density (OD) of the solution read at 652 nm on a UV-Vis spectrophotometer (UV-201; Shimadzu). The chlorophyll concentration was calculated as $C = OD_{652}/34.5$ (mg ml$^{-1}$).

Active Fe

Active Fe concentration was measured using a method modified from Abadia, *et al.* (1984). Approximately 50 mg of the YEL was collected for each replicate plant at harvests and combined. The sample was finely chopped with scissors, weighed and added to a 10 ml vial. Five ml of 1.5% 2'-2'-dipyridyl was immediately added. The tubes were incubated for 24 hours at room temperature. A 3 ml subsample was pipetted into a cuvette and the optical density of the solution read at 522 nm on a UV-Vis spectrophotometer. The concentration of active Fe in the sample was calculated by reading from a plot of a series of standards from 0 to 100 mM $FeSO_4.7H_2O$.

Shoot dry weight

The YELs and shoots of harvested plants were dried in an oven at $80^{O}C$ for 48 hours and dry weights taken. The combined dry weight of the YEL and whole shoots was recorded as the shoot dry weight.

Nutrient concentrations

Before drying the YELs were washed in 0.1 M HCl for 30 seconds and rinsed in RO water for 60 seconds to wash off any surface contamination. The dried YELs were digested in concentrated $HNO_3$ and analysed for nutrient concentrations by ICP-AES (Section 3.2).

*Statistical analyses*

In all experiments an analysis of variance (Genstat 5.41) was conducted on interactions and main effects of genotypes, treatments and DAS (were relevant) to test for significance of all measurements. Individual means were compared using LSD.

### 4.3.3 Results

*Effect of soil moisture (Expt. 2a)*

Chlorosis score, chlorophyll meter readings, number of leaves, shoot dry weight and chlorophyll concentration

There was a significant three-way interaction between soil moisture, genotype and DAS for chlorosis score ($P < 0.05$) and chlorophyll meter readings ($P < 0.05$). For shoot dry weight the interactions between genotype and DAS, soil moisture and DAS and genotype and soil moisture were significant ($P < 0.05$), while for number of leaves and chlorophyll concentration, only the interaction between soil moisture and DAS was significant ($P < 0.05$).

Genotype and soil moisture had no effect on the rate of emergence or final emergence percentage. P23370 showed the most vigorous early growth with the fastest overall growth rate (34 mg $day^{-1}$), while P20954 and P22932 were similar (21 and 20 mg $day^{-1}$, respectively; Fig. 4.4). There was no difference between shoot dry weights at any of the soil moisture treatments at 14 DAS and 21 DAS. At 28 DAS shoot dry weights were similar at 80% and 100% soil moisture contents and these were significantly greater than at 120% soil moisture and the control (Fig. 4.5). Thirty five DAS, the 80% soil moisture pots produced plants with the greatest dry weights, while there were no significant differences between the other moisture treatments and the control. When the data were averaged over the four harvests, the shoot dry weights of P20954 and P22932 decreased significantly as soil moisture increased, but P23370 was not significantly affected (Fig. 4.5). The plants of P20954 and P22932 in the control treatment were significantly lighter than those at the 80% moisture treatment and similar to those at 100% and 120% moisture treatments. P23370 had less biomass in the control (Glenthorne soil) than at any moisture treatment in the Wangary soil (Fig 4.5).

All genotypes showed a similar increasing trend in number of leaves over time although P20954 had significantly fewer leaves than P23370 and P22932 at all harvests (Fig. 4.4). The number of leaves in all treatments increased by approximately two per week, except the control which had a lower rate of increase at 28 DAS and 35 DAS (Fig. 4.4). The data averaged over the four harvests showed no significant interaction between soil moisture and genotype (Fig. 4.5).

Some of the root systems were inspected and it was noted that proteoid roots had been formed. There appeared to be no difference between genotypes in the extent of proteiod root formation, but as the moisture level increased, the number of proteoid roots increased in all genotypes.

Chlorotic symptoms in the YEL were first visible at 10 DAS, and by 14 DAS there were clear genotypic differences at all of the moisture levels. Chlorotic symptoms increased with higher soil moisture, especially in the moderately intolerant and moderately tolerant genotypes, P22932 and P20954 (Fig. 4.6). The plants in the control pots remained a healthy green although showing slower growth than plants in the calcareous soil. The chlorosis score continued to increase for all treatments and genotypes until 21 DAS. In the following weeks chlorosis score increased for P22932 at 100% and 120% soil moisture and P20954 at 120% soil moisture and decreased almost to zero at 80% for all genotypes and at 100% for P23370 (Fig. 4.6, Plate 4.1).

**Figure 4.4** The main effects of genotype (P23370 - tolerant, P20954 - moderately tolerant and P22932 - moderately intolerant) and soil moisture in the Wangary calcareous soil (% of field capacity) compared with the Glenthorne non-calcareous soil (control) on the total plant dry weight and number of leaves from 14 to 35 days after sowing. The three-way interaction between genotype, soil moisture and time was not significant. Bars indicate LSD values ($P < 0.05$).

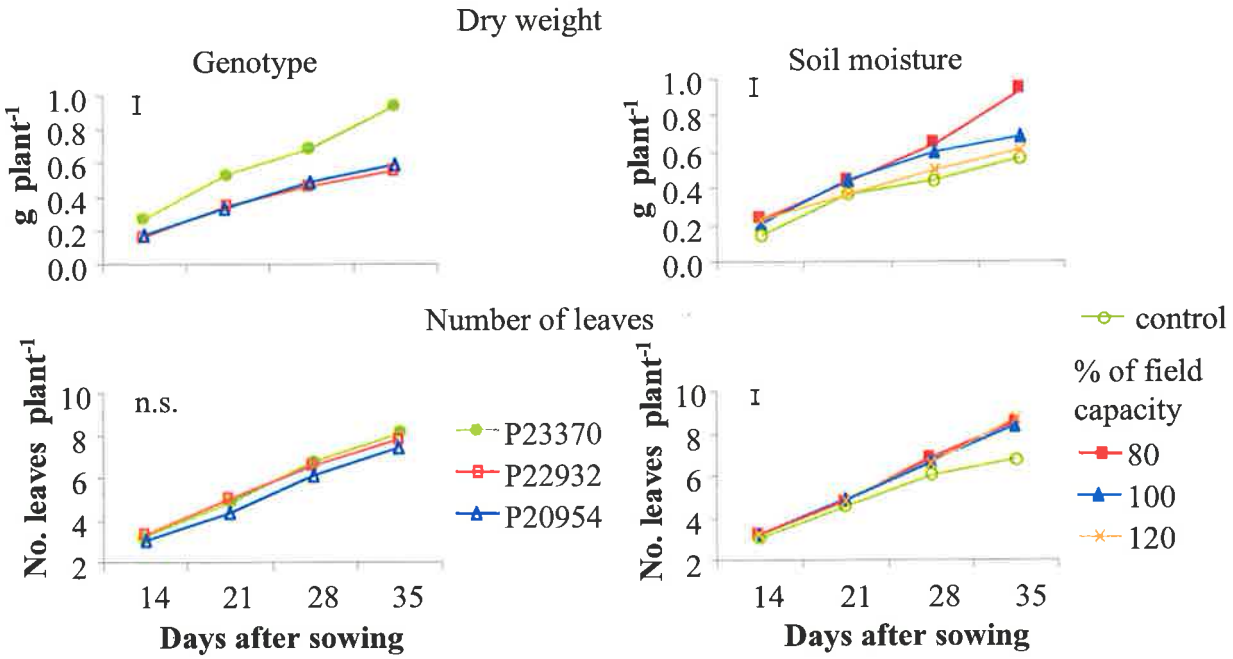


**Figure 4.5** The effect of increased soil moisture (% of field capacity) in the Wangary calcareous soil compared with the Glenthorne non-calcareous soil (control) on the total plant dry weight and number of leaves of genotypes of *L. pilosus* tolerant (P23370), moderately tolerant (P20954) and a moderately intolerant (P22932) of calcareous soils (average of the four weeks harvests as there were no significant interactions at individual harvests). Bar indicates LSD value ($P < 0.05$), n.s. - not significant.

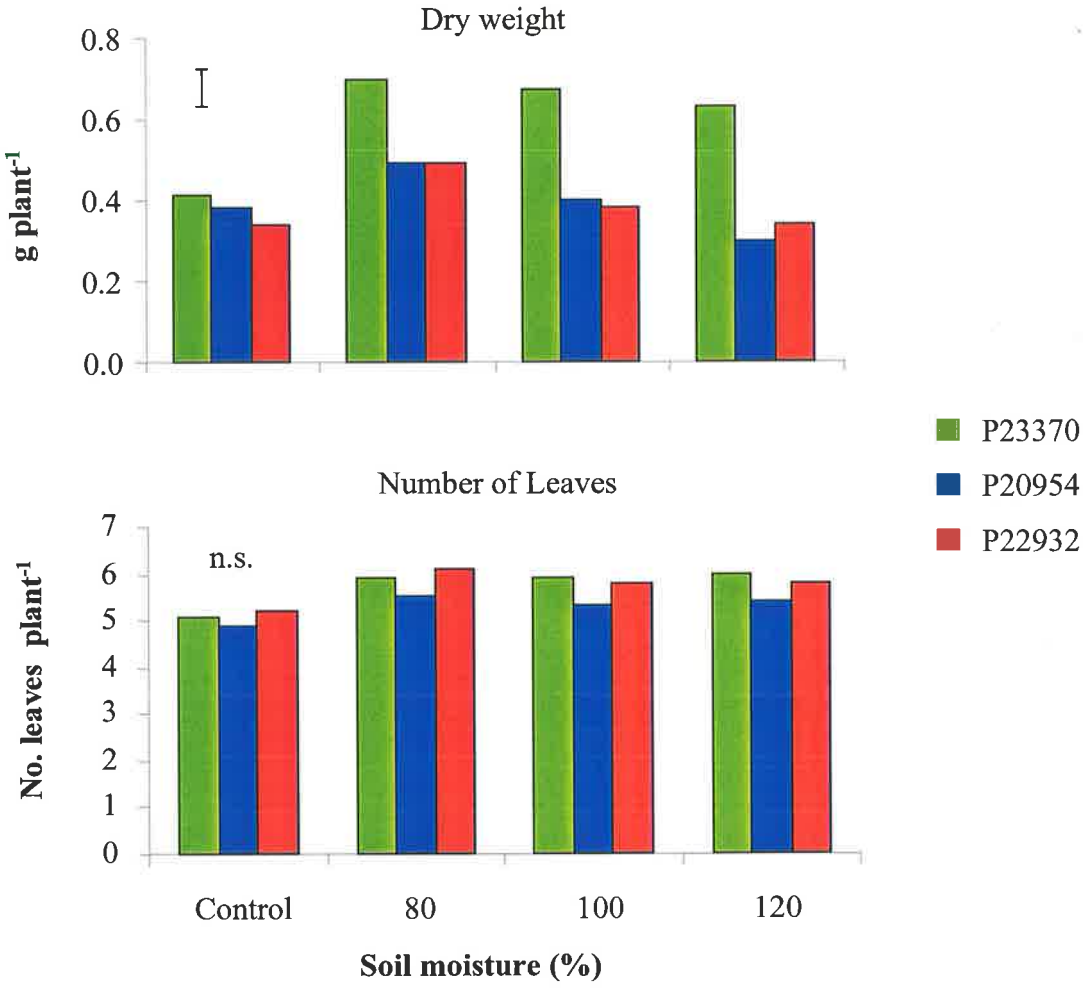


**Figure 4.6** The effect of increased soil moisture in the Wangary calcareous soil compared with the Glenthorne non-calcareous soil (control) on chlorosis score, chlorophyll meter readings and chlorophyll concentration in the YEL of a tolerant (P23370), moderately tolerant (P20954) and a moderately intolerant (P22932) genotype of *L. pilosus* from 14 to 35 days after sowing. Bars indicate LSD ($P < 0.05$); n.s. not significant.

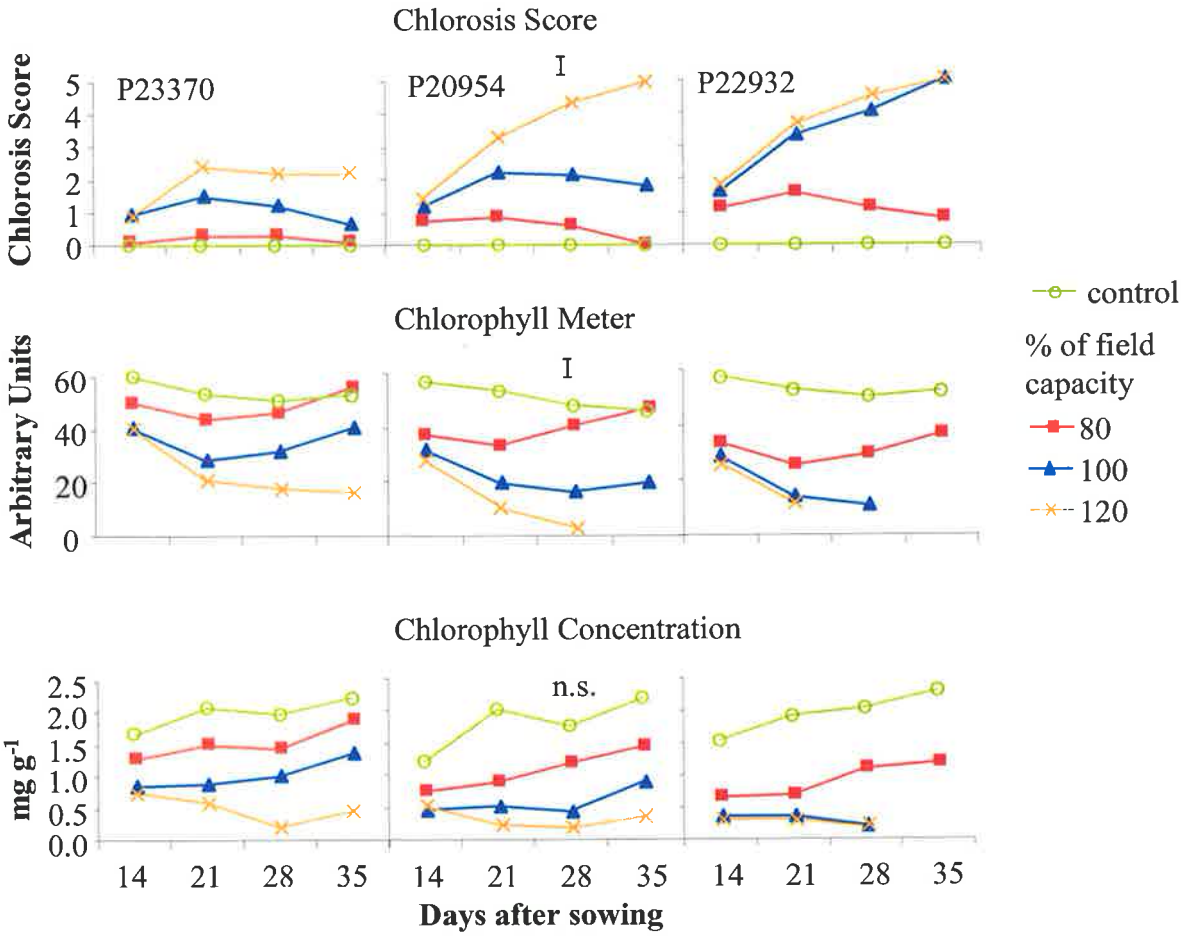


**Plate 4.1** The effect of increased soil moisture (top - 80%, middle - 100%, bottom - 120% of field capacity) in the calcareous Wangary soil on chlorotic symptoms of *L. pilosus* genotypes 21 days after sowing.
a. Tolerant (P23370).

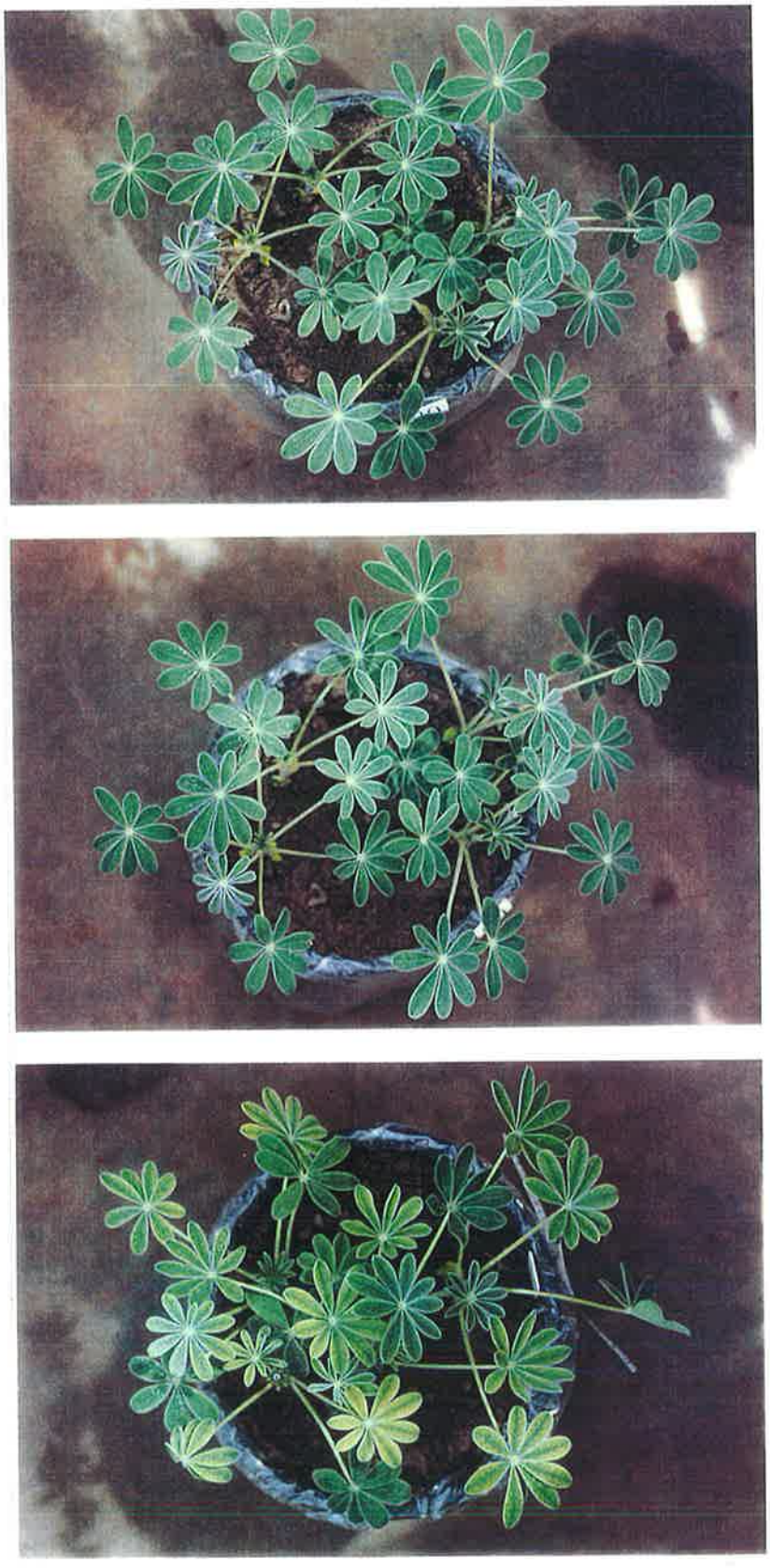


**Plate 4.1** The effect of increased soil moisture (top - 80%, middle - 100%, bottom - 120% of field capacity) in the calcareous Wangary soil on chlorotic symptoms of *L. pilosus* genotypes 21 days after sowing.
b. Moderately tolerant (P20954).

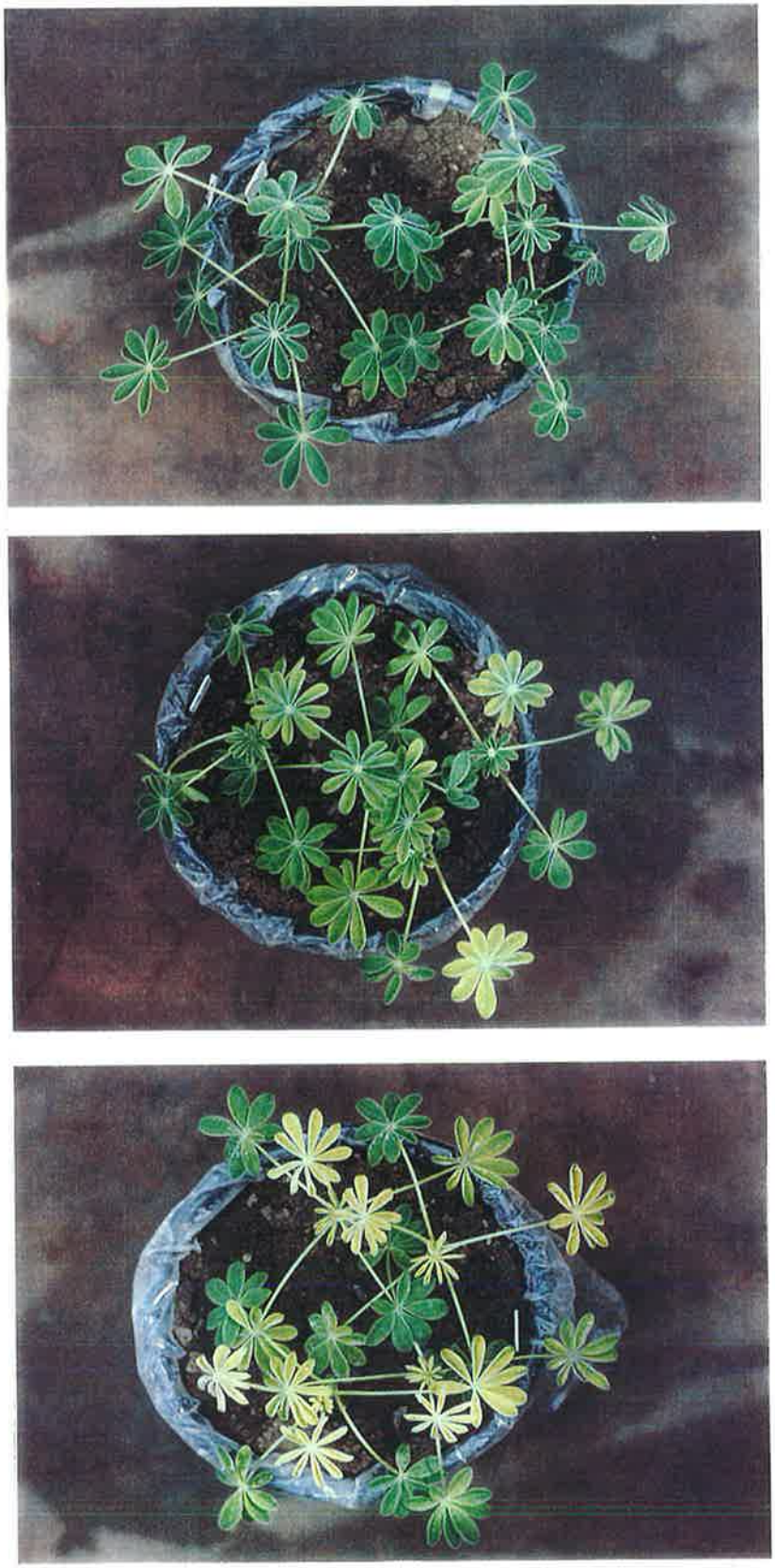


**Plate 4.1** The effect of increased soil moisture (top - 80%, middle - 100%, bottom - 120% of field capacity) in the calcareous Wangary soil on chlorotic symptoms of *L. pilosus* genotypes 21 days after sowing.
c. Moderately intolerant (P22932).

The trends indicated by the chlorophyll meter readings were the inverse of the chlorosis score. The control treatment was similar for all genotypes with a slight decrease during the experiment. For P23370 at 80% and 100% soil moisture, P20954 at 80% soil moisture and P22932 at 80% soil moisture these readings clearly show the regreening (increase in arbitrary units) that occurred from 21 DAS to 35 DAS (Fig. 4.6). By 35 DAS in the 80% moisture treatment, readings were similar to the control for P23370 and P20954.

The trends in chlorophyll concentration for all soil moisture treatments, except for the control, were similar to those of chlorophyll meter readings (Fig. 4.6). For all genotypes, the highest chlorophyll concentrations were recorded on plants grown in the control soil. As soil moisture levels increased in the calcareous soil, chlorophyll concentrations decreased. Over time there was an increase in chlorophyll concentration for all the genotypes at the lower two moisture treatments, except P22932 in the 100% moisture treatment. The moderately intolerant genotype, P22932, showed lower chlorophyll concentrations than the other genotypes at all soil moisture contents, but not in the control. The moderately tolerant genotype, P20954, had reduced chlorophyll concentrations compared with the tolerant genotype, P23370, at both 80% and 100% soil moisture levels (Table 4.6).

Chlorosis score was significantly negatively correlated with chlorophyll meter readings and chlorophyll concentration, whilst chlorophyll meter readings positively correlated with chlorophyll concentration (Table 4.6). Relative shoot dry weight and relative number of leaves were positively correlated, but they did not correlate with chlorosis, chlorophyll meter readings or chlorophyll concentrations (Table 4.6).

Nutritional concentrations

There were no significant three-way interactions for any of the nutrient concentrations. However, the two-way interactions between genotype and DAS, for active Fe ($P < 0.05$), Ca ($P < 0.05$) and Mg ($P < 0.05$) and, soil moisture and DAS, for active Fe ($P < 0.05$), total Fe ($P < 0.05$), Mn ($P < 0.05$), Mg ($P < 0.05$), Na ($P < 0.05$), P ($P < 0.05$) and S ($P < 0.05$) were significant. B, Cu and Zn had no significant interactions and did not correlate with any other measurements, hence data for these elements is not shown.

**Table 4.6** Correlation coefficients (r) between chlorosis score (CS), relative chlorophyll meter readings (CM %), relative number of leaves (NL %), relative total shoot dry weight (SDW %), relative chlorophyll concentrations (CC %), active iron (aFe), total Fe (tFe), manganese (Mn), calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), phosphorus (P) and sulphur (S) in the YEL of three genotypes of *L. pilosus* grown in the Wangary soil at moisture contents of 80, 100 and 120% of field capacity.

| | CS | CM % | NL % | SDW % | CC % | aFe | tFe | Mn | Ca | Mg | Na | K | P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **CM %** | -0·95** | | | | | | | | | | | | |
| **NL %** | 0·07 | -0·37* | | | | | | | | | | | |
| **SDW %** | -0·17 | -0·08 | 0·65** | | | | | | | | | | |
| **CC %** | -0·89** | 0·97** | -0·31* | -0·11 | | | | | | | | | |
| **AFe** | -0·76** | 0·90** | -0·25 | -0·10 | 0·89** | | | | | | | | |
| **TFe** | -0·74** | 0·88** | -0·44** | -0·21 | 0·90** | 0·82** | | | | | | | |
| **Mn** | -0·56** | 0·74** | -0·53** | -0·34* | 0·79** | 0·70** | 0·84** | | | | | | |
| **Ca** | 0·03 | -0·36* | -0·14 | -0·29 | -0·16 | -0·32* | -0·22 | -0·10 | | | | | |
| **Mg** | 0·66** | -0·80** | -0·05 | -0·26 | -0·69** | -0·69** | -0·68** | -0·54** | 0·66** | | | | |
| **Na** | 0·21 | 0·06 | -0·27 | -0·55** | 0·15 | 0·25 | 0·21 | 0·52** | 0·03 | 0·09 | | | |
| **K** | 0·70** | -0·84** | 0·07 | -0·06 | -0·79** | -0·81** | -0·74** | -0·65** | 0·62** | 0·91** | -0·12 | | |
| **P** | 0·39* | -0·24 | -0·30 | 0·12 | -0·35* | -0·35* | -0·11 | -0·13 | -0·32* | 0·10 | -0·22 | 0·20 | |
| **S** | -0·01 | -0·04 | -0·26 | 0·26 | -0·05 | -0·23 | 0·08 | 0·14 | -0·05 | -0·04 | -0·27 | 0·11 | 0·70** |

* $P < 0·05$, ** $P < 0·01$

P23370 had the highest active Fe concentration in the YEL, significantly greater than P20954 and P22932 at 14 and 21 DAS after sowing, but at 28 and 35 DAS there was little difference among genotypes (Fig. 4.7). Plants grown in control pots had significantly higher active Fe concentrations than plants in the calcareous soil at any soil moisture treatment. Increased soil moisture decreased active Fe concentrations. Total Fe concentration decreased slightly over time for all genotypes (Fig. 4.7). At 14 DAS there were no differences between genotypes, but at 21 and 28 DAS the total Fe concentration in P23370 was slightly greater than for P22932 and P20954. Also at 28 DAS P20954 had a slightly higher Fe concentration than P22932. For the calcareous soil treatments, as the moisture increased the Fe concentration decreased. The plants grown in the control soil had 100% more Fe at 14 DAS and 50% more at 35 DAS than at the 80% moisture treatment of the calcareous soil (Fig. 4.7).

Mn concentrations were slightly higher in P23370 than other genotypes at all harvests (Fig. 4.7). There was no difference between soil moisture treatments, although the concentrations in the control were significantly greater at all harvests.

The Ca concentration was variable over time for all genotypes and treatments, increasing from 14 to 21 DAS, decreasing from 21 to 28 DAS and then slightly increasing again (Fig. 4.7). Generally the Ca concentration was lowest in P23370 and highest in P20954 although at 28 DAS there was no difference between P23370 and P22932. There were no consistent differences for Ca concentrations between moisture treatments across harvests.

There were no significant differences in Na concentrations between genotypes (Fig. 4.7). Increased soil moisture appeared to result in plants with increased Na levels by 28 DAS, although they were generally lower than those grown in the control soil.

The P concentration in the YEL decreased over time for all genotypes and 80% and 100% soil moisture (Fig. 4.7). P22932 had the highest P concentrations from 21 to 35 DAS and there was no difference between P23370 and P20954. The P concentration appeared to increase as soil moisture increased, especially at 28 DAS (Fig. 4.7). Plants grown in the control had similar concentrations to those grown in 80% soil moisture treatment.

Correlations of all nutrients with growth and chlorophyll measurements and other nutrients are summarised in Table 4.6. It was particularly notable that active and total Fe and Mn were

**Figure 4.7** The main effects of genotype (P23370 - tolerant, P20954 - moderately tolerant and P22932 - moderately intolerant) and soil moisture (% of field capacity) in the Wangary calcareous soil compared with the Glenthorne non-calcareous soil (control) on the nutritional concentrations in the YEL from 14 to 35 days after sowing. Bars indicate LSD values ($P < 0.05$). n.s. - not significant.

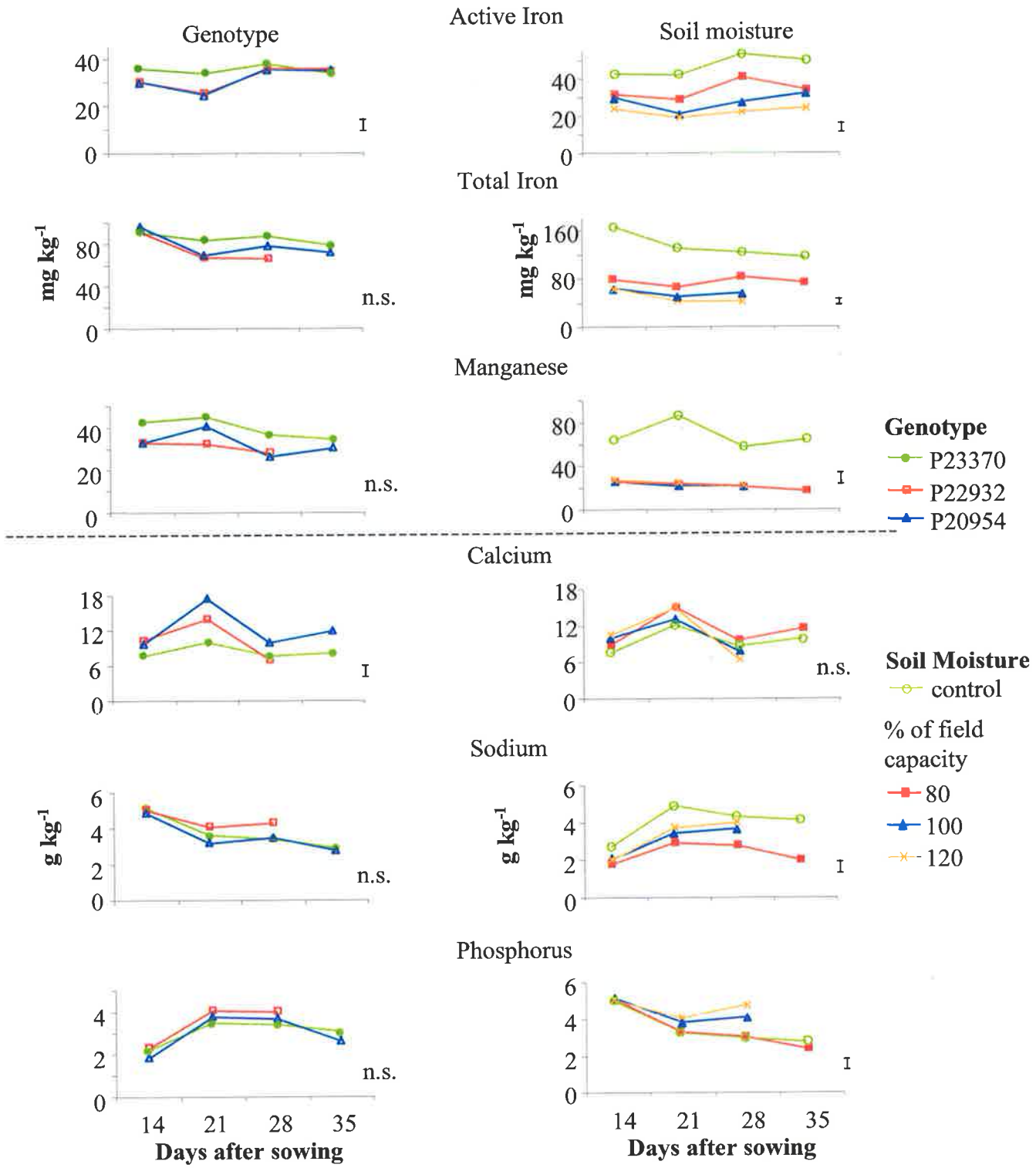
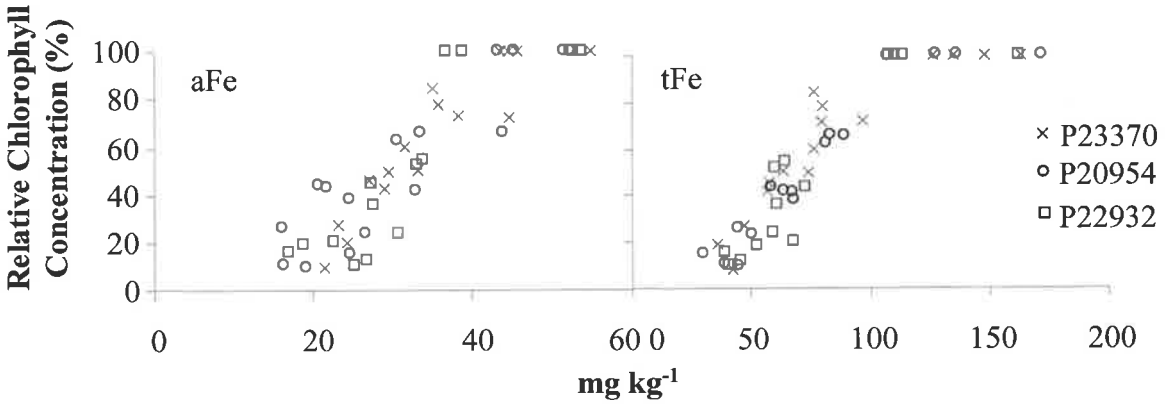


**Figure 4.8** Relationship between relative chlorophyll concentration and active iron (aFe) and total iron (tFe) concentrations for *L. pilosus* genotypes grown on a calcareous soil at three moisture levels (80%, 100% and 120% of field capacity). Relative chlorophyll concentrations of 100% represent those plants grown on the non-calcareous soil.



negatively correlated with chlorosis score and positively correlated with chlorophyll meter readings and chlorophyll concentration. P was negatively correlated with shoot dry weight and number of leaves. Ca had only a minor negative correlation with chlorophyll meter readings and active Fe concentrations. The relationships of active and total Fe with relative chlorophyll concentrations in the YEL are shown in figure 4.8. All genotypes responded similarly to tissue Fe concentrations indicating that these genotypes had similar internal Fe requirement for maximal chlorophyll synthesis, with a critical value of 40 mg $kg^{-1}$ for active Fe and 100 mg $kg^{-1}$ for total Fe in the YEL.

*Effect of nutrient addition (Expt. 2b)*

Chlorosis score, chlorophyll meter readings, shoot dry weight and chlorophyll concentration

There was a three-way interaction between genotype, nutrient treatment and DAS for chlorosis score ($P < 0.05$) and chlorophyll meter readings ($P < 0.05$). For shoot dry weight the interactions between genotype and DAS, and nutrient treatment and DAS were significant ($P < 0.05$), while for chlorophyll concentration, only the interaction between nutrient treatment and DAS was significant ($P < 0.05$).

Genotype and nutrient treatment had no effect on the emergence rate or final emergence percentage. P23370 showed more vigorous growth than P20954 and P22932 over the course of the experiment (Fig. 4.9). There was no difference in shoot dry weights at any of the soil nutrient treatments at 21 DAS. Twenty one DAS FeEDDHA treated plants were stunted and showed a toxic effect of FeEDDHA. All other treatments showed similar growth. At the final harvest plants sprayed with $FeSO_4$ had shoot dry weights significantly greater than those of the control (Fig. 4.9).

Chlorosis symptoms in the YEL were first visible 14 DAS and by 21 DAS there were clear genotypic differences at all of the treatments, except the 'nutrients with FeEDDHA' treatment in which there were no symptoms (Fig. 4.10). As observed in experiment 2a, P23370 did not show chlorosis, P22932 had slight to moderate symptoms while P22932 had moderate to severe symptoms (Plate 4.2). Throughout the experiment chlorosis rankings of genotypes remained similar in both the control and the 'nutrients without Fe' treatment. The chlorosis score of P22932 sprayed with $FeSO_4$ was significantly reduced compared with other treatments from 42 DAS.

**Figure 4.9** The main effects of genotype (P23370 - tolerant, P20954 - moderately tolerant and P22932 - moderately intolerant) and nutrient treatment on the total shoot dry weight of plants grown in the Wangary calcareous soil from 21 to 84 days after sowing. The three-way interaction between genotype, soil moisture and DAS was not significant. Bars indicate LSD values ($P < 0.05$) for two-way interactions.

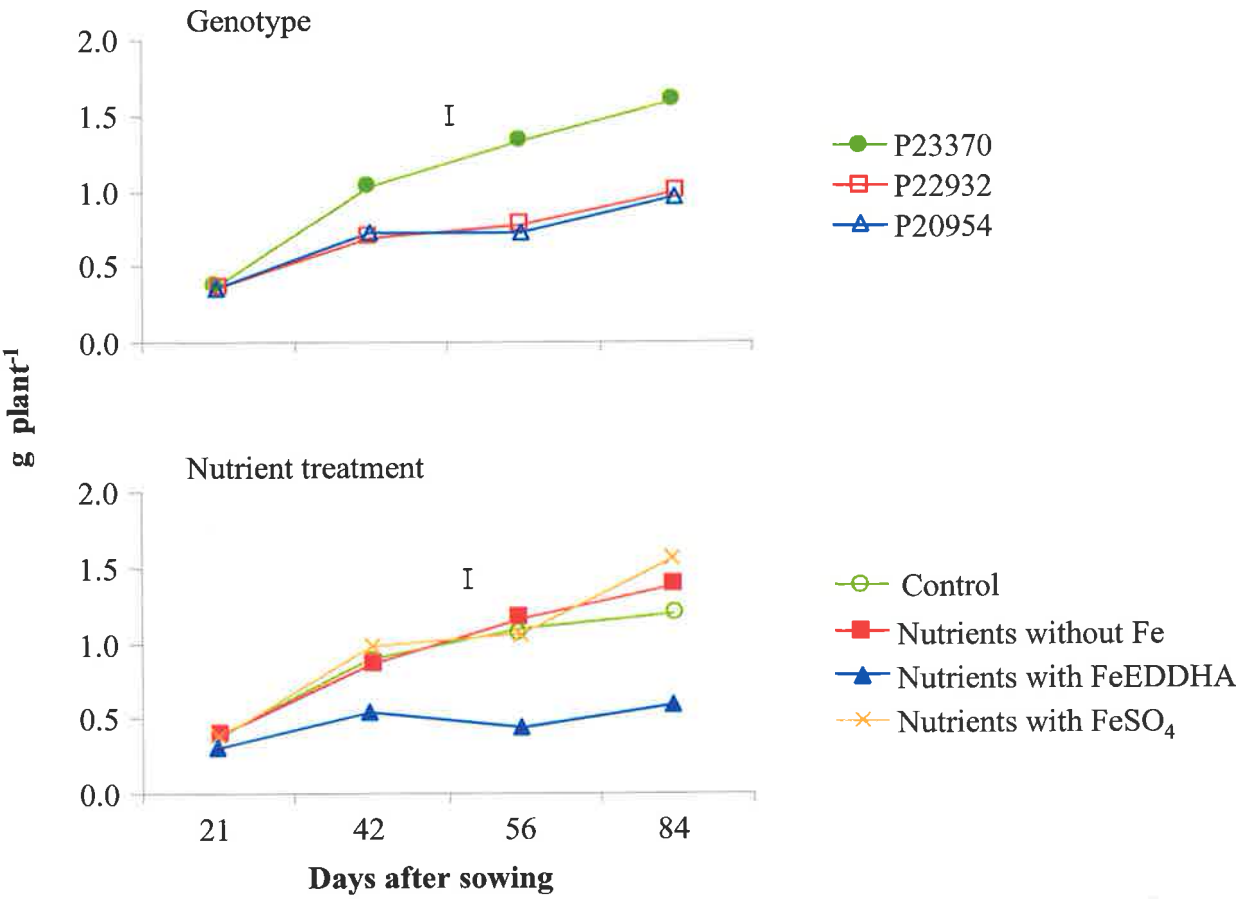


**Figure 4.10** The effect of soil nutrition and Fe fertilization on chlorosis score chlorophyll meter readings and chlorophyll concentration of a tolerant (P23370), moderately tolerant (P20954) and a moderately intolerant (P22932) genotype of *L. pilosus* grown in the Wangary calcareous soil from 21 to 84 days after sowing. Bars indicate LSD ($P < 0.05$); n.s. not significant.

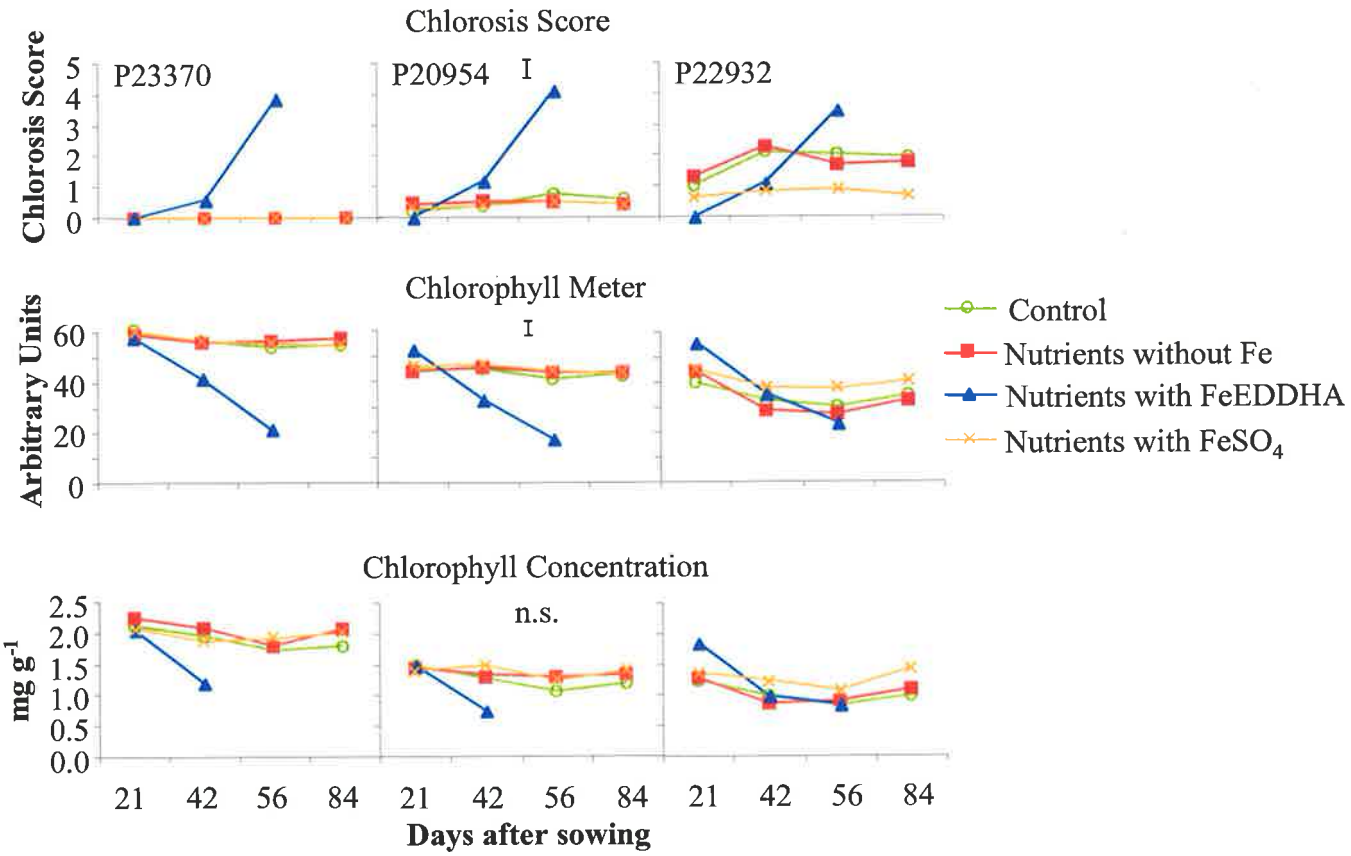


**Plate 4.2** The effect of soil nutrition on chlorotic symptoms of a tolerant (P23370), moderately tolerant (P20954) and a moderately intolerant (P22932) genotype of *L. pilosus* grown in the calcareous Wangary soil 21 days after sowing.


**Plate 4.2** The effect of soil nutrition on chlorotic symptoms of a tolerant (P23370), moderately tolerant (P20954) and a moderately intolerant (P22932) genotype of *L. pilosus* grown in the calcareous Wangary soil 21 days after sowing.


Chlorophyll meter readings and chlorophyll concentrations displayed an inverse trend to that of chlorosis score and were negatively correlated with chlorosis score (Table 4.7). The chlorophyll meter readings and chlorophyll concentrations in the YEL were generally highest for P23370 and lowest for P22932 (Fig. 4.10).

Nutritional concentrations

There was a three-way interaction between genotype, nutrient treatment and DAS for active Fe, total Fe and Mn concentrations ($P < 0.05$). The two-way interactions between genotype and DAS for B, Zn, Mg, Na, K and S, and nutrient treatment and DAS, for B, Cu, Zn, Ca, Mg, Na, K, P and S were significant ($P < 0.05$).

P23370 had the highest active Fe concentration in the YEL at all harvests and in all treatments, except 'nutrients with $FeSO_4$' for P20954 and P22932 and 'nutrients with FeEDDHA' for P22932. Foliar spray of $FeSO_4$ increased the active Fe concentration in all genotypes and the addition of FeEDDHA increased the concentration in P22932 (Fig. 4.11). Active Fe concentrations increased slightly from 21 to 56 DAS, but decreased from 56 to 84 DAS for all genotypes and treatments. Total Fe concentrations showed similar trends to active Fe, except that the addition of FeEDDHA increased total Fe concentrations compared with other treatments. There were no significant differences in the concentrations between genotypes in any treatment (Fig. 4.11).

The Mn concentrations in the YEL generally decreased from 21 to 42 DAS and remained constant or increased slightly from 42 to 84 days for the genotypes grown in all treatments except the FeEDDHA treatment (Fig. 4.11). In the FeEDDHA treatment Mn concentrations were significantly lower than other treatments and decreased with time.

Concentrations of Ca, Mg, Na, K, P, S, B, Cu and Zn appeared to be in above critical concentrations described for *L. angustifolius* (Table 3.3; Reuter and Robinson, 1997). Both the addition of basal nutrients and $FeSO_4$ foliar spray did not affect the concentrations of these elements. The addition of FeEDDHA increased concentrations of B, Zn, Cu and P and decreased the concentration of Ca. P23370 had lower concentrations of Ca, Mg, K and Na than the other two genotypes (data not shown).

**Figure 4.11** The effect of soil nutrition on active and total Fe and Mn concentrations of a tolerant (P23370), moderately tolerant (P20954) and a moderately intolerant (P22932) genotype of *L. pilosus* grown in the Wangary calcareous soil from 21 to 84 days after sowing. Bars indicate LSD ($P < 0.05$).

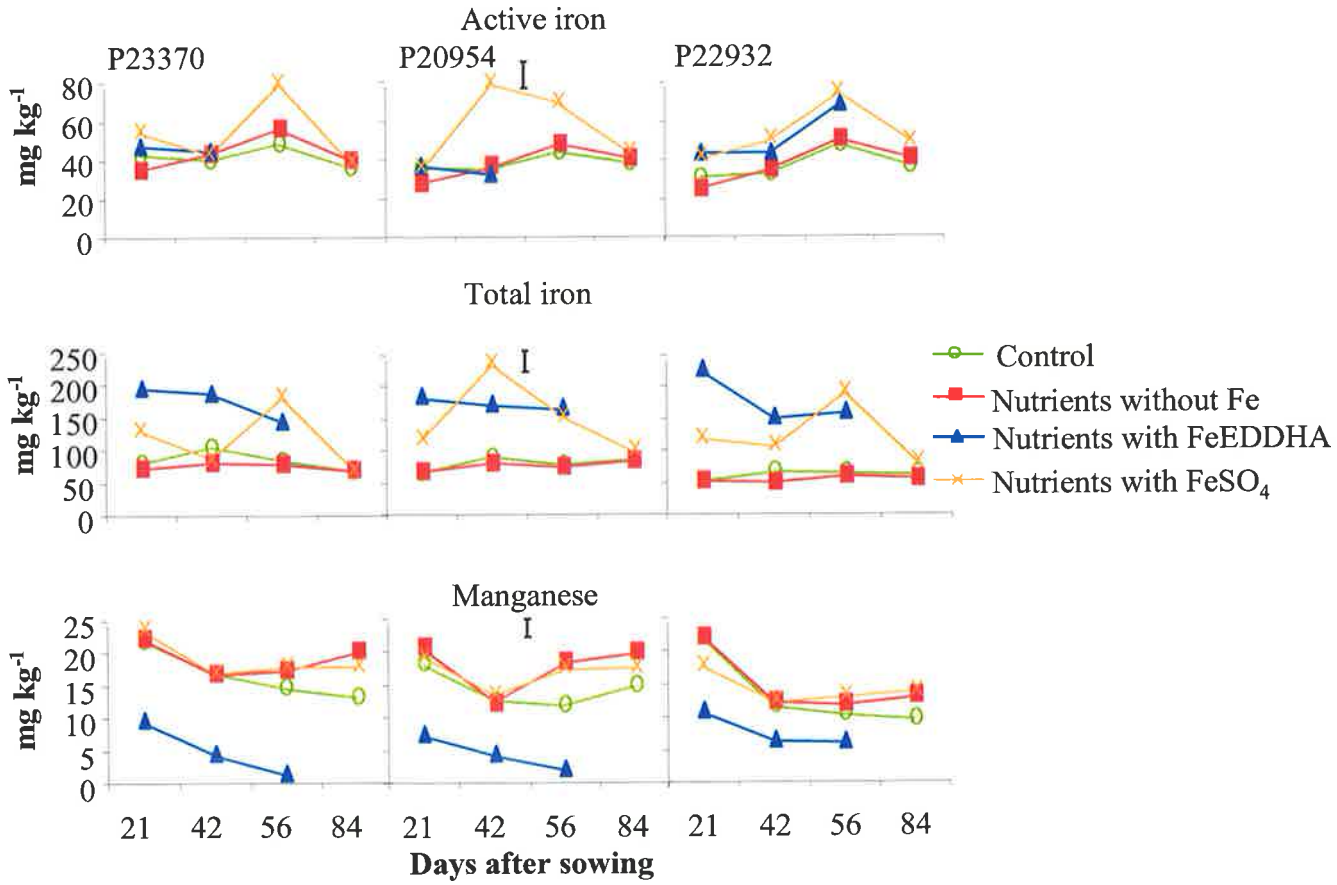


**Table 4.7** Correlation's coefficients (r) between chlorosis score (CS), chlorophyll meter readings (CM), total shoot dry weight (SDW), chlorophyll concentrations (CC), active Fe (aFe), total Fe (tFe), manganese (Mn), boron (B), copper (Cu), zinc (Zn), calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), phosphorus (P) and sulphur (S) in the YEL of three genotypes of *L. pilosus* grown on the Wangary soil with four nutrient regimes.

| | CM | CS | SDW | CC | aFe | tFe | Mn | B | Cu | Zn | Ca | Mg | Na | K | P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **CS** | -0.93** | | | | | | | | | | | | | | |
| **SDW** | 0.35* | -0.35* | | | | | | | | | | | | | |
| **CC** | 0.90** | -0.82** | 0.26 | | | | | | | | | | | | |
| **aFe** | 0.01 | -0.02 | 0.15 | 0.02 | | | | | | | | | | | |
| **tFe** | -0.15 | 0.16 | -0.30* | 0.00 | 0.58** | | | | | | | | | | |
| **Mn** | 0.66** | -0.64** | -0.09 | 0.59** | -0.10 | 0.43** | | | | | | | | | |
| **B** | -0.60** | 0.63** | -0.33* | -0.48** | -0.04 | 0.09 | -0.27 | | | | | | | | |
| **Cu** | -0.22 | 0.28* | -0.60** | -0.04 | 0.00 | 0.26 | -0.17 | 0.45** | | | | | | | |
| **Zn** | -0.49** | 0.48** | -0.51** | -0.41** | 0.01 | 0.32* | -0.21 | 0.56** | 0.68** | | | | | | |
| **Ca** | -0.01 | -0.17 | -0.11 | -0.16 | -0.05 | -0.15 | 0.04 | -0.39** | -0.12 | -0.10 | | | | | |
| **Mg** | -0.49** | 0.37** | -0.45** | -0.50** | -0.16 | -0.05 | 0.07 | 0.09 | -0.01 | 0.09 | 0.50** | | | | |
| **Na** | -0.65** | 0.55** | 0.09 | -0.70** | 0.00 | -0.16 | -0.09 | 0.45** | -0.22 | 0.08 | 0.13 | 0.30* | | | |
| **K** | -0.57** | 0.48** | -0.49** | -0.58** | -0.05 | 0.02 | -0.06 | 0.16 | 0.09 | 0.31* | 0.40** | 0.80** | 0.23 | | |
| **P** | -0.42** | 0.51** | -0.70** | -0.23 | -0.13 | 0.35* | -0.05 | 0.61** | 0.67** | 0.67** | -0.43** | 0.19 | -0.11 | 0.30* | |
| **S** | 0.13 | -0.03 | -0.69** | 0.30* | -0.14 | 0.30* | -0.05 | 0.19 | 0.63** | 0.40** | -0.17 | 0.05 | -0.54** | 0.12 | 0.71** |

* $P < 0.05$, ** $P < 0.01$

Correlations between concentrations of nutrients, shoot dry weight and chlorophyll measurements are summarised in Table 4.7. Particularly notable were that Mn, B, Zn, Mg, Na, K and P all correlate significantly with chlorophyll meter readings, chlorosis score and chlorophyll concentrations. Relative shoot dry weight was correlated with Cu, Zn, Mg, K, P and S and to a lesser extent with total Fe and B.

*Effect of $N_2$ fixation (Expt. 2c)*

There was a three-way interaction between genotype, treatment and DAS for chlorosis score ($P < 0.05$). For chlorophyll meter readings, active Fe and chlorophyll concentrations, and shoot dry weight, the interactions between genotype and treatment were not significant, although the main effect of genotype was significant ($P < 0.05$).

Chlorosis symptoms in the YEL were first visible 10 DAS and by 14 DAS there were clear genotypic differences in each of the treatments (Fig. 4.12). Similar to previous experiments P23370 showed the least chlorotic symptoms followed by P20954 while P22932 had the most severe chlorosis. The severity of chlorosis increased in P22932, slightly increased in P20954 and was unchanged in P23370 with DAS. There was no difference between the two nitrogen treatments for all genotypes. (Fig. 4.12). At harvest (28 DAS) chlorophyll meter readings, and active Fe and chlorophyll concentrations showed the opposite trend to that of chlorosis score (Fig. 4.12 and 4.13). Chlorosis score, chlorophyll meter readings, and active Fe and chlorophyll concentrations were all highly correlated at harvest (Table 4.8).

The growth response showed different trends, possibly due to the short duration of the experiment. Shoot dry weights of P23370 and P22932 were significantly greater than P20954 (Fig. 4.13).

**Table 4.8** Correlation coefficients (r) between chlorosis score (CS), chlorophyll meter readings (CM), total shoot dry weight (SDW), chlorophyll concentrations (CC), and active Fe (aFe) in the YEL of three genotypes of *L. pilosus* grown in the Wangary soil with two nitrogen regimes (with inoculation compared $NH_4NO_3$ fertilization).

| | CS | CM | SDW | aFe |
|---|---|---|---|---|
| **CM** | -0.98** | | | |
| **SDW** | 0.02 | 0.15 | | |
| **aFe** | -0.88** | 0.95** | 0.39 | |
| **CC** | -0.95** | 0.99** | 0.23 | 0.98** |

**Figure 4.12** The effect of N source (inoculation (-N) *v.* the addition of $NH_4NO_3$ (+N)) on chlorosis score of P23370, P20954, and P22932 grown in the Wangary calcareous soil from 14 to 28 DAS. Bar indicates LSD ($P < 0.05$).

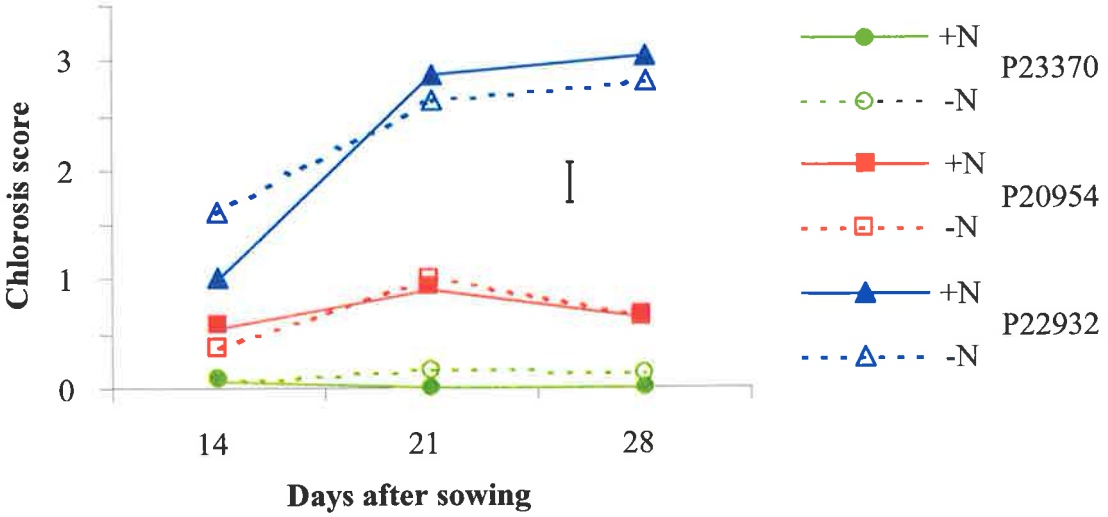
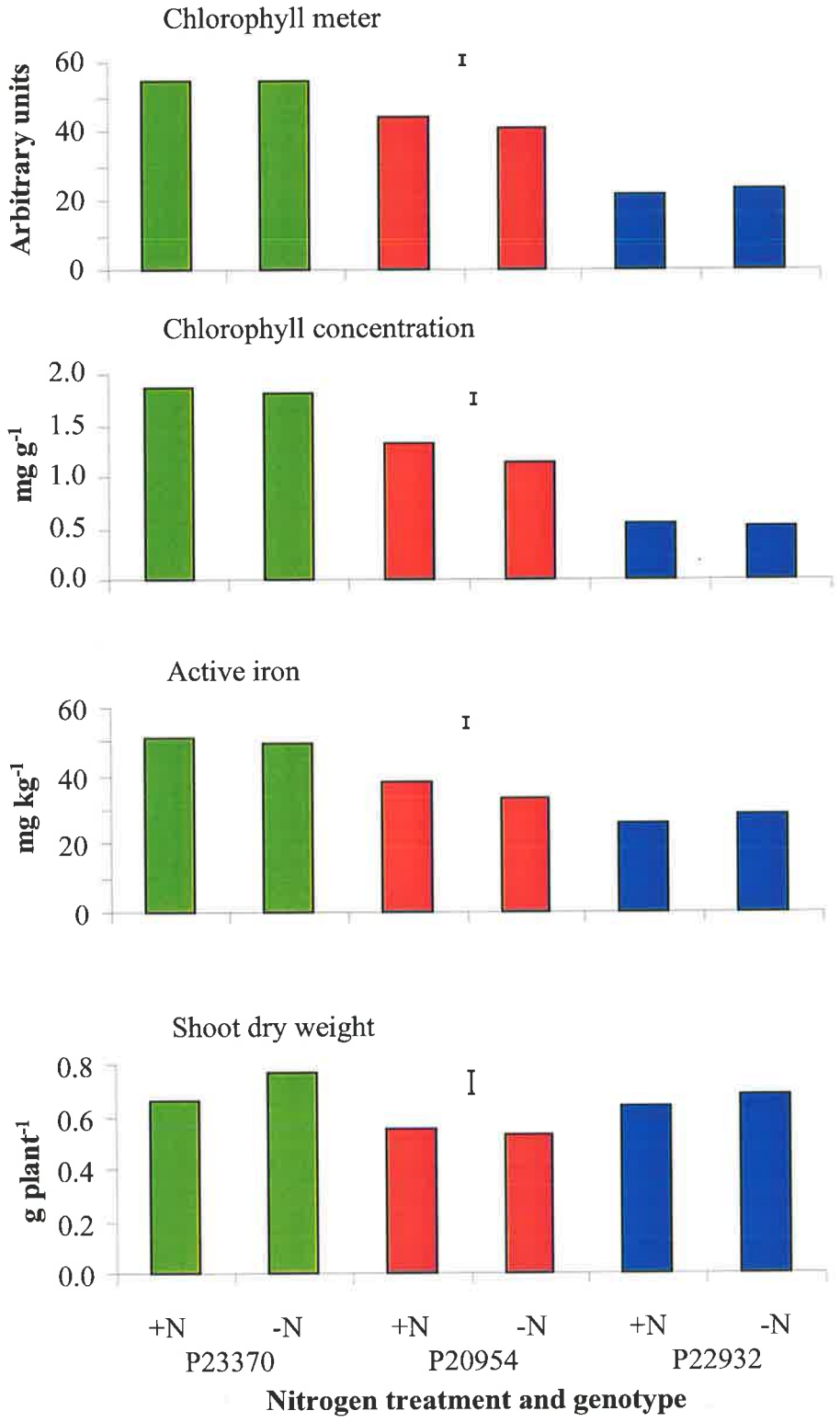


**Figure 4.13** Chlorophyll meter readings, active iron and chlorophyll concentration in the YEL and total shoot dry weight at harvest for P23370, P20954, and P22932 with rhizobia inoculation (-N) or with the addition of $NH_4NO_3$ (+N) grown in the Wangary calcareous soil from 14 to 28 DAS. The two-way interaction between genotype and treatment was not significant. Bars indicate LSD for comparison between genotypes only.



## 4.4 Discussion

The principal aim of this chapter was to develop a soil screening system that enables the selection of genotypes of lupins tolerant to calcareous soils. The first experiment indicated that, the addition of $CaCO_3$ to the non-calcareous Glenthorne soil was not suitable for a screening procedure. A preliminary trial in the Wangary calcareous soil (Section 4.3.1) indicated genetic variation in *L. pilosus.* Thus the following three experiments focussed on the effects of moisture, nutrition and inoculation on the ability to identify the tolerance of tolerant, moderately tolerant and moderately intolerant genotypes of *L. pilosus* in the Wangary soil.

*Genotypic variation*

Both interspecific and intraspecific variation, in terms of chlorotic symptoms, for tolerance to calcareous soils was observed in these experiments. As expected from previous research (White and Robson, 1989b; Jessop, *et al.*, 1990; Tang, *et al.*, 1995b), in experiment 1 Gungurru showed the most severe chlorotic symptoms, whilst Alma and P20954 showed few or no symptoms when $CaCO_3$ was added to the soil. The *L. atlanticus* genotype, P22927, also showed significant chlorosis, despite previous research indicating that the species has potential as an agricultural crop on calcareous soils (Buirchell, 1993; Egan and Hawthorne, 1994). *L. albus* also showed some genotypic variation on the soil with added $CaCO_3$ with P27486 and P27797 appearing more tolerant of the calcareous soil than Hamburg at 21 DAS. However, the symptoms on Hamburg decreased from 21 to 42 DAS so that it was unclear which genotype was more tolerant at 42 DAS. Also relative shoot dry weights were similar for the three genotypes in all treatments further indicating that there was little difference in tolerance between these genotypes.

In the experiments on the calcareous soil, the tolerance of the three genotypes of *L. pilosus* could be clearly identified at any moisture content (Expt. 2a), nutrient treatment (Expt. 2b) and with and without inoculation (Expt. 2c) using chlorosis score and chlorophyll meter readings (Fig. 4.6). The measurements of shoot dry weight and number of leaves were not related to chlorosis although the tolerant genotype P23370 generally produced the heaviest plants.

In experiment 2a (soil moisture) the moderately intolerant genotype, P22932, was more sensitive to an increase in soil moisture than the moderately tolerant genotype, P20954, which was more sensitive than the tolerant genotype P23370. In experiment 2b (soil nutrients) there were no differences in chlorotic symptoms between treatments, except FeEDDHA, for P23370 and

P20954. The intolerant genotype, P22932, showed a slight reduction in chlorosis, but not in chlorophyll concentration, with the application of a foliar spray of $FeSO_4$.

*Factors affecting lupin growth on calcareous soil*

The addition of $CaCO_3$ to a non-calcareous soil induced chlorotic symptoms resembling Fe deficiency and reduced shoot dry weight in intolerant genotypes. The photosynthetic activity of affected plants, was impaired and likely to result in reduced grain yields at the end of the season. Previous studies indicated that high pH was a major factor restricting the growth of narrow-leafed lupins on calcareous soil (Tang, 1995; Tang, *et al.*, 1995b). These results indicate that $CaCO_3$ in the soil *per se* is the major causative factor of chlorosis and not the pH, as chlorotic symptoms on the intolerant genotypes increased proportionally with $CaCO_3$ content. The pH of soils at each $CaCO_3$ content was constant (approximately 7.5; Table 4.2), however the buffering capacity of the soil was likely to increase with increased $CaCO_3$ content.

As discussed in Section 2.2.3, in calcareous soils the $CaCO_3$ reacts with soil water and $CO_2$ and produces $HCO_3^-$ which is likely to be the causative factor of symptoms resembling Fe deficiency (Coulombe, *et al.*, 1984a) seen in these experiments. High concentrations of $HCO_3^-$ can impair Fe absorption and utilization in plants (Marschner, 1995) and are likely to affect the availability of many other nutrients (Section 2.3). In solution culture it was found that increasing the $HCO_3^-$ concentration increased chlorotic symptoms and decreased the Fe concentration in soybeans (Coulombe, *et al.*, 1984b).

A number of factors including $CaCO_3$ content, moisture, pH can influence the $HCO_3^-$ concentration in soil solution. In the first experiment an increase in chlorosis was related to increased $CaCO_3$, although results may be confounded by the effects poor soil structure, which caused waterlogging. At 20% $CaCO_3$ the soil was 'hard setting' when dry and became relatively impermeable to water (similar to sodic soils). The unamended Glenthorne soil when dry was crumbly and very permeable to water. Allowing the soil to equilibrate for a longer period and pass through several wetting and drying cycles may redevelop the structure and thereby overcome the waterlogging problems. In previous research waterlogging induced symptoms resembling Fe deficiency in *L. angustifolius* and *L. atlanticus* (Broué, *et al.*, 1976; Phuphak and Setter, 1989; White and Robson, 1989b), whilst both *L. pilosus* and *P. sativum* are more tolerant giving them an adaptive advantage (B Buirchell, pers. comm.).

Increased soil moisture in the Wangary calcareous soil resulted in an increase in the chlorotic symptoms on *L. pilosus* similar to other intolerant crops (Ao, *et al.*, 1987; Fairbanks, *et al.*, 1987; Expt. 2a). As moisture content increases $P_{CO_2}$ increases, thus more $HCO_3^-$ is formed (Section 2.2.2). All *L. pilosus* genotypes appeared to recover from chlorotic symptoms at low soil moisture, even though the symptoms at 14 DAS were more severe in P20954 and P22932. At high moisture only the tolerant genotype, P23370, had recovered.

Shoot dry weight was decreased as the soil moisture increased (Fig. 4.4) indicating that there was a relationship with chlorosis score, but it was not genotype specific within a moisture treatment. Apart from its increased tolerance, P23370 had the highest shoot dry weight across all treatments indicating that this genotype has good early vigour. Early vigour is important when breeding a crop for adaptation to the drier areas and competitiveness against weeds. Leaf number was highly correlated with shoot dry weight, but showed some different trends. All soil moisture treatments were very similar in terms of leaf number, suggesting that dry weight differences were due to the difference in leaf size.

In soil with high concentrations of $CaCO_3$, such as the Wangary soil used in experiment 2 and the 20% $CaCO_3$ treatment in experiment 1, the soil is also likely to have a high buffering capacity. The buffering capacity of the soil may be especially important for *L. angustifolius*, which relies on strong acidification of the rhizosphere for uptake of nutrients. Soils with high buffering capacity will neutralize the $H^+$ ions excreted from the plant, thus the plant is unable to efficiently acidify the rhizosphere.

The chlorosis observed on plants grown in calcareous soils has commonly been attribute to Fe deficiency induced by high $HCO_3^-$ concentrations (Mengel and Geurtzen, 1986; Ao, *et al.*, 1987; Plessner, *et al.*, 1992), but results in these experiments confirm results of chapter 3 showing that total Fe was not deficient. In experiment 2a and 2b both active Fe and total Fe were measured to indicate whether the Fe inside the YEL's was being inactivated in intolerant genotypes in response to the stress associated with calcareous soils. Results from this study indicated that inactivation of Fe in the leaf was unlikely to be the reason for chlorotic symptoms resembling Fe deficiency in previous experiments and high concentrations which have been previously observed on calcareous soils (Kaur, *et al.*, 1984). Firstly, there was a negative correlation of both active and total Fe with chlorosis measurements. Secondly, in experiment 2a the proportion of active to total Fe was increased in the calcareous soil compared with the control, not decreased,

as would have been expected if inactivation in the plant grown in the calcareous soil treatments had occurred. In addition, in the Wangary calcareous soil the Fe concentrations in the shoots decreased compared with the Glenthorne control soil, not increased as previously reported in (Kaur, *et al.*, 1984).

In experiment 2b the addition of Fe to a calcareous soil or as a foliar spray reduced $HCO_3^-$ induced chlorosis consistent with previous findings (Anderson, 1983; Fouda, 1984; Moraghan, 1987; Plessner, *et al.*, 1992), but chlorosis was not completely eliminated which supports the findings in chapter 3 and previous suggestions that Fe deficiency was not the only reason for chlorosis on calcareous soils. It also showed that the addition of FeEDDHA to a soil at 5 mg $kg^{-1}$ was toxic to plant growth causing severe chlorosis and stunted growth. Mn concentrations were significantly reduced, whilst total Fe increased and active Fe remained constant compared with other treatments. This shows that plants are taking up the FeEDDHA, but were not able to convert it to an active form, which may be a result of the toxicity of EDDHA. The chlorotic symptoms may also be a result of the severe Mn deficiency.

The application of a foliar spray of $FeSO_4$ increased active and total Fe in the YEL, despite symptoms remaining similar to other treatments. This confirms that chlorosis was due to a deficiency other than Fe or a toxicity, such as $HCO_3^-$, or there was contamination resulting from Fe sprayed onto the leaves.

Alternatively, chapter 3 indicated that Mn deficiency might be contributing to chlorosis in plants grown on calcareous soils. In experiment 1, although Mn concentrations were extremely low in the $CaCO_3$ treatments, particularly for Gungurru, P22927, P20954 and Alma, they were not significantly different from 0% $CaCO_3$ in which plants showed no symptoms. In *L. albus* there was generally a decrease in Mn with increasing $CaCO_3$, but concentrations were above critical concentrations (Reuter and Robinson, 1997).

Also the Mn concentrations in the YELs of *L. pilosus* genotypes in experiment 2a were significantly correlated with chlorosis measurements. However, there were no differences in Mn concentrations among the moisture treatments, indicating that Mn is not likely to be a major factor in the differences in chlorotic symptoms between the treatments.

Another nutrient which has been indicated as possibly toxic in calcareous soils is Ca (Tang, 1995). Data showed that Ca was not toxic in the Wangary calcareous soil as there appeared to be no difference to the control and there are no highly significant correlations with chlorosis or growth measurements. When comparing *L. pilosus* genotypes in experiment 2a and 2b the tolerant genotype, P23370, was able to maintain lower concentrations of Ca in the YEL, which corresponds with experiment 1 in that P20954 and Alma had proportionally (c.f. 0% $CaCO_3$) lower Ca concentrations than the other intolerant genotypes.

Poor nodulation (Tang and Robson, 1995) has also been indicated as a factor causing poor growth of lupins grown on alkaline soils. As there were no differences between inoculation and nitrate fertilization for all measurements recorded, particularly chlorosis score and chlorophyll meter readings, it can be concluded that the success of inoculation did not confound chlorosis symptoms in this study.

Overall results indicate that $HCO_3^-$ induced deficiencies of Fe and Mn, and toxicity of Ca was unlikely to be the full cause of chlorosis. Alternatively, $HCO_3^-$ might have a direct effect on chlorophyll concentration. The $HCO_3^-$ taken up into the plant could be transported towards the leaves through the xylem and might increase the pH if not regulated through neutralization by protons or carbonic acid formed in the leaves from the intake of $CO_2$ through the stomata. Protons are involved in various stages of chlorophyll formation, thus if they are being consumed through neutralization of $HCO_3^-$, chlorophyll synthesis is expected to be impaired.

*Mechanisms of tolerance*

To define mechanisms of tolerance to calcareous soils, physiological or nutritional reasons for the chlorosis must be defined. From the experiments in this chapter, evidence in nutritional results indicate that chlorosis was partly related to lower concentrations of Fe and Mn and higher concentrations of Ca, although appearing to be in the adequate range (Reuter and Robinson, 1997; Table 3.3). As discussed previously, $HCO_3^-$ may also have a direct effect on reducing chlorophyll concentrations in the plant in addition to inducing chlorosis via the reduction in the uptake and transport.

Possible mechanisms of tolerance in P20954 compared with Gungurru were discussed in chapter 3. These experiments indicate that the most probable mechanism of tolerance is related to an ability to prevent uptake of $HCO_3^-$ or efficiently sequester it once inside the root which prevents

increases in internal pH and transport to the shoots. In these experiments, at lower moisture contents, the moderately tolerant and moderately intolerant genotypes of *L. pilosus* were able to partly recover from chlorosis. From this, it appears that there is a mechanism activated in response to the soil, and that there is a critical $HCO_3^-$ concentration, above which the mechanism does not function. In the tolerant genotype the mechanism functions from germination.

*Conditions for soil screening*

The major aim of this chapter was to define a suitable screening method for identifying tolerance to calcareous soils in lupins, particularly the rough-seeded species, *L. pilosus* and *L. atlanticus*. These experiments indicate that the use of a calcareous field soil is preferable to the addition of $CaCO_3$ to a non-calcareous soil. The addition of $CaCO_3$ to the Glenthorne was not a suitable method for screening lupins for tolerance to calcareous soil as there is a large confounding effect associated with the loss of soil structure and waterlogging.

In the calcareous soil, moisture content, the addition of nutrients and the use of nitrate rather than inoculation as a nitrogen source did not affect the ranking of genotypes. The most efficient and effective methods for identifying the tolerance of *L. pilosus* genotypes were chlorosis score and chlorophyll meter readings, both being highly correlated with chlorophyll concentrations, but faster and cheaper.

The ideal soil moisture content was between 80% and 100% as at these contents plants show chlorosis, but the intolerant genotypes were not killed. In experiments 2b and 2c 90% moisture was used and gave a consistent chlorosis ranking over time. If the aim was to screen for tolerance between moderately tolerant and tolerant genotypes moisture content could be increased to enhance the separation between tolerant and intolerant genotype. The results in experiment 2a indicate that 21 DAS is the best time for scoring chlorosis, showing the greatest differentiation between genotypes.

# CHAPTER 5

## Development of a solution screening method to identify lupins tolerant to calcareous soils

## 5.1 Introduction

Solution screening systems have been used successfully to identify tolerance to nutritional deficiencies and toxicities (Coulombe, *et al.*, 1984b; Chaney, *et al.*, 1992a; Chaney, *et al.*, 1992b; Campbell, *et al.*, 1998). For example, nutrient solutions containing high concentrations of $HCO_3^-$ were used to identify tolerance to lime-induced Fe chlorosis in soybeans (Coulombe, *et al.*, 1984b; Chaney, *et al.*, 1992a), whilst solutions containing toxic concentrations of B were used to identify tolerance to B toxicity in wheat (Campbell, *et al.*, 1998). In lupins no solution screening systems for nutritional deficiencies and toxicities have been developed, although Tang, *et al.* (1996a) compared a number of species at a range of pH and $HCO_3^-$ concentrations from 1 to 5 mmole. *Lupinus pilosus* was found to be the least affected in terms of root and shoot growth and chlorotic symptoms.

The advantages of a solution system compared with a soil system are:

1. the elimination of problems associated with diseases in the root zone. In soil systems inoculant levels of root pathogens, particularly fungal root rots, can rapidly increase if the soil is used repeatedly. Changing the soil after each screening experiment is laborious and increases the chance of variability in results. In solution systems the nutrients can be changed regularly and equipment sterilized, eliminating root disease problems.
2. the reduction of problems associated with spatial variability in the moisture and nutrient concentration which can confound results in a soil system. In a soil system slight variations in moisture and nutrient concentration between pots or across a large screening box could be expected. Also if the soil has to be changed regularly, variability in the nutritional status between batches of soil may cause inconsistent results.
3. that solution systems are often more efficient. For B toxicity in wheat it was found that the root length 10 days after sowing was a reliable indicator of B tolerance (Campbell, *et al.*, 1998), whilst in a soil system results could not be recorded until 6-8 weeks growth when symptoms were visible (Paull, *et al.*, 1988).

4. solution culture is non destructive and enables plants to be transplanted for seed production after screening.
5. results from a solution system are generally easier to reproduce than soil systems because the screening can be conducted under controlled conditions.

Conversely, a solution system has a major disadvantage if the plant response to calcareous soil depends on reactions occurring in the root rhizosphere. For example, Römheld (1986) and Dinkelaker, *et al.* (1989) showed that plants acidify or release chelates or exudates in the rhizosphere to increase the availability of limiting nutrients. As the solution is being constantly circulated as a result of aeration, these effects are likely to be negated.

Development of a solution-based screening system, firstly requires reliable field or soil screening measurements on calcareous soils to validate results through correlation analysis. Since there had been no previous research identifying genetic variation in rough-seeded lupins for tolerance to calcareous soil, a soil screening system was first developed (Chapter 4). Results from preliminary screening trials in the Wangary soil indicated a wide range of tolerance to calcareous soil between species and among *L. pilosus* genotypes. Detailed screening trials which confirmed these unpublished preliminary results are described in Chapter 6.

Secondly, results from a solution-based system must accurately reflect the results seen in soil, thus the major limiting nutrient or toxicity in calcareous soils must be identified. Bicarbonate has been identified as a major toxic ion in calcareous soils inducing symptoms resembling Fe deficiency in intolerant crops (Coulombe, *et al.*, 1984a). Results in chapter 4 also indicated that $HCO_3^-$ was likely to be the major factor causing chlorosis in *L. pilosus* grown in the Wangary soil. The availability of some nutrients in calcareous soils are also likely to be limited (Section 2.3.1), although chapters 3 and 4 did not indicate any particular deficiencies in plants grown on calcareous soils other than lower concentrations of Fe and Mn compared with a non-calcareous control soil.

Finally, a measurement providing good differentiation between tolerant and intolerant genotypes, that can be recorded early in the growth of the plant, must be determined. In previous research on $HCO_3^-$ induced Fe deficiency in soybeans and chickpeas, the severity of chlorosis in the youngest emerging leaf recorded approximately 21 days after transplanting has been used as an index of tolerance (Coulombe, *et al.*, 1984b; Chaney, *et al.*, 1992a). In solution screening

systems for identifying B tolerance in wheat root length after 10 days was recorded (Campbell, *et al.*, 1998).

To identify tolerance to Fe chlorosis on calcareous soils in soybeans and other dicotyledonous crops detailed methods using $HCO_3^-$ have been described by (Chaney, *et al.*, 1992a). These methods are excellent from a plant physiologist's perspective, but are expensive and relatively laborious to set-up and maintain in a breeding program. Also their method focuses on Fe deficiency induced by $HCO_3^-$, but $HCO_3^-$ may also induce Mn deficiency and/or have a direct effect on chlorophyll synthesis (Chapter 4). Hence, the investigations reported in this chapter focussed on the effects of $HCO_3^-$ in complete nutrient solutions supplied with relatively high Fe concentrations.

The major aims of this chapter were to investigate the effects of $HCO_3^-$ on plant growth and to define a solution screening method which enables the identification of intraspecific variation for tolerance to calcareous soils.

# 5.2 The effects of increasing bicarbonate concentrations in nutrient solutions on the growth and chlorosis of four *Lupinus* species and *P. sativum* (Experiment 1)

## 5.2.1 Introduction

Bicarbonate can directly reduce root and shoot growth and/or induce nutrient deficiencies, particularly Fe, which results in chlorosis of the youngest leaves (Woolhouse, 1966b; Lee and Woolhouse, 1969; Coulombe, *et al.*, 1984a; Bertoni, *et al.*, 1992; Tang and Thomson, 1996) (Section 2.3). In calcareous soils the concentration of $HCO_3^-$ in soil solution can theoretically range from negligible to > 30 mM depending on the moisture content and physical and chemical characteristics of the soil (Fig. 2.3; Russell, 1988).

The concentration at which $HCO_3^-$ impairs growth or induces chlorosis depends on the tolerance of the species or individual genotypes. Work on soybeans (Chaney, *et al.*, 1992a) showed that 10-15 mM $HCO_3^-$ in solution culture caused significant Fe chlorosis in intolerant genotypes. Tang, *et al.* (1996b) also found that 5 mM impaired root elongation in *L. angustifolius*, *L. atlanticus*, *L. luteus* and *L. albus*, but not in *L. pilosus*, *L. cosentinii* and *P. sativum*. However, the effects of higher concentrations, more representative of calcareous soils, have not been investigated in lupins.

This experiment examined the effect of increasing $HCO_3^-$ concentrations in solution on the growth and chlorosis of four species of lupin differing in tolerance to calcareous soils.

## 5.2.2 Materials and methods

This experiment was conducted in two sub-experiments because of limited resources. In the first experiment, *L. angustifolius* and *L. pilosus* were compared (Experiment 1a) whilst in the second sub-experiment, *L. albus* and *L. atlanticus* were compared (Experiment 1b). Each experiment was statistically analysed separately, but results were compiled concurrently for ease of comparison between the sub-experiments.

*Genetic Material*

The genotype of each species used were:

*L. angustifolius* cv. Gungurru, *L. pilosus* P20954, *L. atlanticus* P22927 and *L. albus* cv. Kiev Mutant (see Table 6.1). A field pea cultivar (*P. sativum* cv. Alma) was grown in experiment 1a with Gungurru and P20954 as it is tolerant of calcareous soil.

*Container and solution preparation*

The experiments were conducted in five litre black plastic buckets (210 mm diametre) which had been sterilized by wiping the surfaces with 70% ethanol and soaking in 1 M $HNO_3$ for seven days to remove any nutritional contamination. The buckets had square plastic lids (250 x 250 mm) with 12 holes (27 mm diametre), into which a tapered cup fitted (Fig 5.1). The cups had the bases cut out and replaced with 2 mm diameter plastic mesh on which a single layer of muslin cloth was placed (Fig. 5.1). A plastic tube was inserted through a 10 mm diametre hole in the plastic lids to provide aeration via a silent flo 2000® aquarium pump.

Five litres of basal nutrient solution was required for each bucket (Table 5.1). All water used in solution experiments was purified by filtering through a milli-Q (MQ) system (Millipore corp.). Nine concentrations of $KHCO_3$ (0.2, 0.5, 1.0, 2.0, 5.0, 7.5, 10.0, 15.0, 20.0 mM) were compared with a control (0.0 mM). No treatments higher than 20 mM were used, as in preliminary studies plants died at higher concentrations. In the $HCO_3^-$ treatments, $CaCO_3$ was added to buffer the solution, for example, 0.05 g $L^{-1}$ $CaCO_3$ for the 0.5 mM $KHCO_3$ treatment, 0.1 g $L^{-1}$ $CaCO_3$ for the 1.0 mM $KHCO_3$ treatment (Tang and Thomson, 1996). In the control treatment 1 mM MES (2-[N-Morpholino]ethanesulfonic acid) was used to buffer the solution to pH 5.1 (Tang and Thomson, 1996).

*Plant growth and experimental design*

Prior to pregermination, seeds were surface sterilized (Section 4.2.2) and both *L. pilosus* and *L. atlanticus* genotypes were scarified (Section 3.2.4). Seeds of uniform size (for each genotype) were germinated for five days at 15-20$^{O}$C in the dark, on a plastic mesh (200 x 300 mm) covered with muslin cloth, suspended over an aerated solution containing 0.6 mM $CaCl_2$ and 5 µm $H_3BO_3$.

Eight seedlings of Gungurru and P20954 for experiment 1a, and P22927 and Kiev Mutant for experiment 1b were transferred to individual buckets according to figure 5.1 (holes 1-8). There

was only one major genotype per bucket to prevent interactions between roots of different species. In experiment 1a two seedlings of Alma were transferred to every bucket (holes 11 and 12; Fig. 5.1). To plant each seedling in the cup, the taproot was carefully fed through the mesh into the solution and then the cotyledons were covered with black plastic beads. Unused holes were covered to prevent light entering the solution. Buckets in each sub-experiment were arranged randomly.

Plants were grown in the aerated nutrient solutions for 28 days at constant temperature ($20^{O}C$) in a growth room under a 12 hour day/night regime (550-650 μmol $s^{-1}$ per uA). Nutrient solutions were changed weekly.

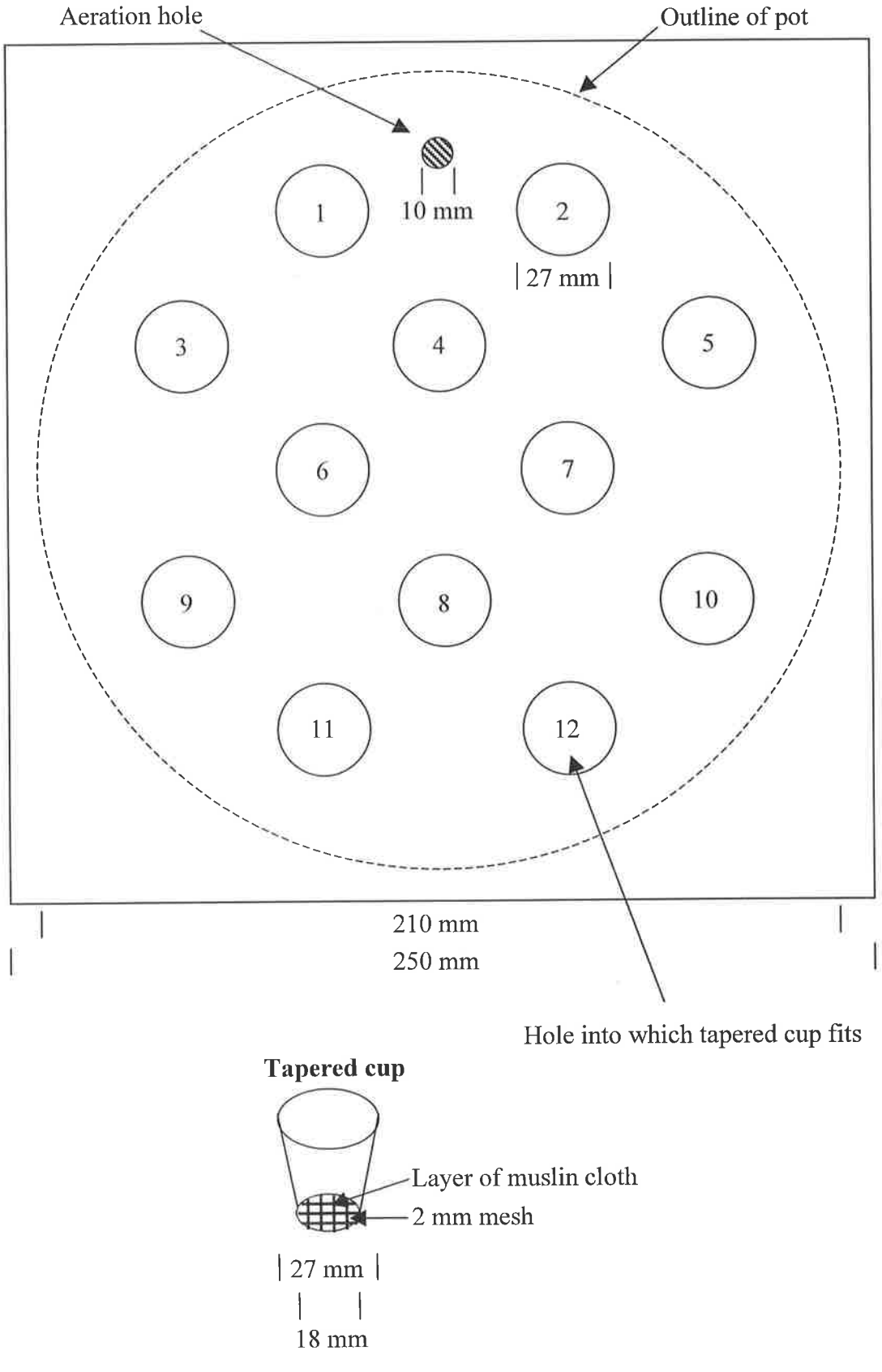
**Table 5.1** Concentrations of basal nutrients in solution culture experiments (Tang and Thomson, 1996).

| Nutrient | Solution μM |
|---|---|
| $CaCl_2.2H_2O$ | 2400 |
| $K_2SO_4$ | 2400 |
| $Ca(NO_3)_2.4H_2O$ | 1600 |
| $MgSO_4.4H_2O$ | 800 |
| $NH_4NO_3$[b] | 400 |
| $KH_2PO_4$ | 80 |
| FeEDDHA | 40 |
| $H_3BO_3$ | 20 |
| $MnSO_4.H_2O$ | 4 |
| $ZnSO_4.H_2O$ | 3 |
| $CoSO_4.7H_2O$ | 0.8 |
| $CuSO_4.5H_2O$ | 0.8 |
| $Na_2MoO_4.2H_2O$ | 0.12 |

*Measurements*

Solution pH was monitored throughout the experiments. Root length was recorded at transplanting and 2, 4 and 28 days after transplanting (DAT) and the root elongation rate (mm $hr^{-1}$) was calculated from 0-2, 0-4 and 2-4 DAT. At 28 DAT chlorosis score of the YEL (Section 3.2; Table 3.2) and number of leaves were recorded before the shoots and roots were harvested. The YELs and roots were washed in 0.1 M HCl for 30 seconds and rinsed in RO water for 60 seconds to remove surface contamination. All samples were dried at $80^{o}C$ for 48

**Figure 5.1** Diagramatic outline of lids and tapered cups used on buckets in experiment 1.



hours. Dry weights were recorded and the YEL and root samples were analysed for nutrient concentrations via ICP-AES.

*Statistical analyses*

As there was no formal replication in these experiments, statistical analysis were only provided as a guide to possible significant differences of measurements and need to be treated with caution. Also, raw data has been presented for growth and chlorosis measurements to provide an indication of the variation among individual plants. The analysis of variance (ANOVA; Genstat 5.41) was conducted using the individual plants within a pot as pseudoreplication. Individual means were compared using LSD. For experiment 1a Alma was omitted from the ANOVA because there was only two plants per pot.

### 5.2.3 Results

*Solution pH*

The solution pH was significantly related to $HCO_3^-$ concentration in both experiments ($r = 0.73$ and $r = 0.76$, in experiments 1a and 1b, respectively; $P < 0.05$). From 0 to 2 mM $KHCO_3$ the pH increased by more than 2 units and by less than 1 unit from 2 to 20 mM $HCO_3^-$ (Table 5.2). Generally in experiment 1a the pH was marginally greater than in 1b.

**Table 5.2** The pH of $KHCO_3$ solutions used in each sub-experiment.

| $KHCO_3$ concentration | Experiment 1a | 1b |
|---|---|---|
| 0.0 | 5.10 | 5.10 |
| 0.2 | 6.30 | 6.00 |
| 0.5 | 7.00 | 6.70 |
| 1.0 | 7.25 | 7.00 |
| 2.0 | 7.65 | 7.45 |
| 5.0 | 8.00 | 7.75 |
| 7.5 | 8.20 | 7.90 |
| 10.0 | 8.30 | 8.05 |
| 15.0 | 8.40 | 8.20 |
| 20.0 | 8.45 | 8.25 |

*Root elongation rate, chlorosis score, number of leaves, root length and shoot and root dry weight*

The ANOVA suggested that there was a two-way interaction between genotype and $HCO_3^-$ concentration for root elongation rate during 0-2 DAT ($P < 0.05$), 0-4 DAT ($P < 0.05$) and 2-4 DAT ($P < 0.05$) and chlorosis score ($P < 0.05$), root length ($P < 0.05$) and shoot dry weight ($P < 0.05$) at 28 DAT in both experiments. In experiment 1b root dry weights ($P < 0.05$) were also significantly different.

The root elongation rate during 0-2 DAT, 0-4 DAT and 2-4 DAT and root length at 28 DAT of P20954 was generally greater than Gungurru and Alma at all $KHCO_3$ concentrations in experiment 1a (Fig. 5.2), whilst in experiment 1b both Kiev Mutant and P22917 were similar (Fig. 5.3). For all genotypes in both experiments the root elongation rate was generally higher from 2-4 DAT than 0-2 DAT. As the $KHCO_3$ concentration increased the root elongation rates of Gungurru were reduced to less than 20% of the control at 20 mM $KHCO_3$ at all time intervals (Fig. 5.2). For P20954, during 0-2 DAT root elongation was greatest at 1 mM $KHCO_3$ and then decreased to 65% of the control in 20 mM $KHCO_3$. From 0-4 and 2-4 DAT increased $KHCO_3$ appeared to stimulate root elongation (Fig. 5.2). For Alma the addition of $KHCO_3$ had little effect on the root elongation rate (Fig. 5.2). For both Kiev Mutant and P22917 root elongation was variable from 0 to 5 mM $KHCO_3$ and appeared to decrease from 7.5 to 20 mM. (Fig. 5.3).

At 28 DAT in experiment 1a total root length of P20954 and Alma was less affected by increased $KHCO_3$ than Gungurru (Fig. 5.2). For example, at 20 mM total root length of Gungurru was reduced by 65% compared with the control whilst in P20954 and Alma the reduction was 20% and 25%, respectively. In experiment 1b both genotypes were similar (Fig. 5.3). For all concentrations from 5 to 20 mM $KHCO_3$ in both experiments, a brown staining was noted on the root surface of all genotypes, whilst in *Lupinus* species the roots also appeared thickened and stunted at high $KHCO_3$ and there was less branching as $KHCO_3$ was increased. On P20954 in experiment 1a and both genotypes in experiment 1b, increasing numbers of proteoid roots were observed at 7.5, 10, 15 and 20 mM $KHCO_3$. Gungurru does not form proteoid roots.

Chlorotic symptoms on the YEL of Gungurru, Kiev Mutant and P22917 were first noted 14 DAT in the 15 and 20 mM $KHCO_3$ treatments. By 21 DAT chlorosis was severe for Gungurru and moderate for Kiev Mutant and P22917 in the 15 and 20 mM. Symptoms were also notable on P20954 at 20 mM $KHCO_3$. At 28 DAT in experiment 1a the chlorosis score of both Gungurru

**Figure 5.2** The effect of increasing $KHCO_3$ concentrations in solution on the root elongation rate (mm $hr^{-1}$) from day 0 to day 2, day 0 to day 4, day 2 to day 4; and total root length (mm) at 28 days after transplanting of *L. angustifolius* cv. Gungurru, *L. pilous* P20954 and *P. sativum* cv. Alma. Bars represent LSD ($P < 0.05$).

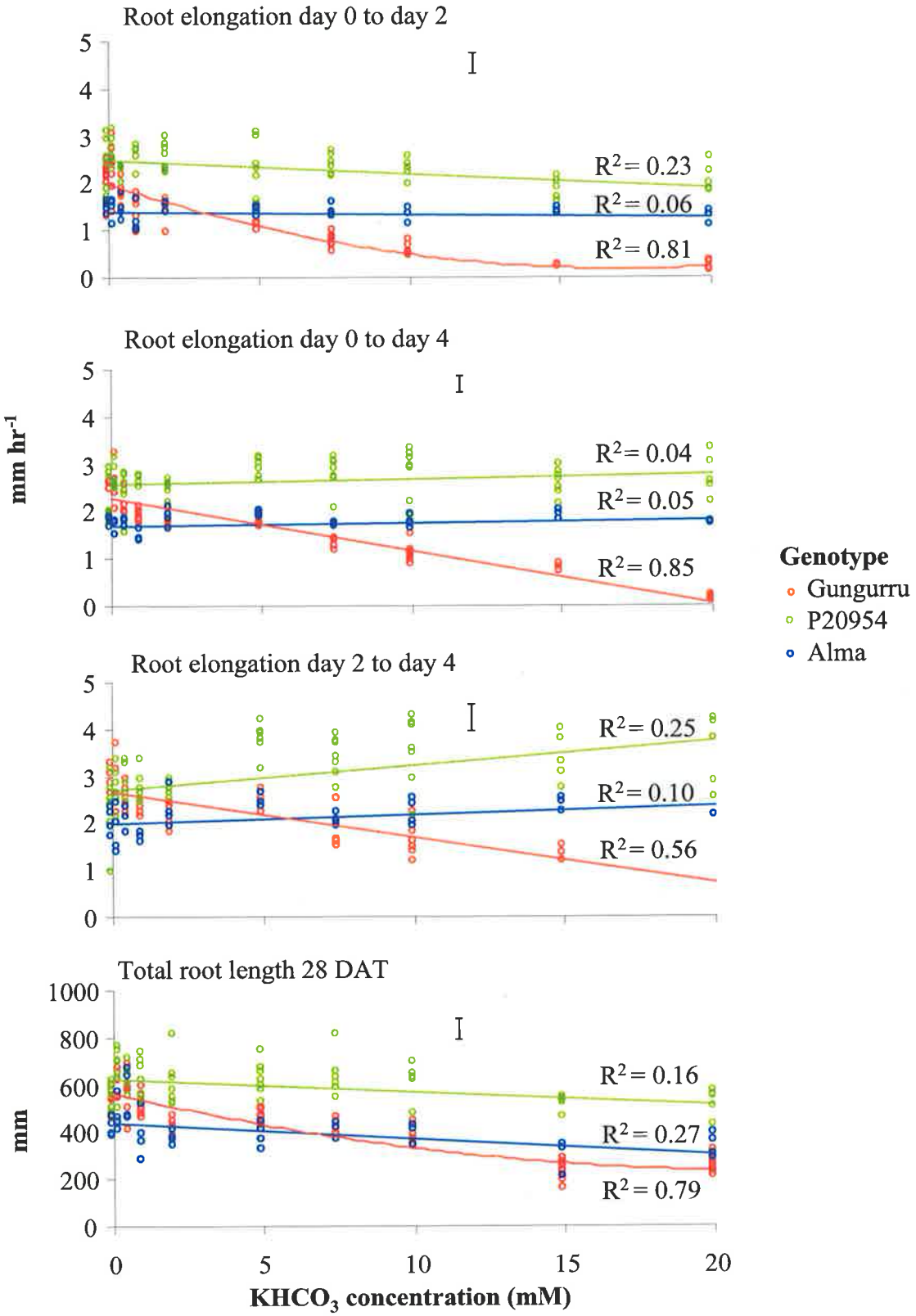
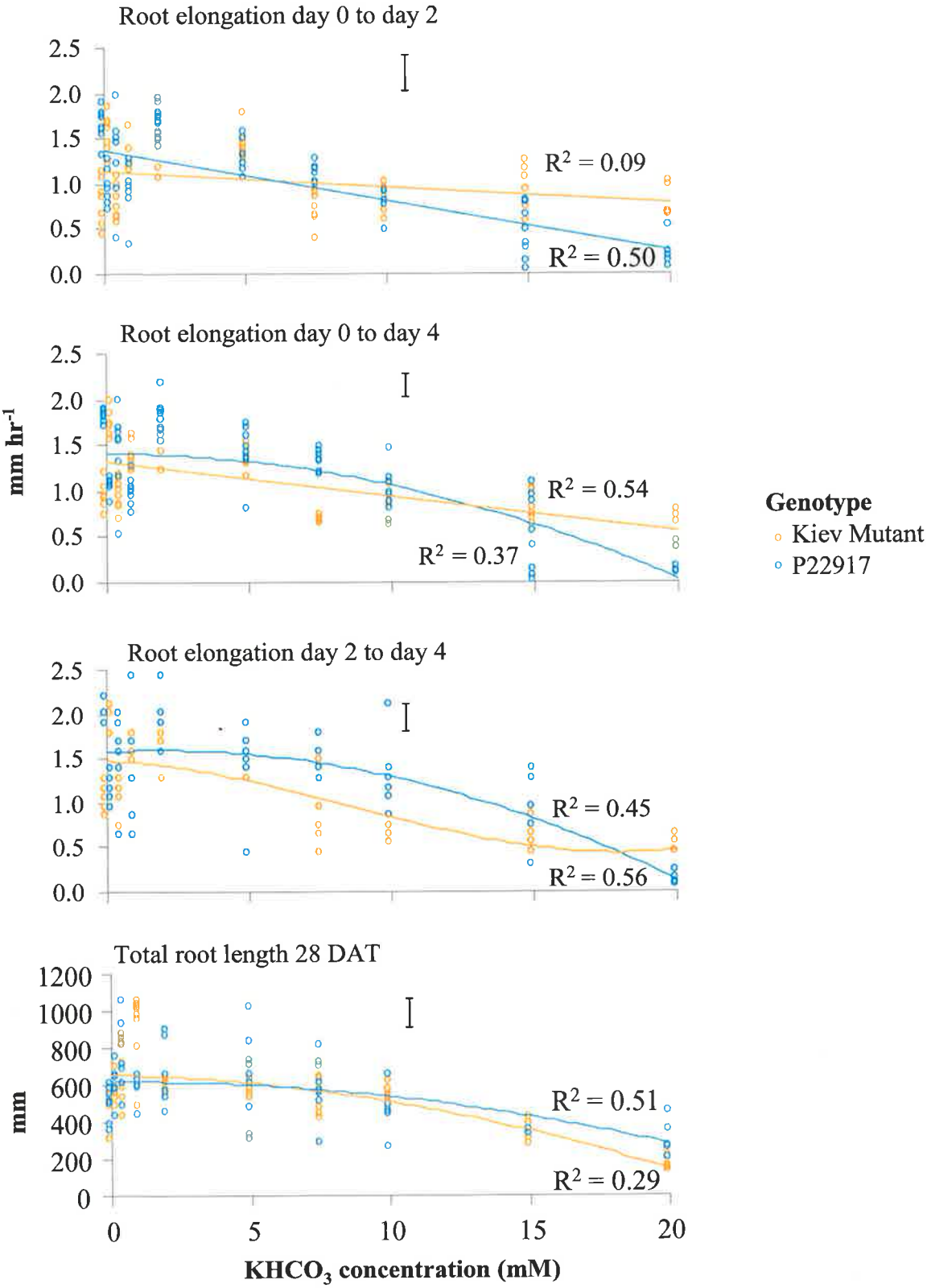


**Figure 5.3** The effect of increasing $KHCO_3$ concentrations in solution on the root elongation rate (mm $hr^{-1}$) from day 0 to day 2, day 0 to day 4, day 2 to day 4; and total root length (mm) at 28 days after transplanting of *L. albus* cv. Kiev Mutant and *L. atlanticus* P22917. Bars represent LSD ($P < 0.05$).



and P20954 increased proportionally with $KHCO_3$ concentrations above 5 mM (Fig. 5.4). Generally P20954 had a chlorosis score 1.5 units lower than that of Gungurru (Fig. 5.4). No chlorosis was noted on Alma. In experiment 1b, chlorosis scores in the YEL increased as $KHCO_3$ concentrations increased. P22917 showed slightly more severe chlorosis than Kiev Mutant at the lower concentrations (5 and 7.5 mM) and less severe at the higher concentrations (Fig. 5.4).

The number of leaves on Gungurru and P20954 in experiment 1a were unaffected with increased $KHCO_3$, whilst for Alma there was a large reduction compared with that at the lower $KHCO_3$ concentrations, particularly at 10, 15 and 20 mM $KHCO_3$ (Fig. 5.4). In experiment 1b the number of leaves on Kiev Mutant was unaffected by $KHCO_3$ up to 15 mM and decreased at the highest concentration, whilst that of P22917 decreased at $KHCO_3$ concentrations greater than 7.5 mM (Fig. 5.4).

Shoot and root dry weights of Gungurru, Kiev Mutant and P22917 were generally unaffected by 0.2 to 2 mM $KHCO_3$ in the solution, whilst in P20954 and Alma these concentrations appeared to slightly increase shoot and root growth (data not shown). At $KHCO_3$ concentrations above 2 mM, the shoot dry weight of all genotypes in both sub-experiments was decreased. In experiment 1a Alma showed the largest decrease in shoot weight as the $KHCO_3$ concentration increased from 2 to 20 mM $KHCO_3$ (1.3 to 0.3 g $plant^{-1}$) compared with Gungurru (0.7 to 0.3 g $plant^{-1}$) and P20954 (1.2 to 0.7 g $plant^{-1}$; Fig. 5.5). In experiment 1b Kiev Mutant showed the largest decrease in shoot weight as the $KHCO_3$ concentration increased from 2 to 20 mM $KHCO_3$ (1.3 to 0.4 g $plant^{-1}$) compared with P22917 (0.8 to 0.4 g $plant^{-1}$; Fig. 5.5).

Root dry weights from 2 to 20 mM $KHCO_3$ showed slightly different trends than the shoot dry weights. In experiment 1a the root dry weight of all genotypes in 2 mM $KHCO_3$ was 0.3 g $plant^{-1}$. As the $KHCO_3$ concentration increased Gungurru showed the largest reduction in root weight (0.30 to 0.15 g $plant^{-1}$) compared with Alma and P20954 (0.3 to 0.2 g $plant^{-1}$; Fig. 5.5). In the experiment 1b, the root weight of P22917 was reduced slightly from 0.25 to 0.15 g $plant^{-1}$ as $KHCO_3$ increased compared with Kiev Mutant, 0.65 to 0.10 g $plant^{-1}$ (Fig. 5.5).

Correlation coefficients indicate that in experiment 1a all measurements except chlorosis score were positively correlated for Gungurru (Table 5.3). The root elongation of P20954 during 0-2 DAT was negatively correlated with root elongation at 2-4 DAT and chlorosis score, and

**Figure 5.4** The effect of increasing $KHCO_3$ concentrations in solution on the chlorosis score and number of leaves at 28 days after transplanting of *L. angustifolius* cv. Gungurru, *L. pilosus* P20954, *P. sativum* cv. Alma, *L. albus* cv. Kiev Mutant and *L. atlanticus* P22917. For chlorosis score Alma was not shown as there was no chlorosis. Bars represent LSD ($P < 0.05$), n.s. - not significant.

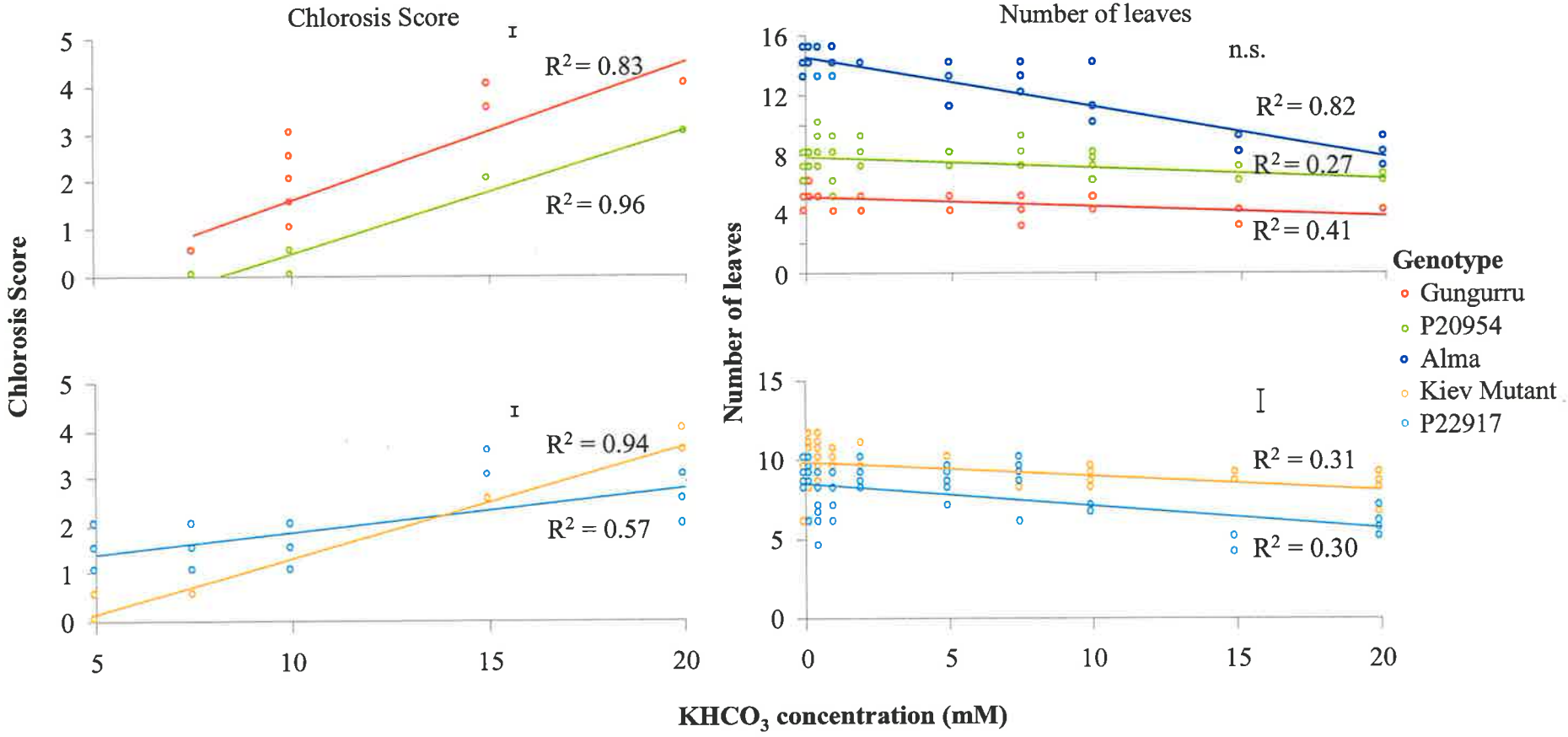
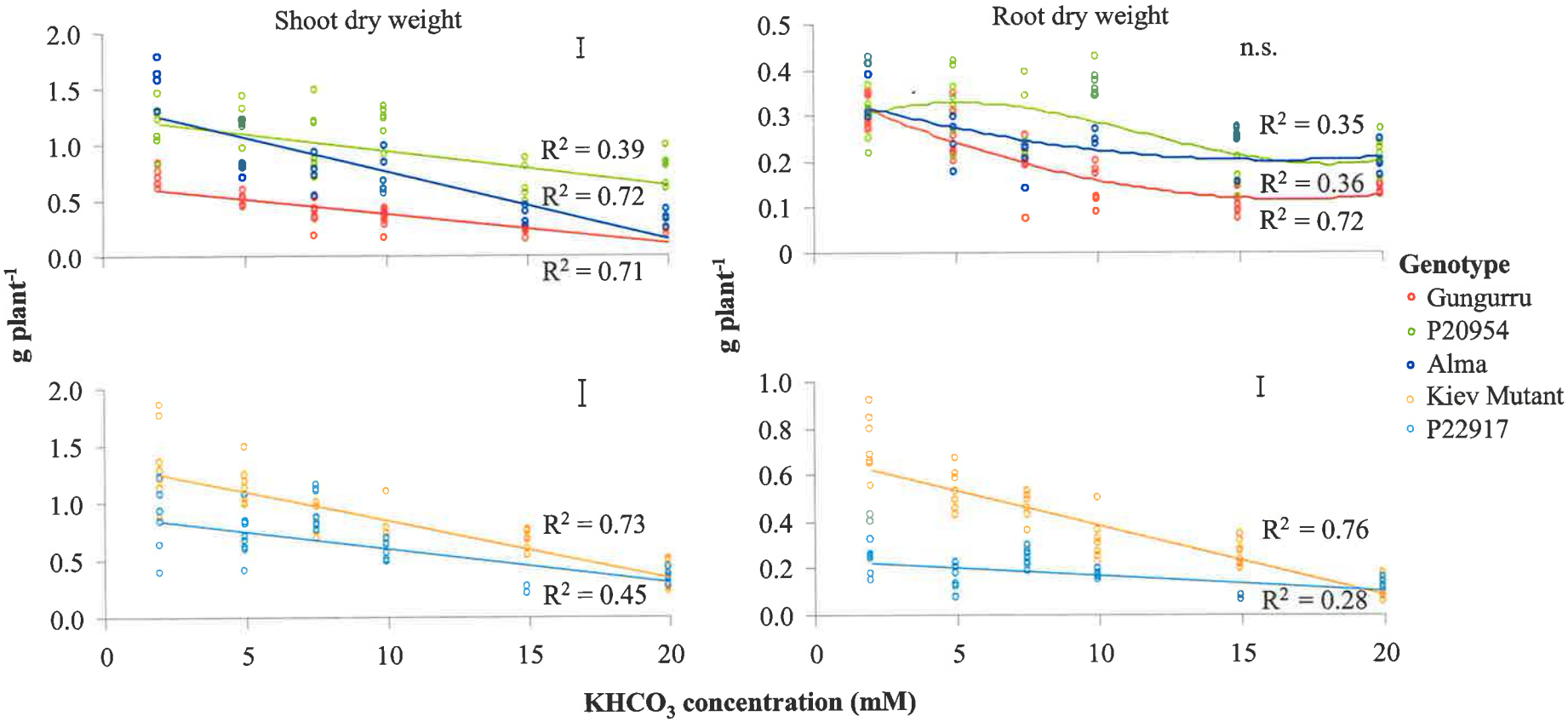


**Figure 5.5** The effect of 2 mM to 20 mM $KHCO_3$ in solution on the shoot and root dry weight at 28 days after transplanting of *L. angustifolius* cv. Gungurru, *L. pilosus* P20954, *P. sativum* cv. Alma, *L. albus* cv. Kiev Mutant and *L. atlanticus* P22917. Bars represent LSD ($P < 0.05$), n.s. - not significant.



positively correlated with final root length and root dry weight. Chlorosis score of P20954 correlated with final root length and shoot and root dry weight (Table 5.3). Root elongation of Alma during 0-2 DAT positively correlates with final root length and shoot dry weight (Table 5.3).

In experiment 1b all measurements except chlorosis score were positively correlated for P22917 (Table 5.3). For Kiev Mutant root elongation 2-4 DAT and root length, shoot dry weight, root dry weight and chlorosis score 28 DAT were all correlated. Root elongation 0-2 DAT only correlated with root elongation rates during 2-4 and 0-4 DAT, whilst root elongation 0-4 DAT correlated with root elongation 2-4 DAT and shoot and root dry weight and root length 28 DAT (Table 5.3).

*Nutrient concentrations*

In both experiments the ANOVA suggested that there was a two-way interaction between genotype and $HCO_3^-$ concentration for concentrations of all nutrients in the roots and shoots ($P < 0.05$) at 28 DAT, except Fe in the roots in experiment 1a. Averaged data was presented and correlation coefficients calculated to provide an indication of the relationship of a nutrient with increasing $KHCO_3$ concentrations in solution.

In experiment 1a, Fe concentrations in the roots were generally highest in the control or 0.2 mM $KHCO_3$ and decreased at higher $KHCO_3$ concentrations, however, there was only minor differences between all $KHCO_3$ concentrations above 1 mM (Table 5.4). Concentrations of Mn, Zn, Ca, Mg, Na and S in Gungurru and P20954 initially increased as $KHCO_3$ increased, but decreased at higher $KHCO_3$ concentrations (Table 5.4). Cu increased and P decreased with increasing $KHCO_3$ concentrations in solution, particularly for Gungurru. Mn, Cu, Zn, Ca and Mg in Alma increased whilst K, P and S decreased with increasing $KHCO_3$ (Table 5.4).

In experiment 1b, the concentration of Fe, Zn, Na and P in the roots of Kiev Mutant decreased, whilst Mn, B, Cu, Ca, Mg, and K increased as $KHCO_3$ concentrations increased (Table 5.5). Phosphorus concentrations decreased at low $KHCO_3$ concentrations and increased at high concentrations. For P22917, Fe, Zn and P decreased and Mn and Ca increased as the $KHCO_3$ concentration increased. Mg and K were increased at low $KHCO_3$ concentrations and decreased at high $KHCO_3$ concentrations (Table 5.5).

**Table 5.3** Correlation coefficients (r) between root elongation rate (0-2 days, 0-4 days and 2-4 days after transplanting) and root length (RL), shoot dry weight (SDW), root dry weight (RDW), visual chlorosis score (CS) and number of leaves (NL) of *L. angustifolius* cv. Gungurru, *L. pilosus* P20954, *P. sativum* cv. Alma, *L. albus* cv. Kiev Mutant and *L. atlanticus* P22917 after 28 days growth in nutrient solutions containing 0 to 20 mM $KHCO_3$.

| | RG0,2 | RG0,4 | RG2,4 | RL | SDW | RDW | CS |
|---|---|---|---|---|---|---|---|
| | | | *Gungurru* | | | | |
| **RG0,4** | 0.96** | | | | | | |
| **RG2,4** | 0.88** | 0.97** | | | | | |
| **RL** | 0.96** | 0.94** | 0.87** | | | | |
| **SDW** | 0.94** | 0.91** | 0.83** | 0.86** | | | |
| **RDW** | 0.85** | 0.80** | 0.72* | 0.78** | 0.96** | | |
| **CS** | -0.85** | -0.90** | -0.89** | -0.88** | -0.85** | -0.80** | |
| **NL** | 0.82** | 0.83** | 0.80** | 0.87** | 0.75* | 0.72* | -0.92** |
| | | | *P20954* | | | | |
| **RG0,4** | 0.01 | | | | | | |
| **RG2,4** | -0.72* | 0.68* | | | | | |
| **RL** | 0.78** | 0.32 | -0.35 | | | | |
| **SDW** | 0.62 | -0.08 | -0.50 | 0.76* | | | |
| **RDW** | 0.66* | -0.01 | -0.49 | 0.80** | 0.96** | | |
| **CS** | -0.77** | 0.08 | 0.62 | -0.85** | -0.71* | -0.82** | |
| **NL** | 0.57 | -0.16 | -0.52 | 0.65* | 0.92** | 0.92** | -0.71* |
| | | | *Alma* | | | | |
| **RG0,4** | 0.81** | | | | | | |
| **RG2,4** | 0.62 | 0.96** | | | | | |
| **RL** | 0.66* | 0.35 | 0.16 | | | | |
| **SDW** | 0.65* | 0.30 | 0.10 | 0.63* | | | |
| **RDW** | 0.38 | 0.13 | -0.01 | 0.29 | 0.87** | | |
| **CS** | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| **NL** | 0.65* | 0.22 | -0.01 | 0.71* | 0.86** | 0.64* | 0.00 |
| | | | *Kiev Mutant* | | | | |
| **RG0,4** | 0.88** | | | | | | |
| **RG2,4** | 0.70* | 0.96** | | | | | |
| **RL** | 0.10 | 0.51 | 0.70* | | | | |
| **SDW** | 0.46 | 0.80** | 0.92** | 0.69* | | | |
| **RDW** | 0.46 | 0.78** | 0.88** | 0.60 | 0.96** | | |
| **CS** | -0.21 | -0.61 | -0.78** | -0.82** | -0.87** | -0.89** | |
| **NL** | 0.37 | 0.70* | 0.82** | 0.81** | 0.86** | 0.72* | -0.77** |
| | | | *P22917* | | | | |
| **RG0,4** | 0.98** | | | | | | |
| **RG2,4** | 0.93** | 0.99** | | | | | |
| **RL** | 0.74* | 0.75* | 0.73* | | | | |
| **SDW** | 0.74* | 0.76* | 0.76* | 0.72* | | | |
| **RDW** | 0.65* | 0.67* | 0.66* | 0.65* | 0.92** | | |
| **CS** | -0.77** | -0.73* | -0.68* | -0.74* | -0.71* | -0.79** | |
| **NL** | 0.84** | 0.83** | 0.80** | 0.69* | 0.95** | 0.84** | -0.75* |

* $P < 0.05$, ** $P < 0.01$

**Table 5.4** Nutrient concentrations (mg $kg^{-1}$) in roots of *L. angustifolius* cv. Gungurru, *L. pilosus* P20954 and *P. sativum* cv. Alma after 28 days growth in nutrient solutions containing 0 to 20 mM $KHCO_3$.

| $KHCO_3$ concentration | Fe | Mn | B | Cu | Zn | Ca | Mg | Na | K | P | S |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | *Gungurru* | | | | | |
| 0.0 | 503 | 257 | 24 | 30 | 336 | 9537 | 8230 | 1879 | 67638 | 6032 | 45728 |
| 0.2 | 280 | 536 | 22 | 26 | 482 | 12246 | 18811 | 1653 | 90472 | 3472 | 68081 |
| 0.5 | 305 | 797 | 23 | 37 | 751 | 14676 | 18705 | 1734 | 84075 | 4593 | 70863 |
| 1.0 | 294 | 798 | 25 | 35 | 653 | 11599 | 16549 | 2101 | 90391 | 3978 | 62979 |
| 2.0 | 243 | 561 | 23 | 31 | 480 | 25621 | 28456 | 1771 | 98438 | 3005 | 68262 |
| 5.0 | 246 | 667 | 22 | 51 | 448 | 33345 | 21280 | 1612 | 94267 | 3039 | 55651 |
| 7.5 | 255 | 511 | 21 | 59 | 265 | 24462 | 16978 | 1174 | 99500 | 2291 | 29466 |
| 10.0 | 309 | 454 | 25 | 76 | 220 | 22943 | 14465 | 1592 | 101214 | 1869 | 23380 |
| 15.0 | 392 | 242 | 25 | 70 | 275 | 12188 | 8351 | 1695 | 119921 | 2097 | 16297 |
| 20.0 | 287 | 117 | 26 | 61 | 188 | 16373 | 8838 | 1426 | 131159 | 1463 | 10344 |
| Correl. coeff.[1] | -0.04 | -0.70* | 0.41 | 0.82** | -0.74* | 0.09 | -0.54 | -0.53 | 0.92** | -0.80** | -0.91 |
| | | | | | | *P20954* | | | | | |
| 0.0 | 475 | 90 | 28 | 52 | 391 | 4761 | 1433 | 4006 | 56645 | 4861 | 13957 |
| 0.2 | 559 | 537 | 28 | 46 | 693 | 6684 | 3452 | 9192 | 67889 | 3915 | 19354 |
| 0.5 | 418 | 402 | 24 | 63 | 561 | 6568 | 4944 | 7031 | 73335 | 5942 | 18722 |
| 1.0 | 406 | 877 | 30 | 55 | 907 | 5011 | 5064 | 9127 | 94028 | 4605 | 23362 |
| 2.0 | 344 | 695 | 30 | 53 | 634 | 11886 | 5736 | 8540 | 107871 | 4249 | 30055 |
| 5.0 | 341 | 840 | 27 | 61 | 580 | 12958 | 5625 | 7380 | 116382 | 4653 | 28261 |
| 7.5 | 360 | 744 | 30 | 63 | 454 | 9990 | 6958 | 7404 | 127446 | 3532 | 27766 |
| 10.0 | 324 | 599 | 25 | 72 | 370 | 14328 | 6563 | 5195 | 126390 | 3563 | 18156 |
| 15.0 | 499 | 899 | 31 | 91 | 404 | 17551 | 5579 | 4957 | 113085 | 3346 | 11759 |
| 20.0 | 440 | 216 | 31 | 95 | 323 | 17946 | 4824 | 4663 | 113553 | 2557 | 9475 |
| Correl. coeff. | -0.03 | 0.00 | 0.37 | 0.95** | -0.66* | 0.90** | 0.39 | -0.58 | 0.65* | -0.82** | -0.52 |
| LSD[1] ($P < 0.05$) | n.s. | 341 | 4 | 12 | 142 | 3955 | 2686 | 1074 | 15380 | 759 | 13148 |
| | | | | | | Alma | | | | | |
| 0.0 | 931 | 551 | 37 | 48 | 282 | 9918 | 2706 | 1092 | 147044 | 5960 | 49999 |
| 0.2 | 1104 | 1232 | 32 | 34 | 512 | 8593 | 4747 | 967 | 135648 | 4545 | 34018 |
| 0.5 | 735 | 1452 | 30 | 62 | 574 | 9750 | 5358 | 1022 | 112936 | 5280 | 36109 |
| 1.0 | 583 | 1581 | 32 | 57 | 683 | 8673 | 7981 | 1286 | 153563 | 5171 | 50941 |
| 2.0 | 457 | 999 | 33 | 50 | 528 | 12047 | 9077 | 1231 | 153455 | 4570 | 44599 |
| 5.0 | 388 | 957 | 33 | 84 | 737 | 24820 | 9905 | 1080 | 154785 | 5092 | 54738 |
| 7.5 | 497 | 1658 | 36 | 102 | 1074 | 14682 | 8509 | 803 | 134706 | 4313 | 31058 |
| 10.0 | 459 | 1692 | 36 | 96 | 966 | 13138 | 9831 | 905 | 138746 | 4236 | 22308 |
| 15.0 | 539 | 1511 | 37 | 91 | 662 | 16655 | 9788 | 1232 | 113034 | 3225 | 9227 |
| 20.0 | 555 | 1803 | 41 | 107 | 713 | 18653 | 9535 | 1081 | 95125 | 2695 | 6889 |
| Correl. coeff. | -0.46 | 0.61 | 0.79** | 0.85** | 0.46 | 0.58 | 0.65* | -0.05 | -0.69* | -0.91** | -0.86** |

[1] Correlation coefficients between nutrient concentrations and $KHCO_3$ concentration. *$P < 0.05$, ** $P < 0.01$

[2] Interaction effect significant ($P < 0.01$) for all nutrient concentrations. LSD only for comparison of Gungurru and P20954 as Alma was omitted from the analysis.

**Table 5.5** Nutrient concentrations (mg $kg^{-1}$) in roots of *L. albus* cv. Kiev Mutant and *L. atlanticus* P22917 after 28 days growth in nutrient solutions containing 0 to 20 mM $KHCO_3$.

| $KHCO_3$ concentration | Fe | Mn | B | Cu | Zn | Ca | Mg | Na | K | P | S |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | *Kiev Mutant* | | | | | |
| 0.0 | 324 | 14 | 22 | 19 | 297 | 7276 | 5302 | 4801 | 72925 | 3888 | 16748 |
| 0.2 | 247 | 16 | 22 | 18 | 265 | 7018 | 7907 | 3463 | 71480 | 2695 | 16355 |
| 0.5 | 253 | 20 | 23 | 22 | 294 | 7543 | 8568 | 2838 | 73801 | 2880 | 17754 |
| 1.0 | 247 | 42 | 21 | 21 | 303 | 7392 | 9955 | 1869 | 63774 | 2705 | 14994 |
| 2.0 | 224 | 21 | 21 | 19 | 237 | 11272 | 7505 | 2492 | 81293 | 2728 | 16590 |
| 5.0 | 244 | 19 | 20 | 19 | 200 | 30140 | 9869 | 1571 | 74223 | 2672 | 12465 |
| 7.5 | 289 | 36 | 21 | 22 | 193 | 51396 | 7921 | 1532 | 76383 | 3153 | 11522 |
| 10.0 | 349 | 49 | 27 | 33 | 168 | 67549 | 9276 | 2014 | 80979 | 3243 | 9675 |
| 15.0 | 313 | 69 | 28 | 58 | 220 | 70904 | 7151 | 1854 | 93443 | 3404 | 7597 |
| 20.0 | 303 | 69 | 30 | 83 | 203 | 78574 | 3049 | 1739 | 107564 | 3148 | 9685 |
| Correl. coeff.[1] | 0.54 | 0.90** | 0.85** | 0.93** | -0.69* | 0.96** | -0.51 | -0.57 | 0.91** | 0.28 | -0.90** |
| | | | | | | *P22917* | | | | | |
| 0.0 | 504 | 112 | 33 | 69 | 431 | 11113 | 3257 | 4323 | 106102 | 4067 | 29891 |
| 0.2 | 447 | 284 | 30 | 61 | 299 | 15369 | 3931 | 4945 | 101750 | 3441 | 32396 |
| 0.5 | 424 | 452 | 29 | 68 | 360 | 13311 | 4963 | 5409 | 123004 | 4397 | 62018 |
| 1.0 | 467 | 557 | 33 | 85 | 439 | 14038 | 4265 | 5608 | 124988 | 4641 | 55130 |
| 2.0 | 301 | 346 | 29 | 47 | 242 | 15524 | 7000 | 5350 | 128079 | 4433 | 38193 |
| 5.0 | 365 | 492 | 29 | 72 | 280 | 99070 | 3395 | 4852 | 101086 | 3811 | 25263 |
| 7.5 | 323 | 440 | 25 | 57 | 260 | 63933 | 3802 | 3881 | 109696 | 3431 | 19229 |
| 10.0 | 345 | 476 | 32 | 65 | 221 | 145537 | 3915 | 4227 | 118519 | 3462 | 25341 |
| 15.0 | 841 | 280 | 50 | 212 | 403 | 199449 | 1695 | 3653 | 67712 | 2817 | 6978 |
| 20.0 | 484 | 205 | 30 | n.d.[3] | n.d. | n.d. | 2209 | 4210 | 99665 | 2080 | n.d. |
| Correl. coeff. | 0.39 | -0.22 | 0.37 | 0.70* | -0.19 | 0.96** | -0.62* | -0.70* | -0.57 | -0.89** | -0.76* |
| LSD[2] ($P < 0.05$) | 69 | 109 | 5 | 9 | 59 | 31430 | 1492 | 753 | 13322 | 516 | 9090 |

[1] Correlation coefficients between nutrient concentrations and $KHCO_3$ concentration. *$P < 0.05$, ** $P < 0.01$

[2] Interaction effect significant ($P < 0.01$) for all nutrient concentrations.

[3] n.d. - not determined

In the YEL, concentrations of Fe, Zn, P and S were generally decreased as $KHCO_3$ increased for all genotypes in experiment 1a, whilst Ca and Mg increased at low concentrations and decreased at high concentrations (Table 5.6). Mn in Gungurru and P20954, but not Alma was only slightly decreased at the highest concentrations. P20954 was generally able to maintain higher concentrations of Fe and Mn than Gungurru (Table 5.6). In experiment 1b the concentration Fe of in the YEL of Kiev Mutant decreased, whilst Mn, Ca, Mg, Na, K, and S increased as $KHCO_3$ increased (Table 5.7). For P22917, Fe, Mn, B, Zn, P and S decreased and K increased as the $KHCO_3$ concentration increased (Table 5.7).

Root and shoot growth measurements and chlorosis scores of Gungurru and P20954 in experiment 1a and both genotypes in the experiment 1b were correlated with a range nutrient concentrations in the YEL (Table 5.8). For Alma root elongation did not correlate with any nutrient. Root length and shoot dry weight 28 DAT positively correlated with Fe concentrations in the YEL, whilst shoot dry weight also positively correlated with Zn and Ca concentrations. Root dry weight positively correlated with Ca concentrations (Table 5.8).

**Table 5.6** Nutrient concentrations (mg $kg^{-1}$) in youngest emerging leaves of *L. angustifolius* cv. Gungurru, *L. pilosus* P20954 and *P. sativum* cv. Alma after 28 days growth in nutrient solutions containing 0 to 20 mM $KHCO_3$.

| $KHCO_3$ concentration | Fe | Mn | B | Cu | Zn | Ca | Mg | Na | K | P | S |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | *Gungurru* | | | | | |
| 0.0 | 155 | 100 | 37 | 9 | 105 | 19999 | 2746 | 329 | 31517 | 4254 | 8325 |
| 0.2 | 59 | 92 | 36 | 6 | 139 | 20705 | 3939 | 288 | 39858 | 3829 | 7829 |
| 0.5 | 58 | 119 | 37 | 7 | 148 | 24736 | 4350 | 329 | 43467 | 5428 | 11753 |
| 1.0 | 58 | 103 | 40 | 6 | 124 | 25176 | 4044 | 361 | 47490 | 5458 | 12679 |
| 2.0 | 51 | 87 | 38 | 5 | 91 | 26737 | 3664 | 316 | 50204 | 3829 | 11657 |
| 5.0 | 43 | 128 | 40 | 5 | 108 | 25021 | 4788 | 324 | 56939 | 4126 | 10450 |
| 7.5 | 42 | 100 | 33 | 8 | 95 | 20883 | 4463 | 313 | 58268 | 2991 | 5655 |
| 10.0 | 39 | 94 | 33 | 8 | 83 | 26749 | 4150 | 358 | 68746 | 2830 | 5787 |
| 15.0 | 39 | 51 | 31 | 8 | 56 | 13580 | 3169 | 350 | 62469 | 2091 | 5223 |
| 20.0 | 33 | 37 | 28 | 7 | 46 | 9661 | 2577 | 366 | 65033 | 1595 | 4500 |
| Correl. coeff.[1] | -0.53 | -0.79** | -0.87** | 0.23 | -0.89** | -0.73* | -0.42 | 0.59 | 0.82** | -0.89** | -0.78** |
| | | | | | | *P20954* | | | | | |
| 0.0 | 200 | 138 | 34 | 14 | 170 | 11842 | 3182 | 439 | 44280 | 3863 | 5054 |
| 0.2 | 85 | 172 | 26 | 9 | 165 | 10641 | 2731 | 863 | 35569 | 2687 | 3833 |
| 0.5 | 47 | 127 | 30 | 8 | 126 | 14330 | 2887 | 568 | 41993 | 3119 | 3887 |
| 1.0 | 54 | 194 | 34 | 10 | 199 | 14932 | 3640 | 669 | 51802 | 3535 | 4144 |
| 2.0 | 46 | 136 | 34 | 8 | 160 | 15406 | 3551 | 693 | 51514 | 3398 | 3987 |
| 5.0 | 40 | 166 | 31 | 8 | 149 | 15086 | 3775 | 726 | 57393 | 3733 | 4001 |
| 7.5 | 42 | 169 | 31 | 7 | 131 | 16310 | 4048 | 1009 | 69181 | 3020 | 3770 |
| 10.0 | 34 | 154 | 23 | 6 | 88 | 16777 | 3692 | 806 | 64859 | 2323 | 3108 |
| 15.0 | 40 | 99 | 24 | 6 | 62 | 14754 | 3714 | 1012 | 88150 | 1855 | 2765 |
| 20.0 | 38 | 108 | 23 | 7 | 63 | 12132 | 3657 | 915 | 91315 | 1558 | 2666 |
| Correl. coeff. | -0.45 | -0.58 | -0.73* | -0.66* | -0.89** | 0.11 | 0.55 | 0.69* | 0.96** | -0.85** | -0.87** |
| LSD[2] ($P < 0.05$) | 16 | 25 | 3 | 1 | 17 | 3040 | 393 | 205 | 5566 | 434 | 1309 |
| | | | | | | *Alma* | | | | | |
| 0.0 | 179 | 41 | 33 | 8 | 99 | 14157 | 1716 | 337 | 86166 | 5102 | 4757 |
| 0.2 | 289 | 43 | 28 | 6 | 107 | 15013 | 2482 | 286 | 77478 | 3305 | 4495 |
| 0.5 | 217 | 51 | 27 | 7 | 141 | 16071 | 2002 | 261 | 80076 | 4815 | 5253 |
| 1.0 | 175 | 52 | 28 | 9 | 147 | 17794 | 2072 | 287 | 90609 | 4661 | 6592 |
| 2.0 | 117 | 60 | 30 | 7 | 131 | 16673 | 2257 | 272 | 88597 | 3571 | 6982 |
| 5.0 | 67 | 100 | 37 | 9 | 146 | 17945 | 2572 | 333 | 101478 | 5659 | 7595 |
| 7.5 | 45 | 112 | 31 | 6 | 123 | 12772 | 2565 | 228 | 77792 | 4164 | 4204 |
| 10.0 | 45 | 110 | 35 | 7 | 95 | 13572 | 2844 | 289 | 85630 | 3902 | 3780 |
| 15.0 | 45 | 64 | 40 | 7 | 51 | 8574 | 2155 | 334 | 84071 | 2217 | 2851 |
| 20.0 | 48 | 70 | 44 | 8 | 43 | 6957 | 2146 | 360 | 100170 | 1778 | 2806 |
| Correl. coeff. | -0.76* | 0.41 | 0.88** | 0.16 | -0.81** | -0.87** | 0.20 | 0.47 | 0.38 | -0.74* | -0.68* |

[1] Correlation coefficients between nutrient concentrations and $KHCO_3$ concentration. *$P < 0.05$, ** $P < 0.01$

[2] Interaction effect significant ($P < 0.05$) for all nutrient concentrations. LSD only for comparison of Gungurru and P20954 as Alma was omitted from the analysis.

**Table 5.7** Nutrient concentrations (mg $kg^{-1}$) in youngest emerging leaves of *L. albus* cv. Kiev Mutant and *L. atlanticus* P22917 after 28 days growth in nutrient solutions containing 0 to 20 mM $KHCO_3$.

| $KHCO_3$ concentration | Fe | Mn | B | Cu | Zn | Ca | Mg | Na | K | P | S |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | *Kiev Mutant* | | | | | |
| 0.0 | 111 | 163 | 36 | 4 | 47 | 10486 | 1314 | 231 | 53192 | 2598 | 2185 |
| 0.2 | 86 | 160 | 34 | 4 | 52 | 10882 | 1368 | 186 | 58683 | 2138 | 1902 |
| 0.5 | 63 | 183 | 34 | 3 | 53 | 12178 | 1588 | 178 | 62515 | 2285 | 1840 |
| 1.0 | 54 | 202 | 35 | 2 | 64 | 12587 | 1937 | 226 | 71999 | 2352 | 2081 |
| 2.0 | 38 | 147 | 32 | 2 | 46 | 12571 | 1424 | 185 | 62602 | 1747 | 1762 |
| 5.0 | 39 | 219 | 38 | 2 | 58 | 14358 | 1749 | 257 | 90307 | 2051 | 2282 |
| 7.5 | 39 | 247 | 41 | 2 | 58 | 15852 | 2207 | 307 | 102244 | 2330 | 2619 |
| 10.0 | 35 | 282 | 40 | 3 | 58 | 16990 | 2272 | 368 | 123730 | 2208 | 2505 |
| 15.0 | 28 | 286 | 40 | 3 | 51 | 14835 | 2228 | 344 | 127681 | 2146 | 2375 |
| 20.0 | 27 | 168 | 36 | 5 | 36 | 5465 | 1885 | 391 | 102426 | 2172 | 3485 |
| Correl. coeff.[1] | -0.69** | 0.46 | 0.51 | 0.49 | -0.41 | -0.17 | 0.65* | 0.92** | 0.82** | -0.14 | 0.87** |
| | | | | | | *P22917* | | | | | |
| 0.0 | 61 | 179 | 38 | 8 | 144 | 38343 | 4921 | 337 | 50406 | 3805 | 12586 |
| 0.2 | 44 | 126 | 36 | 7 | 125 | 47676 | 5186 | 268 | 49043 | 3092 | 10271 |
| 0.5 | 24 | 87 | 34 | 5 | 115 | 42139 | 5501 | 370 | 56962 | 3189 | 10358 |
| 1.0 | 17 | 102 | 37 | 6 | 120 | 39679 | 5692 | 375 | 58815 | 3804 | 10460 |
| 2.0 | 26 | 98 | 36 | 5 | 113 | 39230 | 5540 | 294 | 69791 | 2914 | 8874 |
| 5.0 | 22 | 149 | 35 | 6 | 98 | 38056 | 6619 | 362 | 89442 | 2916 | 8447 |
| 7.5 | 31 | 120 | 30 | 6 | 80 | 36847 | 7010 | 320 | 98698 | 2084 | 6043 |
| 10.0 | 25 | 141 | 30 | 5 | 67 | 52483 | 8023 | 327 | 114977 | 2238 | 6335 |
| 15.0 | 42 | 62 | 26 | 10 | 41 | 23276 | 5209 | 304 | 128187 | 1120 | 4994 |
| 20.0 | 30 | 33 | 29 | 7 | 39 | 16568 | 5674 | 401 | 165446 | 1252 | 5346 |
| Correl. coeff. | -0.07 | -0.63* | -0.87** | 0.38 | -0.96** | -0.71* | 0.24 | 0.27 | 0.99** | -0.93** | -0.89** |
| LSD[1] ($P < 0.05$) | 11 | 42 | 5 | 1 | 12 | 8358 | 491 | 53 | 9496 | 464 | 1889 |

[1] Correlation coefficients between nutrient concentrations and $KHCO_3$ concentration. $*P < 0.05$, $** P < 0.01$

[2] Interaction effect significant ($P < 0.05$) for all nutrient concentrations.

**Table 5.8** Correlation coefficients (r) between nutrient concentrations in the YEL 21 days after transplanting and root elongation rate (RG)(0-2 days, 0-4 days and 2-4 days after transplanting), and root length (RL), shoot dry weight (SDW), root dry weight (RDW), visual chlorosis score (CS) and number of leaves (NL) of *L. angustifolius* cv. Gungurru, *L. pilosus* P20954, *P. sativum* cv. Alma, *L. albus* cv. Kiev Mutant and *L. atlanticus* P22917 after 28 days growth in nutrient solutions containing 0 to 20 mM $KHCO_3$.

| | RG0,2 | RG0,4 | RG2,4 | RL | SDW | RDW | CS | NL |
|---|---|---|---|---|---|---|---|---|
| | | | | *Gungurru* | | | | |
| **Fe** | 0.64* | 0.62 | 0.57 | 0.51 | 0.50 | 0.31 | -0.41 | 0.42 |
| **Mn** | 0.62 | 0.75* | 0.82** | 0.73* | 0.60 | 0.54 | -0.88** | 0.76* |
| **B** | 0.71* | 0.80** | 0.82** | 0.73* | 0.76* | 0.65* | -0.88** | 0.65* |
| **Cu** | -0.23 | -0.16 | -0.09 | -0.21 | -0.47 | -0.60 | 0.29 | -0.10 |
| **Zn** | 0.84** | 0.87** | 0.84** | 0.94** | 0.74* | 0.66* | -0.85** | 0.80** |
| **Ca** | 0.48 | 0.60 | 0.67* | 0.57 | 0.59 | 0.55 | -0.80** | 0.64* |
| **Mg** | 0.18 | 0.33 | 0.45 | 0.37 | 0.23 | 0.31 | -0.58 | 0.50 |
| **Na** | -0.65* | -0.67* | -0.65* | -0.61 | -0.70* | -0.80** | 0.61 | -0.67* |
| **K** | -0.93** | -0.88** | -0.79** | -0.84** | -0.82** | -0.66* | 0.68* | -0.67* |
| **P** | 0.76* | 0.81** | 0.80** | 0.83** | 0.70* | 0.54 | -0.84** | 0.68* |
| **S** | 0.62 | 0.65* | 0.64* | 0.64* | 0.70* | 0.58 | -0.75* | 0.48 |
| | | | | *P20954* | | | | |
| **Fe** | 0.31 | -0.61 | -0.65* | -0.07 | 0.03 | 0.04 | -0.25 | -0.03 |
| **Mn** | 0.81** | 0.47 | -0.26 | 0.84** | 0.45 | 0.57 | -0.71* | 0.38 |
| **B** | 0.69* | -0.34 | -0.73* | 0.35 | 0.29 | 0.41 | -0.65* | 0.53 |
| **Cu** | 0.62 | -0.50 | -0.79** | 0.11 | 0.18 | 0.23 | -0.41 | 0.18 |
| **Zn** | 0.96** | -0.12 | -0.79** | 0.66* | 0.56 | 0.63* | -0.77** | 0.59 |
| **Ca** | -0.14 | 0.30 | 0.31 | 0.18 | -0.14 | 0.04 | -0.25 | 0.06 |
| **Mg** | -0.31 | 0.51 | 0.58 | -0.20 | -0.67* | -0.53 | 0.24 | -0.48 |
| **Na** | -0.42 | 0.57 | 0.70* | -0.09 | -0.43 | -0.53 | 0.51 | -0.53 |
| **K** | -0.81** | 0.31 | 0.81** | -0.71* | -0.86** | -0.88** | 0.84** | -0.79** |
| **P** | 0.76* | -0.20 | -0.69* | 0.53 | 0.48 | 0.62 | -0.82** | 0.62 |
| **S** | 0.75* | -0.41 | -0.83** | 0.44 | 0.46 | 0.55 | -0.75* | 0.53 |
| | | | | *Alma* | | | | |
| **Fe** | 0.49 | -0.02 | -0.25 | 0.70* | 0.82** | 0.62 | 0.00 | 0.74* |
| **Mn** | -0.13 | 0.30 | 0.47 | -0.25 | -0.48 | -0.32 | 0.00 | -0.38 |
| **Cu** | -0.21 | 0.13 | 0.28 | -0.35 | -0.31 | -0.37 | 0.00 | -0.39 |
| **Zn** | 0.52 | 0.43 | 0.33 | 0.56 | 0.68* | 0.49 | 0.00 | 0.72* |
| **Ca** | 0.53 | 0.41 | 0.31 | 0.51 | 0.76* | 0.65* | 0.00 | 0.72* |
| **Mg** | -0.04 | 0.24 | 0.34 | -0.08 | -0.07 | 0.22 | 0.00 | -0.18 |
| **Na** | -0.32 | -0.13 | -0.03 | -0.54 | -0.49 | -0.36 | 0.00 | -0.68* |
| **K** | -0.17 | 0.26 | 0.43 | -0.53 | -0.34 | -0.29 | 0.00 | -0.56 |
| **P** | 0.53 | 0.48 | 0.40 | 0.52 | 0.40 | 0.20 | 0.00 | 0.56 |
| **S** | 0.44 | 0.47 | 0.42 | 0.22 | 0.61 | 0.50 | 0.00 | 0.48 |
| | | | | *Kiev Mutant* | | | | |
| **Fe** | -0.05 | 0.27 | 0.43 | 0.29 | 0.67* | 0.70* | -0.57 | 0.32 |
| **Mn** | -0.45 | -0.52 | -0.50 | -0.08 | -0.54 | -0.56 | 0.37 | -0.21 |
| **B** | -0.57 | -0.68* | -0.66* | -0.27 | -0.63* | -0.58 | 0.40 | -0.42 |
| **Cu** | -0.24 | -0.43 | -0.50 | -0.69* | -0.38 | -0.40 | 0.61 | -0.58 |
| **Zn** | -0.12 | 0.16 | 0.31 | 0.76* | 0.28 | 0.21 | -0.50 | 0.57 |
| **Ca** | -0.21 | -0.05 | 0.05 | 0.41 | 0.03 | 0.06 | -0.28 | 0.29 |
| **Mg** | -0.45 | -0.60 | -0.62 | -0.16 | -0.73* | -0.78** | 0.56 | -0.36 |
| **Na** | -0.39 | -0.73* | -0.84** | -0.68* | -0.94** | -0.91** | 0.86** | -0.81** |
| **K** | -0.32 | -0.61 | -0.71* | -0.48 | -0.83** | -0.83** | 0.74* | -0.54 |
| **P** | -0.72* | -0.49 | -0.29 | 0.11 | -0.04 | -0.06 | -0.08 | -0.14 |
| **S** | -0.31 | -0.66* | -0.80** | -0.73* | -0.86** | -0.84** | 0.81** | -0.83** |
| | | | | *P22917* | | | | |
| **Fe** | 0.11 | 0.13 | 0.14 | -0.37 | 0.09 | 0.19 | -0.02 | 0.11 |
| **Mn** | 0.63* | 0.70* | 0.73* | 0.41 | 0.65* | 0.45 | -0.49 | 0.73* |
| **B** | 0.81** | 0.72* | 0.62 | 0.62 | 0.62 | 0.61 | -0.90** | 0.76* |
| **Cu** | -0.39 | -0.45 | -0.48 | -0.70* | -0.58 | -0.49 | 0.46 | -0.55 |
| **Zn** | 0.85** | 0.81** | 0.74* | 0.70* | 0.72* | 0.73* | -0.95** | 0.79** |
| **Ca** | 0.45 | 0.56 | 0.62 | 0.65* | 0.66* | 0.59 | -0.66* | 0.60 |
| **Mg** | -0.21 | -0.08 | 0.03 | 0.06 | 0.10 | -0.14 | 0.27 | 0.04 |
| **Na** | -0.18 | -0.28 | -0.34 | -0.09 | -0.41 | -0.42 | 0.15 | -0.30 |
| **K** | -0.80** | -0.78** | -0.74* | -0.75* | -0.70* | -0.71* | 0.92** | -0.72* |
| **P** | 0.79** | 0.73* | 0.67* | 0.69* | 0.62 | 0.59 | -0.93** | 0.72* |
| **S** | 0.75* | 0.69* | 0.61 | 0.58 | 0.54 | 0.61 | -0.90** | 0.64* |

* $P < 0.05$, ** $P < 0.01$

# 5.3 Determination of a bicarbonate concentration in solution for identifying the tolerance of *L. pilosus* to calcareous soil (Experiment 2)

## 5.3.1 Introduction

The previous experiment compared interspecific differences in growth. A screening method must be able to identify intraspecific variation to be useful in a breeding program. Thus, the following experiments focussed on developing a method that could discriminate the tolerance of a range of *L. pilosus* genotypes selected from the preliminary screening in the Wangary soil (Chapter 4 and 6), showing variation for tolerance to calcareous soil.

In the previous experiment it was shown that 10-15 mM $KHCO_3$ may be suitable concentrations for screening lupin species for tolerance to calcareous soil; the concentrations were similar to those used for soybeans (Chaney, *et al.*, 1992a). However, it was unknown if these concentrations could be used to identify intraspecific tolerance. In addition to determining the concentration of $HCO_3^-$ suitable for screening, the form of $HCO_3^-$ used (K vs. $NaHCO_3$) may influence the level of tolerance of genotypes. In experiment 1 and 2a (see below), $KHCO_3$ was used to induce chlorosis rather than $NaHCO_3$, to prevent possible interactions with sodicity tolerance that may be apparent in *L. pilosus* and other lupin species. High concentrations of K are less likely to affect the growth of lupins than equivalent levels of Na. Alternatively, in other research on $HCO_3^-$ in screening systems, $NaHCO_3$ has been commonly used (Coulombe, *et al.*, 1984b; Chaney, *et al.*, 1992a).

After identifying a suitable concentration and form of $HCO_3^-$ for screening it may be necessary to alter the concentration of FeEDDHA in solution. In initial experiments 40 µM FeEDDHA was used, but Chaney, *et al.* (1992a) and Coulombe, *et al.* (1984b) used much lower Fe concentrations in their screening of soybean. The high concentrations of Fe in solutions may be limiting the differential between tolerant and intolerant genotypes, thus making it more difficult to select tolerant genotypes.

In this experiment a range of $KHCO_3$ concentrations (*Experiment 2a*), the form of $HCO_3^-$ (K *vs.* $NaHCO_3$; *Experiment 2b*), and a range FeEDDHA concentrations (*Experiment 2c*) were compared to develop a solution screening method that could identify the level of tolerance of *L. pilosus* genotypes to calcareous soil.

### 5.3.2 Materials and methods

*Genetic Material*

The genotypes of *L. pilosus* grown in these experiments are listed in table 5.9 (see Table 6.1 for collection data). They were selected from preliminary screening trials on the Wangary calcareous soil (Chapter 4). In experiments 2a and 2b, P22937 and P24036 were used as the tolerant and intolerant controls, respectively. In experiment 2c, P23370 and P24036 were used as the tolerant and intolerant controls, respectively.

**Table 5.9** Genotypes of *L. pilosus* grown in experiments 2a, 2b and 2c of this chapter. Tolerance indicates susceptibility to chlorosis as identified in the preliminary soil screen (see Chapter 6 for chlorosis scores). T - tolerant, MT - moderately tolerant, MI - moderately intolerant, I - intolerant.

| | | Experiment | | |
|---|---|---|---|---|
| **Genotype** | **Tolerance** | **2a** | **2b** | **2c** |
| P22937 | T | X | X | |
| P23370 | T | | | X |
| P20954 | MT | X | | X |
| P23029 | MT | X | X | |
| P23345 | MT | X | X | |
| P25776 | MT | X | | |
| P26883 | MI | X | | |
| P22932 | MI | X | | X |
| P24036 | I | X | X | X |

*Container and solution preparation*

The experiments were conducted in 25 litre plastic tanks (280 mm x 410 mm x 250 mm) which had been surface sterilized by wiping with 70% ethanol and then soaked in 1M $HNO_3$ for seven days to remove nutritional contamination (Plate 6.2a). Plastic germination trays (280 mm x 350 mm) with eight columns and six rows (48) of tapered square holes (top - 37 mm x 37 mm, bottom - 10 mm x 10 mm) were used to suspend plants over the solution (Plate 6.2b). Black plastic sheeting at each end of the tray prevented light entering the solution. To aerate the solution, aquapore® tubing hooked to the base of tanks and connected through 4 mm plastic tubing to a silent flo 2000 aquarium pump was used.

Twenty two litres of solution (Table 5.1) was required for each tank. The nutrient solutions were changed 14 DAT.

Experiment 2a consisted of four concentrations of $KHCO_3$ (5, 10, 15 and 20 mM) compared with a control (0 mM $KHCO_3$). The $HCO_3^-$ solutions were buffered with 0.5, 1, 1.5 and 2 g $L^{-1}$ $CaCO_3$, respectively. Solution pH in the control tank was maintained at 6 by addition of 0.1 M KOH daily. There were two replicates with six plants of each genotype in each tank.

Experiment 2b compared $KHCO_3$ or $NaHCO_3$ at two concentrations (10 or 15 mM). A control solution (0.0 mM $HCO_3^-$) was included for comparison. The solutions were buffered as described above. The experiment was unreplicated with six plants of each genotype in each tank.

Experiment 2c consisted of four Fe concentrations (0.5, 2, 10, 40 μM) and two $KHCO_3$ concentrations (10, 15 mM). No control solution with 0 mM $KHCO_3$ were included. The experiment was unreplicated with six plants of each genotype in each tank.

*Plant growth*

Seeds of uniform size (for each genotype) were surface sterilized, scarified (Section 4.2.2) and germinated for five days at 15-20$^{O}$C in the dark (Section 5.2.2). Six seedlings of each genotype (root length > 50 mm) were transferred into each tank and arranged randomly in the germination trays, so that each row contained one plant of each genotype. The seedlings were held in the tapered holes of the lids by a piece of foam wrapped around the taproot. Plants were grown for 28 days in experiments 2a and 2b and 21 days in experiment 2c at constant temperature (20$^{O}$C) in a growth room under a 12 hour day/night regime (550-650 μmol $s^{-1}$ per uA).

*Measurements*

The duration of each experiment and the number of days after transplanting at which measurements were recorded are summarised in Table 5.10. Detailed methods of measurements were described in Sections 3.2 and 4.3.2. Root length was recorded in experiment 2a at transplanting and 4 DAT so that the root elongation rate (mm $hr^{-1}$) could be calculated. In experiment 2a there were two harvests, at 21 DAT when all plants from replicate 1 were removed, and at 28 DAT when all plants from replicate 2 were removed. In experiment 2b and 2c plants were harvested 28 DAT. At harvest in the roots for all the plants of each genotype were

combined. Similarly, to determine nutrient concentrations in the YEL, the six plants of each genotype in a tank were combined for analysis.

**Table 5.10** Duration of experiments 2a, 2b and 2c and days after transplanting when measurements of chlorosis score, chlorophyll meter readings, number of leaves, shoot dry weight, root dry weight and nutrient concentrations were recorded. n.d. - not determined.

| Experiment | Duration of experiment (days) | Chlorosis score | Chlorophyll meter | Number of leaves | Shoot dry weight | Root dry weight | Nutrient concentrations |
|---|---|---|---|---|---|---|---|
| 2a | 28 | 14, 21, 28 | 14, 21, 28 | 21, 28 | 21, 28 | 21, 28 | 21, 28 |
| 2b | 28 | 21, 28 | 21, 28 | 28 | 28 | 28 | 28 |
| 2c | 21 | 14, 21 | 14, 21 | n.d. | 28 | 28 | 28 |

*Statistical analyses*

As there were only two replicates in experiment 2a and each replicate was harvested at different stages, and no replication in experiment 2b and 2c, the statistical analysis were only provided as a guide to possible significant differences of measurements and need to be treated with caution. The ANOVA (Genstat 5.41) was conducted using the individual plants within a pot as pseudoreplication. For clarity, only the average was presented as there was little variability between individual plants. Individual means were compared using LSD.

For correlation coefficients the relative root elongation rate in experiment 2a and relative chlorophyll meter readings, relative number of leaves and relative shoot and root dry weight in experiment 2a and 2b (percentage of each treatment relative to the 0.0 mM $HCO_3^-$ treatment) were calculated.

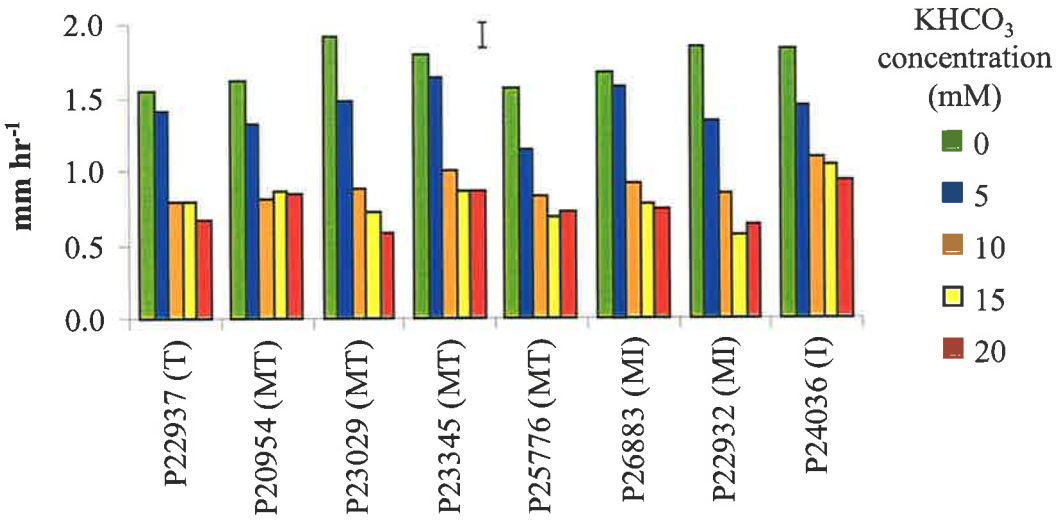
### 5.3.3 Results

*$KHCO_3$ concentrations (Expt. 2a)*

Root elongation and proteoid roots

The root elongation rates during 0 to 4 DAT showed similar trends for all genotypes (Fig. 5.6). Increasing $KHCO_3$ concentrations decreased root elongation, but there was no difference between the 10, 15 and 20 mM $KHCO_3$ treatments for each genotype. Although the ANOVA suggested that there was a significant interaction between genotype and $KHCO_3$ concentration ($P < 0.05$), there was only small differences between genotypes in any one treatment (Fig. 5.6).

**Figure 5.6** The effect of increasing $KHCO_3$ concentrations in solution on the root elongation rate (mm $hr^{-1}$) of eight *L. pilosus* genotypes displaying a range of tolerance to calcareous soils during 0-4 days after transplanting. Bar represents LSD ($P < 0.05$).



Proteoid roots were first observed at 14 DAT on all genotypes at 15 and 20 mM $KHCO_3$ (Plate 5.1). At 21 and 28 DAT, the number of proteoid root clusters increased as $KHCO_3$ increased from 5 to 20 mM. Also there were no visual differences in overall root growth between genotypes, except for P22937 which had a larger root system. Root growth was greatest for most genotypes in the 5 mM $KHCO_3$ treatment.

Chlorosis, number of leaves, shoot and root dry weight

The ANOVA suggested that there was a significant interaction between genotype and $KHCO_3$ concentration for chlorosis score ($P < 0.05$) and chlorophyll meter readings ($P < 0.05$) at 14, 21 and 28 DAT, and shoot dry weight at 21 and 28 DAT ($P < 0.05$). The main effects of genotype and treatment appeared significant for number of leaves ($P < 0.05$). Data of the 20 mM $KHCO_3$ treatment at 28 DAT could not be recorded as the plants had died.

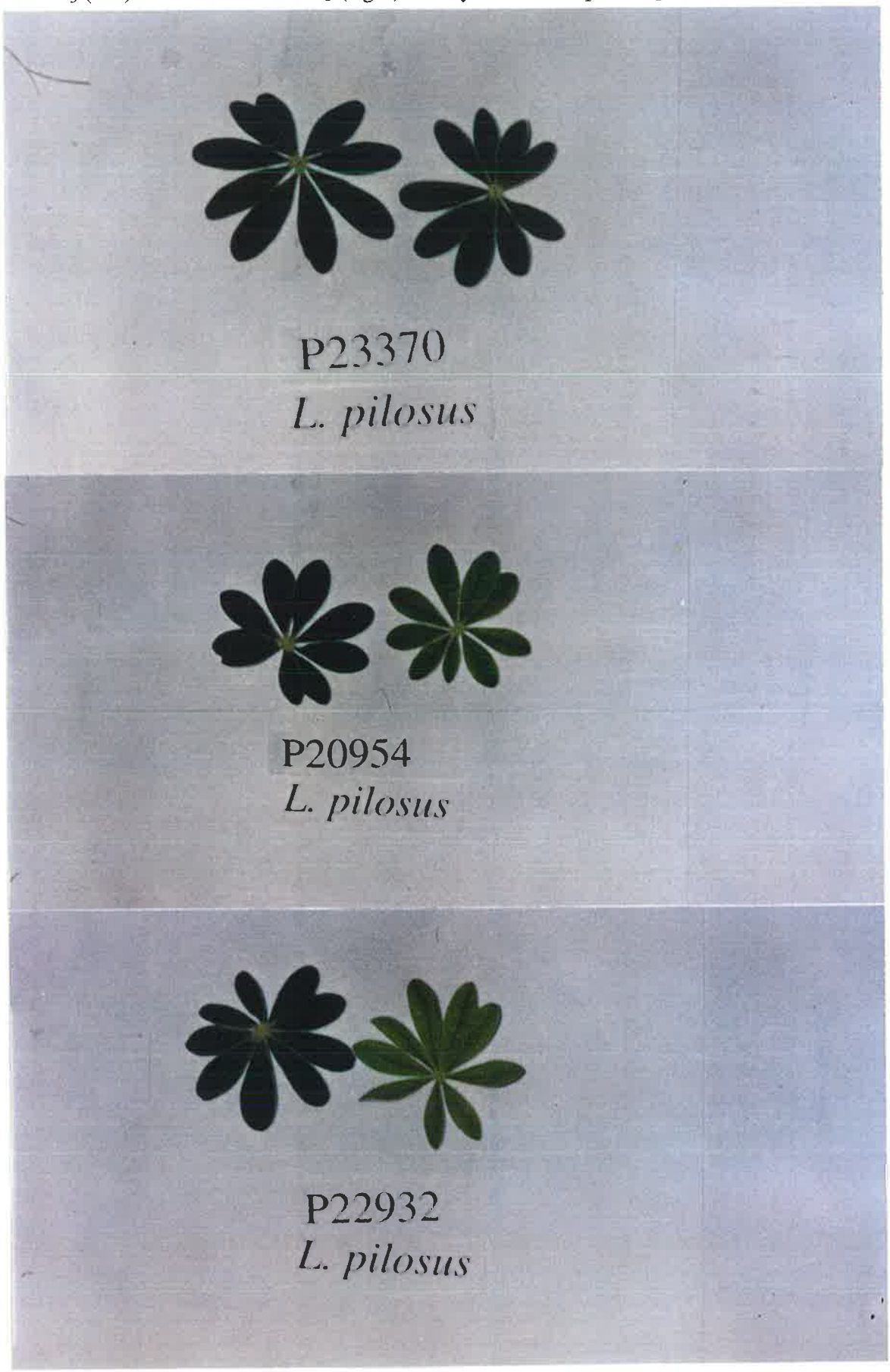
Chlorosis symptoms in the YEL were first noted 7 DAT in the 20 mM $KHCO_3$ treatment, particularly for P22932 (MI) and P24036 (I). By 14 DAT there were large differences in chlorosis score between genotypes and $KHCO_3$ concentrations (Fig. 5.7). For all genotypes there were no visual symptoms in 0 and 5 mM $KHCO_3$. At 10 mM $KHCO_3$ all genotypes showed slight visual chlorosis (chlorosis score 0.1-0.2), except P22932 (MI) and P24036 (I), where chlorosis was clearly visible as indicated by chlorosis scores of 0.5 and 1.5, respectively. In 15 mM $KHCO_3$ chlorosis score increased slightly for all genotypes compared with 10 mM $KHCO_3$. In 20 mM $KHCO_3$ chlorosis scores were greater than 2 for P26883 (MI), P20954 (MT), P22932 (MI) and P24036 (MI) and less than 2 in P22937 (T), P25776 (MT), P23029 (MT) and P23345 (MT)(Fig. 5.7).

By 21 DAT chlorosis score of the YEL had increased for all genotypes grown in solutions with 15 or 20 mM $KHCO_3$, but not in the 5 or 10 mM $KHCO_3$ (Fig. 5.7). At 15 mM $KHCO_3$ the chlorosis score was moderate / severe for both P22932 (MI) and P24036 (I)(Fig. 5.7; Plate 5.2). For the other genotypes, the chlorosis score was less than one and insignificant on the tolerant genotype (Fig. 5.7; Plate 5.2). At 20 mM $KHCO_3$ chlorosis was very severe in P22932 (MI) and P24036 (I), decreasing across genotypes in a similar trend to 15 mM $KHCO_3$ (Fig. 5.7).

All genotypes except P22932 (MI) and P24036 (I) had recovered from chlorosis in the 10 mM $KHCO_3$ treatment by 28 DAT. The chlorosis score of all genotypes was similar to 21 DAT in 15 mM $KHCO_3$ (Fig. 5.7).

**Plate 5.1** Proteoid root clusters formed on the roots of *L. pilosus* at high concentrations of $HCO_3^-$ in solution or in calcareous soil.

**Plate 5.2** Chlorotic symptoms in a tolerant (P23370), moderately tolerant (P20954) and a moderately intolerant (P22937) genotype of *L. pilosus* grown in solution culture with 0 mM $KHCO_3$ (left) and 15 mM $KHCO_3$ (right) 21 days after transplanting.



Generally chlorophyll meter readings showed an inverse trend of chlorosis score (Fig. 5.7). At 14, 21 and 28 DAT chlorophyll meter readings generally decreased as the $KHCO_3$ concentration in solution increased. There was little difference among genotypes except P22932 (MI) and P24036 (I) which showed lower readings, particularly at 15 and 20 $KHCO_3$ (Fig. 5.7).

The number of leaves at 21 and 28 DAT showed no significant trends, although there appeared to be a slight reduction at 15 and 20 mM $KHCO_3$ (data not shown). However, shoot and root dry weights showed significant differences between genotypes and $KHCO_3$ treatments (Fig. 5.8). At 21 DAT shoot dry weights were generally lowest for P23029 (MT) and P24036 (I) and similar for all other genotypes. All genotypes showed the highest shoot dry weight in 5 mM $KHCO_3$ (Fig. 5.8). The lowest shoot dry weight was at 15 or 20 mM $KHCO_3$. Root dry weights showed a similar trend. At 28 DAT shoot and root dry weights followed a similar trend to that at 21 DAT, although differences among genotypes were less notable (Fig. 5.8).

Correlation coefficients indicated that all measurements except relative shoot and root dry weight 28 DAT were correlated (Table 5.11). All measurements also correlated with increased $KHCO_3$ concentrations in solution (Table 5.11). Chlorosis score at 14, 21 and 28 DAT and relative chlorophyll meter readings at 21 DAT of plants grown in 10 and 15 mM were also correlated with chlorosis rankings in the preliminary soil screening (Table 5.12).

Nutrient concentrations

Nutrient concentrations in the YEL of all genotypes at 21 DAT are summarised in Table 5.13. Generally there was a large reduction in Fe and Cu concentrations from 0 to 5 mM $KHCO_3$, but there was little difference for the other elements (Table 5.13). The concentrations of Mn, Zn, P and S decreased and Mg increased as $KHCO_3$ increased. None of the changes in nutrient concentrations appeared to reflect differences between genotypes in chlorotic symptoms and shoot or root dry weight (Table 5.13).

Correlation coefficients showed that root elongation rate correlated positively with Fe, Mn, B, Cu, Zn, Ca, P and S and negatively with Mg and K (Table 5.14). The correlations of nutrients with $KHCO_3$ concentrations show inverse trends to that with root elongation rate. Chlorosis score at 14, 21 and 28 DAT correlates positively with Mg and K and negatively with Mn, Zn, P and S. Relative chlorophyll meter readings show the opposite trend and also correlate negatively

**Figure 5.7** The effect of increasing $KHCO_3$ concentrations in solution on the visual chlorosis score and chlorophyll meter readings of eight *L. pilosus* genotypes displaying a range of tolerance to calcareous soils 21 and 28 days after transplanting. At 28 DAT there were no measurements in 20mM $KHCO_3$ as plants had died. Bars indicate LSD ($P < 0.05$).

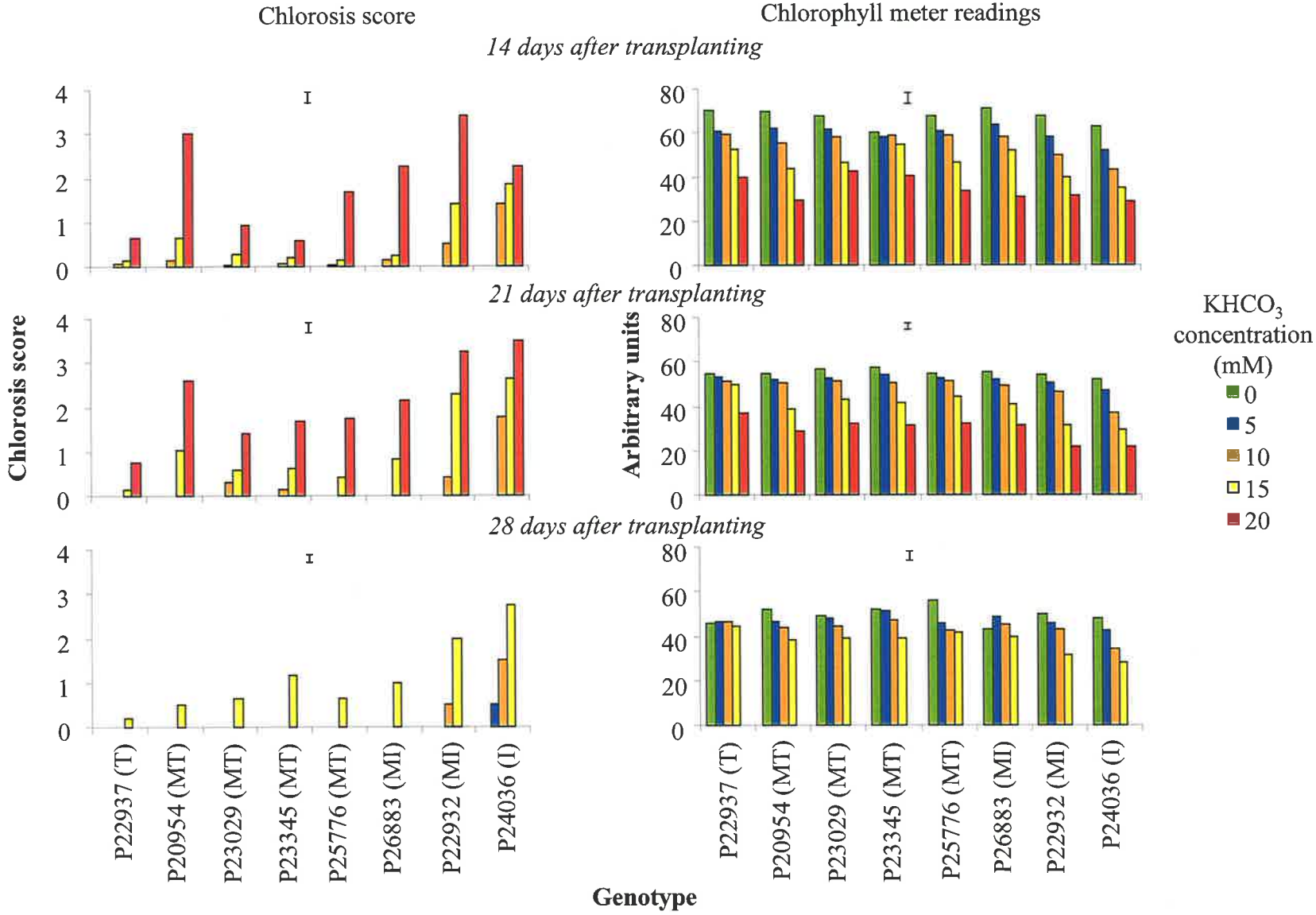


**Figure 5.8** The effect of increasing $KHCO_3$ concentrations in solution on the shoot and root dry weight of eight *L. pilosus* genotypes displaying a range of tolerance to calcareous soils (P22937 - tolerant, P24036 - intolerant) 21 and 28 days after transplanting. At 28 DAT there were no measurements in 20mM $KHCO_3$ as plants had died. Bars indicate LSD ($P < 0.05$). No statistical analysis (n.d.) were completed for root dry weight as the six plants of each genotype within the treatment were combined for weighing.

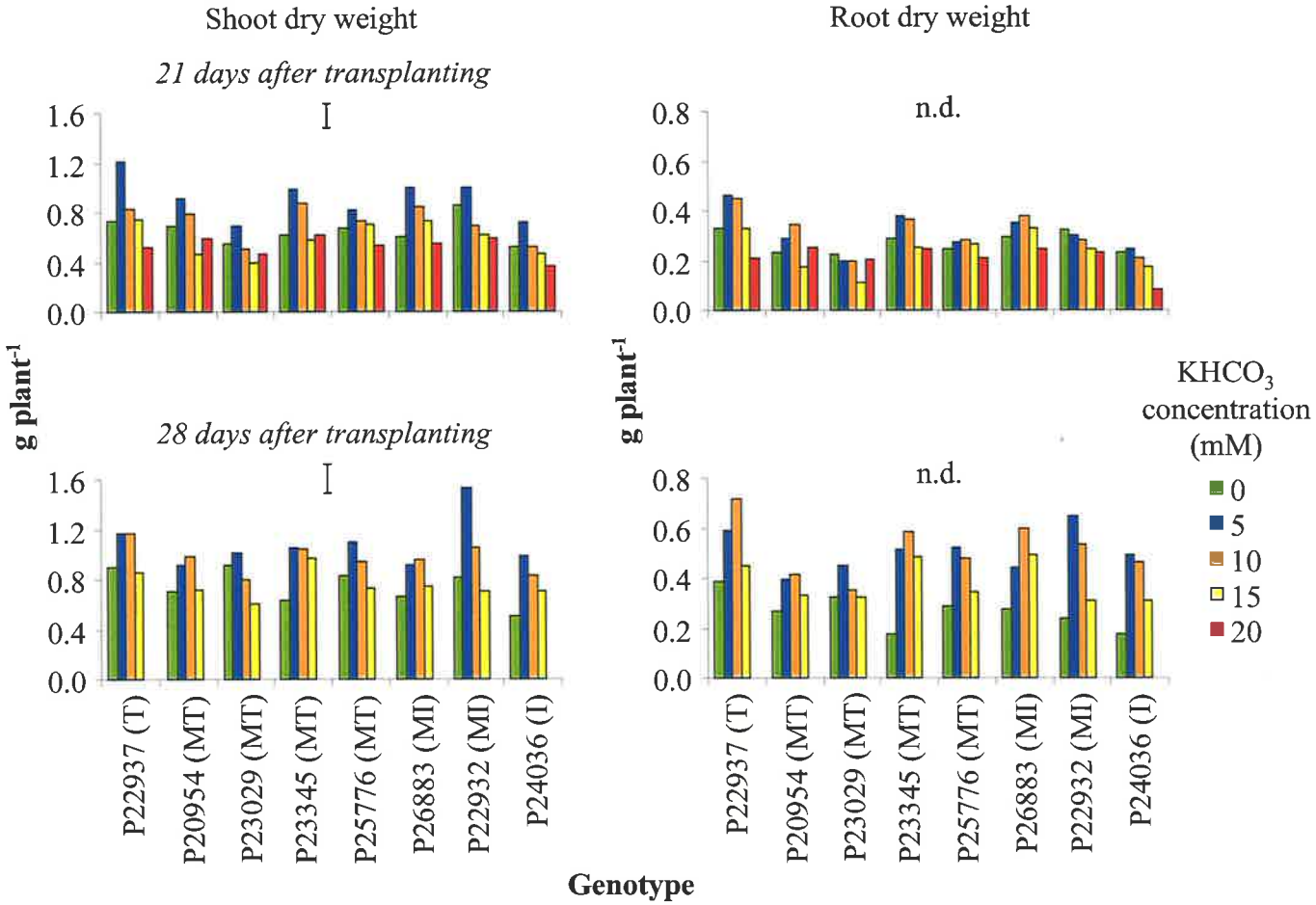


**Table 5.11** Correlation coefficients (r) between concentration of $KHCO_3$ in solution ($KHCO_3$), relative root elongation rate from 0-4 DAT (RG%), chlorosis score (CS) and relative chlorophyll meter readings 14, 21 and 28 DAT (CM%), relative shoot dry weight (SDW%) and relative root dry (SDW%) 21 and 28 DAT of eight *L. pilosus* genotypes grown in complete nutrient solutions containing 5, 10, 15 or 20 mM $KHCO_3$ to induce chlorosis.

| | $KHCO_3$ | RG% | CS14 | CS21 | CS28 | CM%14 | CM%21 | CM%28 | SDW%21 | SDW%28 | RDW%21 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **RG%** | -0.90** | | | | | | | | | | |
| **CS14** | 0.69** | -0.51** | | | | | | | | | |
| **CS21** | 0.75** | -0.57** | 0.94** | | | | | | | | |
| **CS28** | 0.60** | -0.50** | 0.91** | 0.96** | | | | | | | |
| **CM%14** | -0.90** | 0.76** | -0.86** | -0.87** | -0.75** | | | | | | |
| **CM%21** | -0.90** | 0.73** | -0.88** | -0.95** | -0.91** | 0.92** | | | | | |
| **CM%28** | -0.67** | 0.68** | -0.75** | -0.78** | -0.78** | 0.74** | 0.81** | | | | |
| **SDW%21** | -0.48** | 0.49** | -0.54** | -0.59** | -0.42** | 0.55** | 0.57** | 0.53** | | | |
| **SDW%28** | 0.02 | 0.01 | 0.06 | -0.02 | 0.06 | 0.01 | -0.05 | -0.04 | 0.55** | | |
| **RDW%21** | -0.44** | 0.35* | -0.48** | -0.61** | -0.51** | 0.52** | 0.60** | 0.47** | 0.77** | 0.37* | |
| **RDW%28** | 0.26 | -0.25 | 0.12 | 0.09 | 0.16 | -0.09 | -0.23 | -0.19 | 0.42** | 0.89** | 0.25 |

* $P < 0.05$, ** $P < 0.01$

with Fe and Cu. Shoot and root dry weights correlated positively with Mn and negatively with Mg and K concentrations (Table 5.14). No nutrient concentrations correlated with the chlorosis measurements in the preliminary soil screening (Table 5.12).

**Table 5.12** Correlation coefficients (r) between chlorosis score rankings of eight *L. pilosus* genotypes (Chapter 6) in the preliminary screening trial and relative root elongation rate from 0-4 DAT (RG%), chlorosis score (CS) and relative chlorophyll meter readings 14, 21 and 28 DAT (CM%), relative number of leaves (NL%), relative shoot dry weight (SDW%) and relative root dry weight (RDW%) 21 and 28 DAT and relative nutrient concentrations in the YEL 21 DAT in complete nutrient solutions containing 5, 10, 15 or 20 mM $KHCO_3$ to induce chlorosis. n.d. - not determined.

| | **Solution $KHCO_3$ concentration** | | | |
|---|---|---|---|---|
| | **5** | **10** | **15** | **20** |
| **RG%** | -0.19 | 0.35 | -0.09 | 0.03 |
| **CS14** | 0.00 | 0.79* | 0.84** | 0.54 |
| **CS21** | 0.00 | 0.77* | 0.91** | 0.89** |
| **CS28** | 0.67 | 0.79* | 0.94** | n.d. |
| **CM%14** | -0.37 | -0.60 | -0.49 | -0.34 |
| **CM%21** | -0.82* | -0.87** | -0.96** | -0.92** |
| **CM%28** | -0.13 | -0.44 | -0.75* | n.d. |
| **NL%21** | 0.17 | -0.04 | -0.28 | 0.15 |
| **NL%28** | 0.38 | 0.13 | -0.04 | n.d. |
| **SDW%21** | -0.29 | -0.25 | -0.21 | -0.17 |
| **SDW%28** | 0.80* | 0.47 | 0.41 | n.d. |
| **RDW%21** | -0.60 | -0.62 | -0.35 | -0.46 |
| **RDW%28** | 0.67 | 0.45 | 0.32 | n.d. |
| **Fe** | 0.03 | -0.05 | -0.38 | -0.07 |
| **Mn** | -0.45 | -0.11 | -0.26 | 0.26 |
| **B** | -0.07 | -0.12 | 0.10 | 0.03 |
| **Cu** | 0.45 | 0.35 | 0.58 | 0.63 |
| **Zn** | -0.23 | 0.14 | 0.14 | 0.48 |
| **Ca** | -0.66 | -0.04 | -0.10 | -0.17 |
| **Mg** | -0.39 | 0.51 | 0.34 | -0.08 |
| **Na** | 0.02 | -0.15 | -0.07 | -0.05 |
| **K** | -0.50 | 0.27 | 0.37 | -0.05 |
| **P** | 0.50 | 0.00 | 0.34 | 0.47 |
| **S** | -0.04 | -0.27 | 0.12 | 0.10 |

* $P < 0.05$, ** $P < 0.01$

**Table 5.13** Nutrient concentrations (mg $kg^{-1}$) in youngest emerging leaves of eight *L. pilosus* genotypes after 21 days growth in nutrient solutions containing 0 to 20 mM $KHCO_3$. Replicate samples were bulked and thus no statistical analyses could be calculated.

| $KHCO_3$ concentration | Fe | Mn | B | Cu | Zn | Ca | Mg | Na | K | P | S |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | *P22937 (T)* | | | | | |
| 0 | 400 | 66 | 45 | 15.8 | 89 | 11900 | 2600 | 560 | 21000 | 4400 | 6500 |
| 5 | 68 | 90 | 33 | 4.1 | 74 | 14500 | 3400 | 910 | 31000 | 3800 | 4000 |
| 10 | 60 | 56 | 36 | 3.9 | 49 | 8400 | 3000 | 810 | 29000 | 4200 | 3400 |
| 15 | 67 | 28 | 25 | 3.5 | 36 | 5600 | 3300 | 700 | 33000 | 2600 | 2500 |
| 20 | 38 | 7 | 31 | 4.5 | 37 | 1140 | 3700 | 640 | 51000 | 2900 | 2500 |
| | | | | | | *P20954 (MT)* | | | | | |
| 0 | 230 | 50 | 45 | 17.3 | 107 | 9900 | 3100 | 790 | 24000 | 4900 | 4500 |
| 5 | 29 | 74 | 36 | 5.1 | 95 | 15700 | 4100 | 920 | 31000 | 4600 | 3500 |
| 10 | 43 | 62 | 38 | 4.7 | 73 | 11700 | 3900 | 910 | 33000 | 4400 | 3000 |
| 15 | 62 | 33 | 34 | 6.0 | 54 | 10600 | 4900 | 1050 | 50000 | 3200 | 2400 |
| 20 | 30 | 8 | 31 | 5.1 | 41 | 2200 | 4800 | 890 | 66000 | 2400 | 2100 |
| | | | | | | *P23029 (MT)* | | | | | |
| 0 | 197 | 61 | 35 | 23.0 | 118 | 6800 | 2800 | 1820 | 28000 | 6200 | 8300 |
| 5 | 39 | 81 | 26 | 7.1 | 69 | 6600 | 3100 | 1350 | 28000 | 4900 | 4200 |
| 10 | 37 | 80 | 29 | 8.4 | 66 | 7300 | 3300 | 1030 | 29000 | 4600 | 3900 |
| 15 | 52 | 43 | 30 | 9.7 | 60 | 5900 | 4200 | 1130 | 35000 | 4200 | 3600 |
| 20 | 16 | 14 | 27 | 7.6 | 44 | 1520 | 4700 | 980 | 55000 | 3600 | 2900 |
| | | | | | | *P23345 (MT)* | | | | | |
| 0 | 200 | 46 | 37 | 18.0 | 100 | 8300 | 2400 | 450 | 21000 | 4800 | 9900 |
| 5 | 69 | 69 | 28 | 7.3 | 91 | 7300 | 2800 | 580 | 27000 | 4500 | 5300 |
| 10 | 66 | 70 | 34 | 7.6 | 84 | 7300 | 3400 | 680 | 33000 | 4600 | 4800 |
| 15 | 57 | 35 | 28 | 6.9 | 63 | 6400 | 4200 | 650 | 42000 | 3100 | 3800 |
| 20 | 15 | 10 | 26 | 7.2 | 46 | 1490 | 4400 | 570 | 46000 | 3000 | 3300 |

**Table 5.13** *(continued)* Nutrient concentrations (mg $kg^{-1}$) in youngest emerging leaves of eight *L. pilosus* genotypes after 21 days growth in nutrient solutions containing 0 to 20 mM $KHCO_3$.

| $KHCO_3$ concentration | Fe | Mn | B | Cu | Zn | Ca | Mg | Na | K | P | S |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | *P25776 (MT)* | | | | | |
| 0 | 340 | 56 | 45 | 18.7 | 85 | 8700 | 2800 | 780 | 22000 | 4700 | 3600 |
| 5 | 42 | 50 | 40 | 5.4 | 74 | 8400 | 3100 | 740 | 28000 | 5300 | 3200 |
| 10 | 53 | 49 | 36 | 5.9 | 67 | 7700 | 3300 | 830 | 29000 | 5100 | 2900 |
| 15 | 74 | 23 | 32 | 5.0 | 49 | 6000 | 3600 | 780 | 34000 | 3700 | 2100 |
| 20 | 16 | 9 | 34 | 6.1 | 38 | 1710 | 4300 | 690 | 60000 | 3000 | 1990 |
| | | | | | | *P26883 (MI)* | | | | | |
| 0 | 370 | 69 | 47 | 23.0 | 106 | 8400 | 3100 | 880 | 26000 | 5500 | 6200 |
| 5 | 66 | 74 | 37 | 7.3 | 94 | 8000 | 3200 | 1300 | 31000 | 5100 | 4600 |
| 10 | 60 | 65 | 39 | 7.2 | 75 | 7400 | 3500 | 1150 | 31000 | 5000 | 3700 |
| 15 | 49 | 33 | 36 | 8.7 | 62 | 5200 | 4200 | 1240 | 37000 | 3700 | 3300 |
| 20 | 27 | 14 | 35 | 8.1 | 46 | 1440 | 4400 | 890 | 45000 | 3500 | 2900 |
| | | | | | | *P22932 (MI)* | | | | | |
| 0 | 470 | 99 | 51 | 21.0 | 113 | 11900 | 3700 | 1160 | 28000 | 5800 | 5600 |
| 5 | 37 | 57 | 39 | 4.4 | 67 | 10600 | 3900 | 1320 | 31000 | 5600 | 3700 |
| 10 | 49 | 65 | 33 | 4.3 | 57 | 15300 | 4400 | 1070 | 38000 | 4000 | 2700 |
| 15 | 50 | 24 | 32 | 5.9 | 41 | 9200 | 4500 | 1170 | 48000 | 3300 | 2300 |
| 20 | 13 | 6 | 35 | 5.1 | 37 | 2000 | 4800 | 1100 | 69000 | 2900 | 2000 |
| | | | | | | *P24036 (I)* | | | | | |
| 0 | 330 | 65 | 64 | 16.2 | 92 | 15700 | 2400 | 870 | 20000 | 4300 | 5600 |
| 5 | 67 | 67 | 50 | 7.3 | 76 | 8500 | 2900 | 1260 | 25000 | 5200 | 3900 |
| 10 | 57 | 60 | 54 | 7.0 | 70 | 8300 | 3400 | 1070 | 31000 | 4900 | 3100 |
| 15 | 49 | 25 | 43 | 6.6 | 51 | 7200 | 4000 | 960 | 41000 | 3600 | 2900 |
| 20 | 37 | 16 | 46 | 11.9 | 62 | 1460 | 3700 | 900 | 52000 | 4500 | 2900 |

**Table 5.14** Correlation coefficients (r) between nutrient concentrations 21 DAT and concentration of $KHCO_3$ in solution ($KHCO_3$), relative root elongation rate from 0-4 DAT (RG%), chlorosis score (CS) and relative chlorophyll meter readings 14, 21 and 28 DAT (CM%), relative shoot dry weight (SDW%) and relative root dry weight (SDW%) 21 and 28 DAT of eight *L. pilosus* genotypes grown in full nutrient solutions containing 5, 10, 15 or 20 mM $KHCO_3$ to induce chlorosis.

| | $KHCO_3$ | RG% | CS14 | CS21 | CS28 | CM%14 | CM%21 | CM%28 | SDW%21 | SDW%28 | RDW%21 | RDW%28 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Fe** | -0.70** | 0.68** | -0.27 | -0.28 | -0.29 | 0.60** | 0.50** | 0.43** | -0.20 | -0.38* | -0.08 | -0.49** |
| **Mn** | -0.63** | 0.54** | -0.48** | -0.56** | -0.60** | 0.60** | 0.63** | 0.62** | 0.51** | 0.20 | 0.39** | 0.06 |
| **B** | -0.52** | 0.48** | 0.07 | 0.00 | 0.00. | 0.26 | 0.22 | 0.12 | -0.05 | 0.14 | 0.00 | -0.08 |
| **Cu** | -0.71** | 0.67** | -0.26 | -0.24 | -0.25 | 0.62** | 0.45** | 0.42** | -0.23 | -0.41** | -0.18 | -0.50** |
| **Zn** | -0.86** | 0.85** | -0.48** | -0.51** | -0.52** | 0.81** | 0.68** | 0.60** | 0.29 | 0.00 | 0.24 | -0.18 |
| **Ca** | -0.40** | 0.32 | -0.09 | -0.18 | -0.24 | 0.19 | 0.29 | 0.15 | 0.03 | 0.05 | 0.20 | -0.13 |
| **Mg** | 0.71** | -0.67** | 0.49** | 0.54** | 0.51** | -0.71** | -0.72** | -0.61** | -0.36* | 0.00 | -0.32* | 0.14 |
| **Na** | 0.01 | -0.03 | 0.12 | 0.13 | 0.11 | -0.21 | -0.14 | 0.03 | 0.00 | 0.05 | -0.22 | -0.05 |
| **K** | 0.82** | -0.72** | 0.62** | 0.68** | 0.64** | -0.78** | -0.84** | -0.68** | -0.35* | 0.01 | -0.32* | 0.19 |
| **P** | -0.72** | 0.57** | -0.40** | -0.46** | -0.48** | 0.59** | 0.60** | 0.43** | 0.29 | 0.19 | 0.18 | 0.00 |
| **S** | -0.70** | 0.69** | -0.37* | -0.38* | -0.38* | 0.69** | 0.53** | 0.54** | 0.10 | -0.13 | 0.05 | -0.23 |

* $P < 0.05$, ** $P < 0.01$

*$KHCO_3$ vs. $NaHCO_3$ (Expt. 2b)*

Chlorosis, number of leaves, shoot and root dry weight

The ANOVA suggested that there was a significant interaction between genotype and treatment for chlorosis score ($P < 0.05$) and chlorophyll meter readings ($P < 0.05$) 21 and 28 DAT. The main effects of genotype and treatment appeared significant for number of leaves ($P < 0.05$) and shoot and root dry weight ($P < 0.05$).

Chlorotic symptoms in the YEL of P24036 (I) was first noted 7 DAT in both 15 mM $KHCO_3$ and 15 mM $NaHCO_3$. All other genotypes displayed chlorosis by 14 DAT. By 21 DAT P24036 (I) had the highest chlorosis score and P22937 (T) least in both $HCO_3^-$ solutions (Fig. 5.9). There was little difference in chlorosis score for any of the genotypes between $HCO_3^-$ sources. P22937 only showed slight symptoms at 15 mM $KHCO_3$. The other genotypes displayed increased chlorosis scores at 15 mM $KHCO_3$ compared with 15 mM $NaHCO_3$ (Fig. 5.9). Chlorosis scores at 28 DAT were similar to 21 DAT.

Chlorophyll meter readings of the YEL displayed the opposite trend to chlorosis scores at both 21 and 28 DAT, although there were differences between the Na and K treatments at 15 mM $HCO_3^-$ (Fig. 5.9). The chlorophyll meter readings were higher on plants grown in 15 mM $NaHCO_3$ than $KHCO_3$ (Fig. 5.9). Also there was a slight decrease in chlorophyll meter readings from 21 to 28 DAT for all the genotypes in all treatments.

Generally, P23345 (MT) and P24036 (I) had the greatest number of leaves and P23029 (MT) least at 28 DAT (data not shown). There appeared to be a slight reduction in leaf number at 15 mM $HCO_3^-$ compared with other treatments for all genotypes and in $NaHCO_3$ compared with $KHCO_3$ at the 15 mM level (data not shown).

The shoot and root dry weights showed similar trends for all the genotypes and treatments. Generally plants of P22937 (T) and P23345 (MT) had the greatest shoot and root dry weights and P23030 lowest (Fig. 5.10). When comparing treatments, shoot dry weight was reduced at 15 mM K or $NaHCO_3$. There were no differences between $KHCO_3$ and $NaHCO_3$ (Fig. 5.10). Root dry weight was increased at 10 mM $HCO_3^-$ and was similar to the control at 15 mM $HCO_3^-$ (Fig. 5.10). At 15 mM $HCO_3^-$ root dry weight appeared to be reduced using $KHCO_3$ rather than $NaHCO_3$ (Fig. 5.10). Shoot and root dry weight correlated significantly with chlorophyll measurements (Table 5.15).

**Figure 5.9** The effect of 10 and 15 mM $KHCO_3$ or $NaHCO_3$ in solution on visual chlorosis score and chlorophyll meter readings of four *L. pilosus* genotypes displaying a range of tolerance to calcareous soils 21 and 28 days after transplanting. Bars indicate LSD ($P < 0.05$).

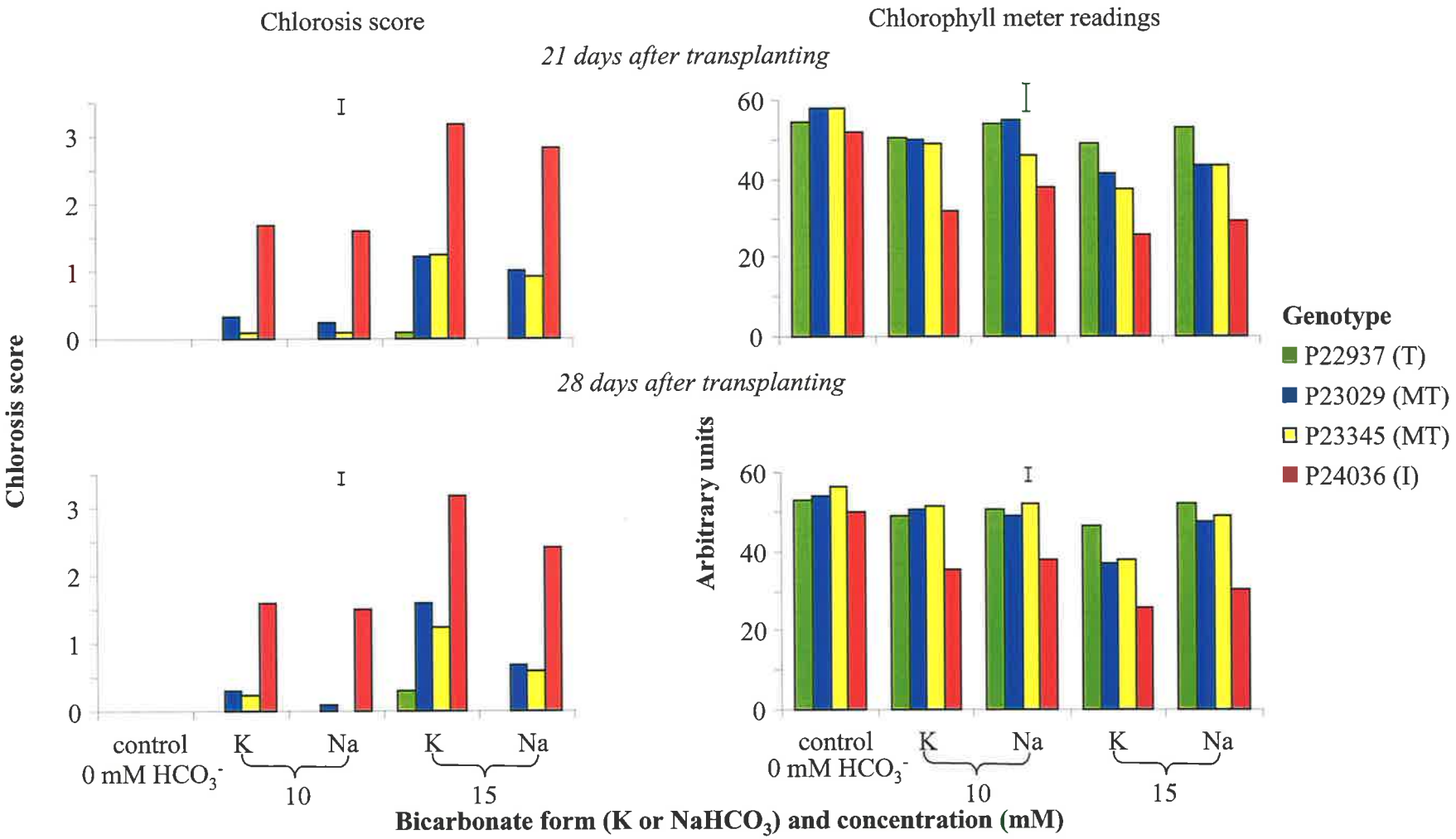


**Figure 5.10** The effect of 10 and 15 mM $KHCO_3$ or $NaHCO_3$ in solution on the shoot dry weight and root dry weight of four *L. pilosus* genotypes displaying a range of tolerance to calcareous soils 28 days after transplanting. n.s. - not significant.

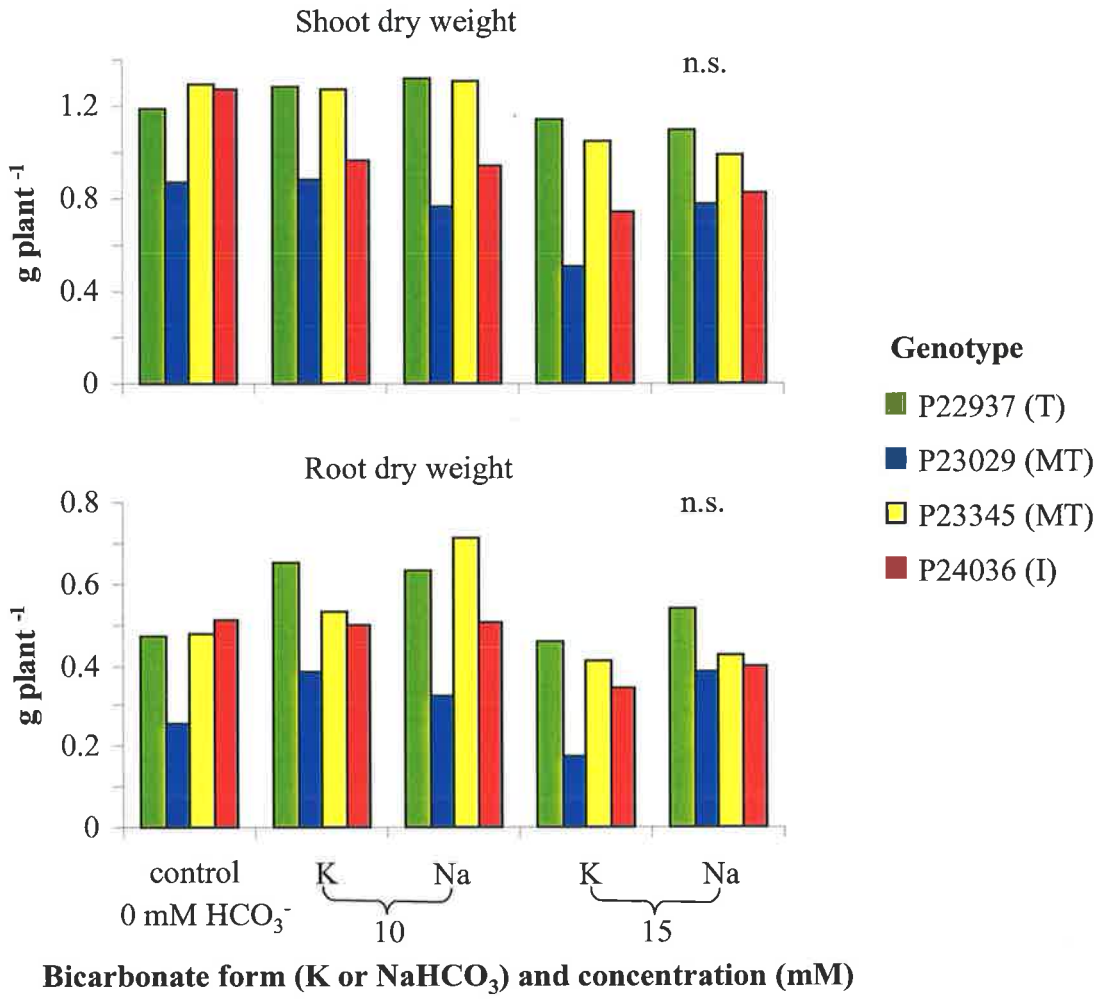


**Table 5.15** Correlation coefficients (r) between chlorosis score (CS) and relative chlorophyll meter readings 21 and 28 DAT (CM%), relative number of leaves (NL), relative shoot dry weight (SDW%) and relative root dry weight (RDW%) 28 DAT of four *L. pilosus* genotypes grown in full nutrient solutions containing 10 or 15 mM $NaHCO_3$ or $KHCO_3$.

| | CS21 | CS28 | CM%21 | CM%28 | NL% | SDW% |
|---|---|---|---|---|---|---|
| **CS28** | 0.98** | | | | | |
| **CM%21** | -0.92** | -0.91** | | | | |
| **CM%28** | -0.94** | -0.96** | 0.94** | | | |
| **NL%** | -0.43 | -0.39 | 0.54* | 0.45* | | |
| **SDW%** | -0.87** | -0.89** | 0.83** | 0.88** | 0.53* | |
| **RDW%** | -0.57** | -0.64** | 0.45* | 0.60** | 0.14 | 0.72** |

* $P < 0.05$, ** $P < 0.01$

*Nutrient concentrations*

The addition of $HCO_3^-$ generally decreased concentrations of Fe, Mn, B, Cu, Zn, Ca, P and S, and increased Mg (Table 5.16). K and Na concentrations were higher in the respective treatment ($KHCO_3$ or $NaHCO_3$). The concentration of Fe decreased and Mn increased in the YEL of plants grown at 15 mM $KHCO_3$ compared with $NaHCO_3$ (Table 5.16).

Chlorosis score was negatively correlated and relative chlorophyll meter readings positively correlated with Fe, Mn and S (Table 5.17). Cu, Mg and K were also positively correlated with relative chlorophyll meter readings. Number of leaves was significantly correlated with all nutrient concentrations except K and shoot dry weight positively correlated with Fe and Mn and negatively correlated with Mg concentrations in the YEL (Table 5.17).

**Table 5.17** Correlation coefficients (r) between nutrient concentrations in the YEL 21 DAT and chlorosis score (CS) and relative chlorophyll meter readings 21 and 28 DAT (CM%), relative number of leaves (NL), relative shoot dry weight (SDW%) and relative root dry (RDW%) 28 DAT of four *L. pilosus* genotypes grown in complete nutrient solutions containing 10 or 15 mM $NaHCO_3$ or $KHCO_3$.

| | CS21 | CS28 | CM%21 | CM%28 | NL% | SDW% | RDW% |
|---|---|---|---|---|---|---|---|
| **Fe** | -0.58** | -0.59** | 0.73** | 0.71** | 0.64** | 0.56** | 0.11 |
| **Mn** | -0.48* | -0.46* | 0.55* | 0.54* | 0.71** | 0.45* | 0.04 |
| **B** | 0.23 | 0.21 | -0.05 | -0.06 | 0.47* | -0.12 | -0.34 |
| **Cu** | -0.32 | -0.29 | 0.51* | 0.45* | 0.58** | 0.26 | -0.22 |
| **Zn** | -0.36 | -0.38 | 0.41 | 0.48* | 0.60** | 0.36 | 0.05 |
| **Ca** | -0.23 | -0.18 | 0.22 | 0.20 | 0.71** | 0.34 | 0.11 |
| **Mg** | 0.43 | 0.44* | -0.66** | -0.60** | -0.76** | -0.49* | -0.04 |
| **Na** | 0.28 | 0.16 | -0.22 | -0.12 | -0.56** | -0.25 | 0.09 |
| **K** | 0.34 | 0.46* | -0.49* | -0.55* | -0.05 | -0.36 | -0.38 |
| **P** | -0.28 | -0.28 | 0.42 | 0.40 | 0.66** | 0.27 | 0.09 |
| **S** | -0.48* | -0.48* | 0.60** | 0.59** | 0.61** | 0.43 | 0.01 |

* $P < 0.05$, ** $P < 0.01$

**Table 5.16** Nutrient concentrations (mg $kg^{-1}$) in youngest emerging leaves of four *L. pilosus* genotypes after 21 days growth in nutrient solutions containing 10 or 15 mM $KHCO_3$ or $NaHCO_3$ compared with a control (0 $HCO_3^-$). n.d. - not determined.

| $HCO_3^-$ concentration (mM) | $HCO_3^-$ form | Fe | Mn | B | Cu | Zn | Ca | Mg | Na | K | P | S |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | *P22937 (T)* | | | | | |
| control | | 134 | 114 | 51 | 18.9 | 81 | 14850 | 2200 | 385 | 21000 | 4000 | 7300 |
| 10 | K | 64 | 48 | 25 | 3.5 | 49 | 13650 | 3850 | 740 | 34000 | 2400 | 2700 |
| 10 | Na | 69 | 36 | 31 | 3.3 | 50 | 11900 | 3500 | 9150 | 19800 | 3200 | 3000 |
| 15 | K | 46 | 29 | 21 | n.d. | 32 | 9800 | 4050 | 535 | 39500 | 1600 | 2075 |
| 15 | Na | 63 | 26 | 21 | 5.1 | 42 | 8950 | 4050 | 14350 | 16300 | 1505 | 2350 |
| | | | | | | | *P23029 (MT)* | | | | | |
| control | | 146 | 108 | 35 | 20.1 | 136 | 12700 | 2600 | 1005 | 27000 | 4850 | 9150 |
| 10 | K | 63 | 50 | 25 | 9.4 | 62 | 9650 | 3900 | 1060 | 34500 | 3450 | 3150 |
| 10 | Na | 62 | 44 | 26 | 7.1 | 61 | 9500 | 3800 | 7550 | 23500 | 4150 | 3700 |
| 15 | K | 45 | 44 | 26 | 7.1 | 49 | 8700 | 4450 | 850 | 44500 | 3050 | 2800 |
| 15 | Na | 56 | 27 | 25 | 5.7 | 49 | 6000 | 4300 | 12250 | 23000 | 2900 | 2900 |
| | | | | | | | *P23345 (MT)* | | | | | |
| control | | 115 | 77 | 40 | 26.2 | 106 | 8750 | 2400 | 385 | 27500 | 5100 | 7500 |
| 10 | K | 62 | 54 | 25 | 5.0 | 74 | 11250 | 4150 | 575 | 41000 | 3050 | 3700 |
| 10 | Na | 60 | 58 | 32 | 4.4 | 87 | 13900 | 4050 | 9000 | 26500 | 3100 | 3800 |
| 15 | K | 44 | 38 | 27 | 5.1 | 54 | 9000 | 4800 | 515 | 50000 | 2190 | 2700 |
| 15 | Na | 55 | 28 | 27 | 4.5 | 56 | 7500 | 4600 | 14350 | 24500 | 1945 | 2950 |
| | | | | | | | *P24036 (I)* | | | | | |
| control | | 100 | 68 | 62 | 18.7 | 84 | 12100 | 2350 | 1095 | 25000 | 5000 | 5200 |
| 10 | K | 46 | 44 | 46 | 4.4 | 59 | 13850 | 4000 | 920 | 39500 | 3200 | 2400 |
| 10 | Na | 54 | 37 | 48 | 5.2 | 62 | 13100 | 3650 | 13450 | 21500 | 4350 | 2900 |
| 15 | K | 33 | 31 | 40 | 7.9 | 49 | 11050 | 4200 | 750 | 54500 | 2250 | 2000 |
| 15 | Na | 39 | 22 | 43 | n.d. | 49 | 7900 | 4000 | 20000 | 17700 | 2600 | 2100 |

*FeEDDHA concentrations (Expt. 2c)*

Chlorosis, number of leaves, shoot and root dry weight

The ANOVA suggested that there was a significant interaction between genotype and treatment for chlorosis score ($P < 0.05$) and chlorophyll meter readings ($P < 0.05$) at 14 and 21 DAT and shoot and root dry weight ($P < 0.05$) at 21 DAT. The main effects of genotype and treatment appeared significant for number of leaves ($P < 0.05$).

Chlorotic symptoms in the YEL were first noted 4 DAT in the 0.5 mM FeEDDHA treatments and 7 DAT in the 2 mM FeEDDHA treatments on all the genotypes. By 14 DAT chlorosis was severe for all genotypes grown in the 0.5 and 2 mM FeEDDHA treatments at 10 and 15 mM $KHCO_3$ (Fig 5.11). The chlorosis score was lowest for P23370 (T), followed by P20954 (MT) and P22932 (MI) and highest for P24036 (I) at 10 mM FeEDDHA and 10 mM $KHCO_3$ 14 DAT. In 10 mM FeEDDHA and 15 mM $KHCO_3$ the chlorosis score was high for P22932 (MI) and P24036 (I) and moderate for P23370 (T) and P20954 (MT). In 40 mM FeEDDHA there was no chlorosis in the 10 mM $KHCO_3$ treatment, whilst the 15 mM $KHCO_3$ treatments showed a similar trend to 10 mM FeEDDHA and 10 mM $KHCO_3$ (Fig. 5.11). Chlorosis score at 21 DAT showed similar trends to 14 DAT except that in 0.5 and 2 mM FeEDDHA most plants were completely chlorotic and dead (Fig. 5.11).

The trends in chlorophyll meter readings were generally the inverse of chlorosis score (Fig. 5.11). Chlorophyll meter readings were not recorded 21 DAT in the 0.5 mM FeEDDHA treatment for both $HCO_3^-$ concentrations and 2 mM FeEDDHA for 15 mM $KHCO_3$ as the plants in these treatments were severely stunted (Fig. 5.11).

Plants grown in 0.5 and 2 mM FeEDDHA had slow growth, which is reflected in the low shoot and root dry weights (Fig. 5.12). Generally the number of leaves increased with increasing FeEDDHA in both $HCO_3^-$ concentrations (Fig. 5.12). There were no genotypic differences.

Shoot and root dry weights were generally increased as FeEDDHA increased and decreased with increasing $KHCO_3$ concentration (Fig. 5.12). There was little difference between the genotypes in response to Fe and $KHCO_3$ (Fig 5.12).

**Figure 5.11** The effect of 10 or 15 mM $KHCO_3$ and 0.5, 2, 10 and 40 mM FeEDDHA in complete nutrient solutions on the visual chlorosis score and chlorophyll meter reading of four *L. pilosus* genotypes, displaying a range of tolerance to calcareous soils 14 and 21 days after transplanting. Bars indicate LSD ($P < 0.05$).

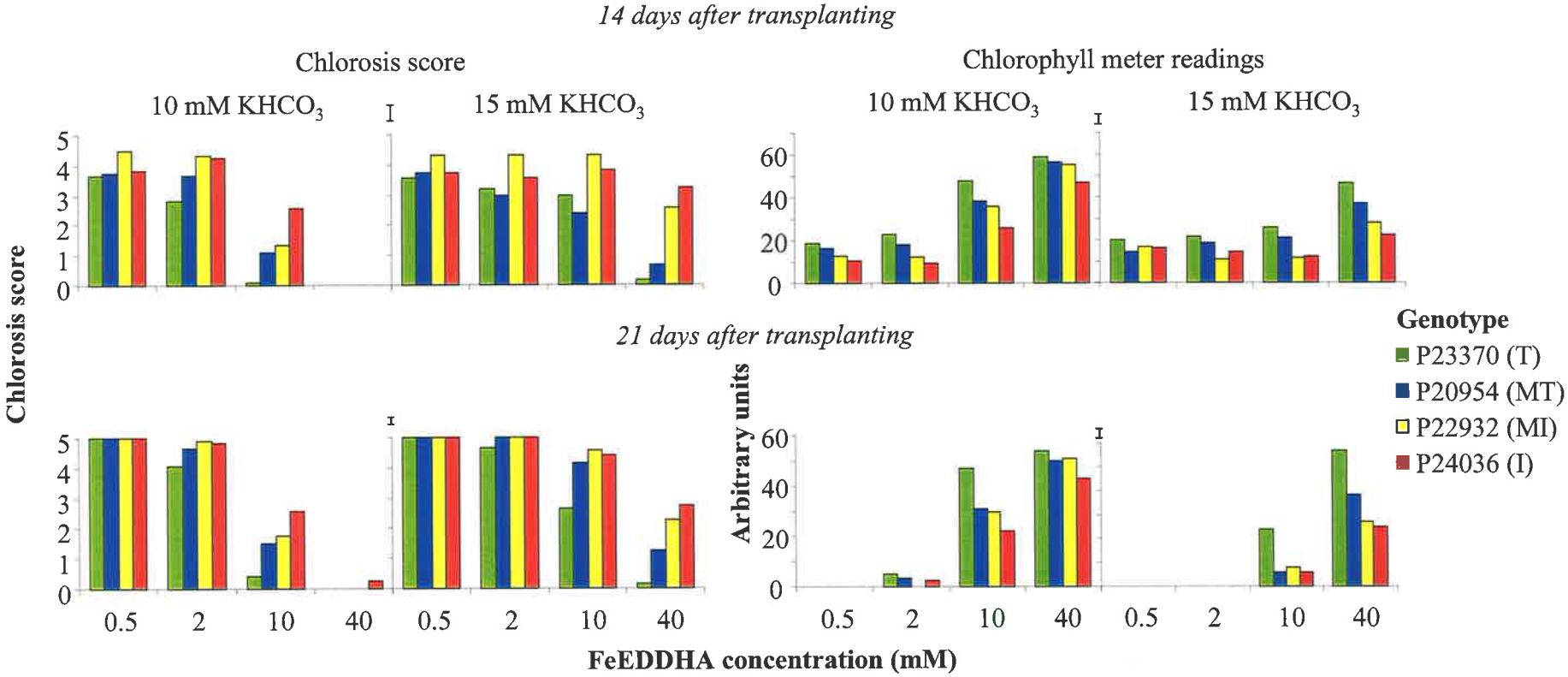
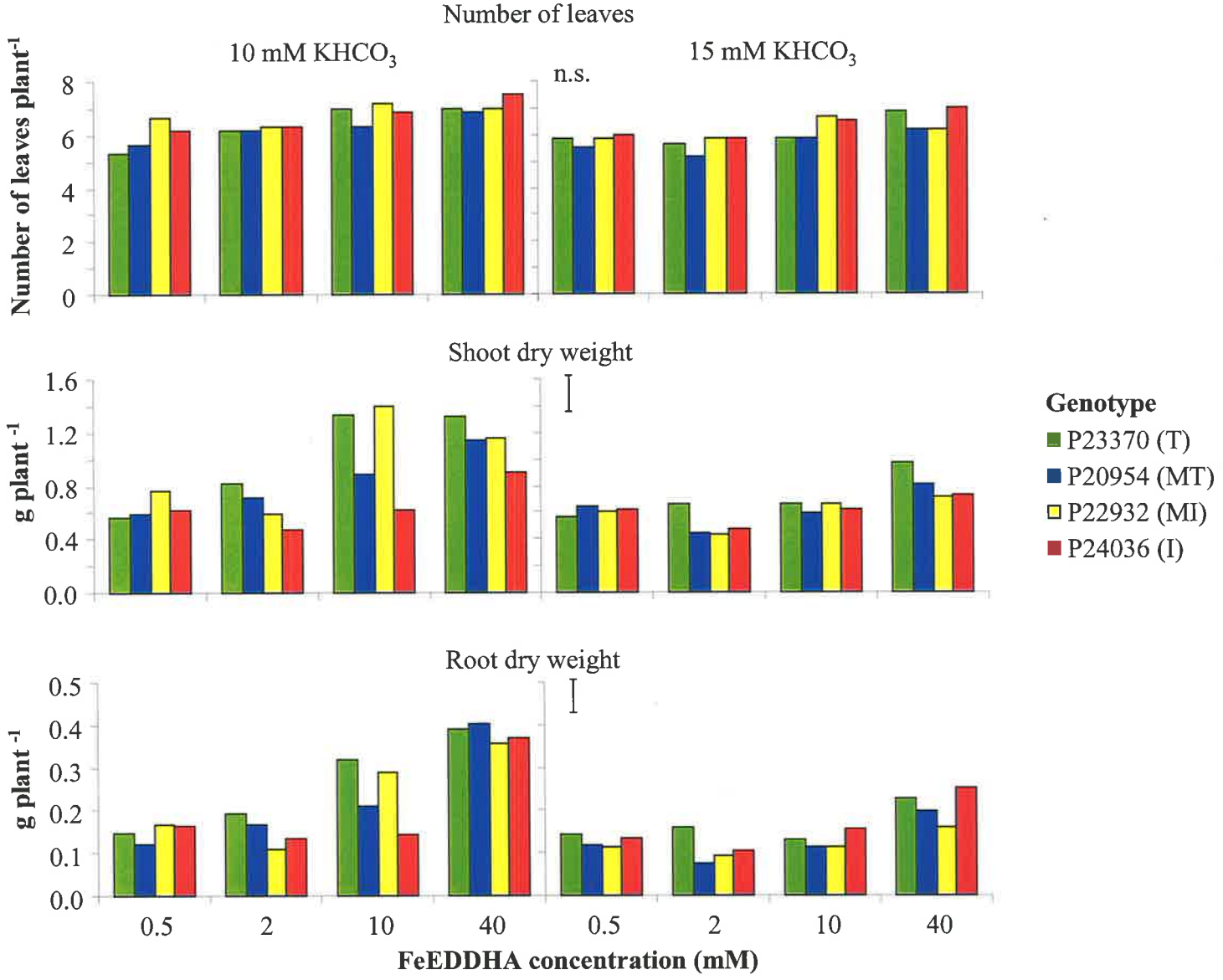


**Figure 5.12** The effect of 10 or 15 mM $KHCO_3$ and 0.5, 2, 10 and 40 mM FeEDDHA in complete nutrient solutions on the number of leaves and shoot and root dry weight four *L. pilosus* genotypes, displaying a range of tolerance to calcareous soils 14 and 21 days after transplanting. Bars indicate LSD ($P < 0.05$); n.s. - not significant.



Growth measurements correlated with chlorophyll measurements (Table 5.18). Generally chlorosis score was negatively correlated and chlorophyll meter readings positively correlated with number of leaves and shoot and root dry weight (Table 5.18).

**Table 5.18** Correlation coefficients (r) between chlorosis score (CS) and chlorophyll meter readings 21 and 28 DAT (CM), number of leaves (NL), shoot dry weight (SDW) and root dry weight (RDW) 21 and 28 DAT for four *L. pilosus* genotypes grown in complete nutrient solutions containing 10 or 15 mM $KHCO_3$ and 0.5, 2, 10 or 20 mM FeEDDHA.

| | CS14 | CS21 | CM14 | CM21 | NL | SDW |
|---|---|---|---|---|---|---|
| **CS21** | 0.95** | | | | | |
| **CM14** | -0.97** | -0.96** | | | | |
| **CM21** | -0.92** | -0.99** | 0.95** | | | |
| **NL** | -0.59** | -0.72** | 0.61** | 0.59** | | |
| **SDW** | -0.81** | -0.83** | 0.85** | 0.75** | 0.72** | |
| **RDW** | -0.84** | -0.86** | 0.89** | 0.79** | 0.77** | 0.88** |

* $P < 0.05$, ** $P < 0.01$

Nutrient concentrations

In both 10 and 15 mM $KHCO_3$ the concentration of Fe in the YEL of all genotypes increased with increasing concentrations of FeEDDHA in the solution (Table 5.19). Differences in the concentration of nutrients in the YEL between the 10 and 15 mM $KHCO_3$ treatments showed similar trends to other experiments.

Correlation coefficients showed significant correlations of Fe, B, Mg, Na, Ca, P, K and P with all chlorosis and most growth measurements (Table 5.20).

**Table 5.20** Correlation coefficients (r) between nutrient concentrations in the YEL 21 DAT and chlorosis score (CS) and relative chlorophyll meter readings 21 and 28 DAT (CM), number of leaves (NL), shoot dry weight (SDW) and root dry weight (RDW) 21 and 28 DAT of four *L. pilosus* genotypes grown in full nutrient solutions containing 10 or 15 mM $KHCO_3$ and 0.5, 2, 10 or 20 mM FeEDDHA.

| | CS14 | CS21 | CM14 | CM21 | NL | SDW | RDW |
|---|---|---|---|---|---|---|---|
| **Fe** | -0.70** | 0.71** | -0.79** | 0.73** | 0.54** | 0.50** | 0.60** |
| **Mn** | -0.11 | 0.12 | -0.08 | -0.35* | 0.11 | 0.21 | 0.21 |
| **B** | 0.37* | -0.44* | 0.39* | -0.51** | -0.05 | -0.48** | -0.24 |
| **Cu** | 0.74** | -0.75** | 0.77** | -0.66** | -0.39* | -0.66** | -0.63** |
| **Zn** | -0.28 | 0.27 | -0.23 | 0.13 | 0.25 | 0.31 | 0.30 |
| **Ca** | -0.52** | 0.53** | -0.55** | 0.25 | 0.63** | 0.60** | 0.71** |
| **Mg** | 0.62** | -0.65** | 0.63** | -0.76** | -0.50** | -0.58** | -0.63** |
| **Na** | -0.50** | 0.52** | -0.64** | 0.45** | 0.51** | 0.43* | 0.52** |
| **K** | 0.49** | -0.55** | 0.44* | -0.61** | -0.30 | -0.54** | -0.54** |
| **P** | 0.43* | -0.38* | 0.48** | -0.46** | -0.34 | -0.25 | -0.36* |
| **S** | 0.00 | 0.06 | 0.05 | -0.15 | -0.07 | 0.18 | 0.13 |

* $P < 0.05$, ** $P < 0.01$

**Table 5.19** Nutrient concentrations (mg $kg^{-1}$) in youngest emerging leaves of four *L. pilosus* genotypes after 21 days growth in nutrient solutions containing 10 or 15 mM $KHCO_3$ and 0.5, 2, 10 or 20 mM FeEDDHA.

| Fe concentration | $KHCO_3$ (mM) | Fe | Mn | B | Cu | Zn | Ca | Mg | Na | K | P | S |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | *P23370 (T)* | | | | | |
| 0.5 | 10 | 13.8 | 184 | 42 | 7.7 | 63 | 9250 | 4300 | 685 | 32500 | 4600 | 4850 |
| 2 | | 10.6 | 340 | 41 | 7.5 | 52 | 10400 | 4450 | 455 | 35000 | 5100 | 4900 |
| 10 | | 37.8 | 187 | 32 | 5.4 | 84 | 11300 | 3450 | 620 | 34000 | 3900 | 3550 |
| 40 | | 55.7 | 199 | 32 | 3.6 | 63 | 16350 | 2950 | 705 | 29000 | 3250 | 2950 |
| 0.5 | 15 | 13.8 | 108 | 40 | n.d. | 57 | 4800 | 5650 | 380 | 48500 | 4150 | 3250 |
| 2 | | 11.0 | 106 | 42 | 7.8 | 55 | 4850 | 5200 | 380 | 46000 | 4100 | 3200 |
| 10 | | 26.4 | 235 | 29 | 4.6 | 48 | 8050 | 4600 | 600 | 46500 | 3400 | 2900 |
| 40 | | 49.0 | 41 | 23 | n.d. | 29 | 4200 | 3100 | 540 | 33000 | 2450 | 2150 |
| | | | | | | | *P20954 (MT)* | | | | | |
| 0.5 | 10 | 16.6 | 135 | 38 | 8.4 | 62 | 7950 | 4300 | 520 | 36000 | 5950 | 3550 |
| 2 | | 10.2 | 212 | 45 | 8.2 | 48 | 9700 | 4450 | 285 | 39000 | 5050 | 3350 |
| 10 | | 15.2 | 152 | 42 | 6.3 | 99 | 14550 | 5150 | 445 | 40500 | 4600 | 3500 |
| 40 | | 29.0 | 118 | 39 | 4.4 | 79 | 17150 | 4050 | 695 | 33000 | 4150 | 3500 |
| 0.5 | 15 | 7.8 | 54 | 40 | n.d. | 55 | 3850 | 5000 | 335 | 44500 | 5400 | 2600 |
| 2 | | 10.0 | 62 | 43 | 9.8 | 62 | 4600 | 5050 | 325 | 47000 | 5800 | 2750 |
| 10 | | 17.2 | 147 | 42 | 5.6 | 56 | 8550 | 5100 | 460 | 53500 | 4550 | 2550 |
| 40 | | 41.9 | 41 | 37 | 4.2 | 42 | 6600 | 4750 | 915 | 49500 | 2750 | 2400 |
| | | | | | | | *P22932 (MI)* | | | | | |
| 0.5 | 10 | 13.9 | 112 | 36 | 10.1 | 64 | 9050 | 4800 | 550 | 39500 | 6450 | 3600 |
| 2 | | 12.5 | 153 | 44 | 10.4 | 55 | 7950 | 4350 | 420 | 37000 | 5650 | 3300 |
| 10 | | 13.6 | 106 | 39 | 5.7 | 80 | 15650 | 5050 | 630 | 44500 | 4550 | 3300 |
| 40 | | 35.3 | 102 | 40 | 4.6 | 59 | 14000 | 3850 | 915 | 30000 | 4500 | 3400 |
| 0.5 | 15 | 17.7 | 23 | 38 | 8.9 | 56 | 3150 | 5150 | 335 | 48000 | 5500 | 2750 |
| 2 | | 15.1 | 37 | 49 | 10.1 | 65 | 4100 | 5350 | 390 | 47000 | 5800 | 2750 |
| 10 | | 14.7 | 100 | 43 | 5.9 | 52 | 7400 | 5000 | 630 | 48500 | 4650 | 2650 |
| 40 | | 61.8 | 34 | 38 | 4.7 | 37 | 6300 | 4750 | 1220 | 51000 | 3200 | 2500 |
| | | | | | | | *P24036 (I)* | | | | | |
| 0.5 | 10 | 17.6 | 96 | 57 | 9.9 | 56 | 13650 | 4000 | 455 | 36500 | 3900 | 2800 |
| 2 | | 11.8 | 196 | 64 | 12.0 | 67 | 15450 | 5050 | 415 | 49500 | 4400 | 3100 |
| 10 | | 27.4 | 106 | 55 | 9.0 | 87 | 11450 | 4450 | 665 | 42000 | 4750 | 3350 |
| 40 | | 52.5 | 162 | 56 | 6.2 | 76 | 16250 | 3700 | 825 | 32000 | 3550 | 2750 |
| 0.5 | 15 | 10.8 | 38 | 51 | 7.9 | 45 | 4700 | 4550 | 270 | 50500 | 3200 | 2300 |
| 2 | | 18.8 | 41 | 59 | 8.2 | 48 | 5550 | 4550 | 300 | 57500 | 2900 | 2200 |
| 10 | | 18.3 | 133 | 43 | 7.2 | 57 | 8850 | 5200 | 590 | 54500 | 3700 | 2750 |
| 40 | | 26.3 | 39 | 45 | 5.8 | 33 | 6850 | 4850 | 880 | 62000 | 1830 | 2050 |

## 5.4 Discussion

The principal aim of this chapter was to develop a solution screening system that enables the selection of genotypes of lupins tolerant to calcareous soils. The first experiment indicated that increased $KHCO_3$ in solution will induce chlorosis in *L. angustifolius*, *L. albus*, *L. atlanticus* and *L. pilosus*. The second (2a), third (2b) and fourth (2c) experiments focussed on developing the screening method for *L. pilosus* and showed that the chlorosis ranking of genotypes in a complete nutrient solution with 15 mM $KHCO_3$ correlates significantly with the ranking achieved on a calcareous soil.

*Genotypic variation*

The results from these experiments indicate that there was variation for tolerance to high $HCO_3^-$ in solution both between species and among *L. pilosus* genotypes. In experiment 1 individual genotypes of four species of lupin and a field pea were compared. Results showed a similar trend to experiment 1 of chapter 4. The most tolerant species of lupin to increasing $KHCO_3$ concentrations in solution, in terms of chlorosis, was *L. pilosus*, followed by *L. albus*, *L. atlanticus* and *L. angustifolius*. The field pea cultivar showed no chlorosis at any of the $KHCO_3$ concentrations. For the growth measurements, the ranking of lupin species was similar, although the number of leaves and shoot and root dry weight of field peas was significantly reduced at high $KHCO_3$ concentrations (15 and 20 mM).

The genetic variation among *L. pilosus* genotypes was consistent across experiments 2a, 2b and 2c. In experiment 2a at $KHCO_3$ concentrations less than 10 mM there was little difference among genotypes for chlorosis measurements. At 10 mM $KHCO_3$ the intolerant genotype P24036 and one of the moderately intolerant genotypes P22932 could be clearly identified in terms of chlorosis, but all other genotypes showed little or no chlorosis. At 15 mM $KHCO_3$ the chlorosis ranking of genotypes was highly correlated with rankings in the preliminary soil screen, particularly at 21 DAT. The 20 mM $KHCO_3$ treatment caused moderate to severe chlorosis in most genotypes, although the ranking of genotypes was similar to 15 mM $KHCO_3$.

There was genotypic variation among *L. pilosus* genotypes for growth measurements and nutrient concentrations, but it was not correlated with chlorosis rankings in this experiment or the preliminary soil screen. Relationships between chlorosis, growth measurements, and nutrient concentrations will be discussed below.

In experiments 2b and 2c the form of $HCO_3^-$ (K or Na) and the concentration of Fe in solution did not affect the ranking of *L. pilosus* genotypes significantly.

*Effect of increasing bicarbonate concentrations in solution on growth and chlorosis*

Increasing the concentration of $KHCO_3$ in solution had a significant effect on the growth and chlorosis of all lupin species and field pea. The first notable effects were a concurrent reduction in root elongation rate at 0-2 DAT or 0-4 DAT in all species, although in experiment 1 there appeared to be no effect of increasing $KHCO_3$ on the root elongation rate of the *L. pilosus* genotype (P20954) from 0-4 DAT. In experiment 2a all *L. pilosus* genotypes showed a concurrent reduction in root elongation rate from 0-4 DAT. Reasons for differences between experiments are unknown, although it may be related to the change in container size, i.e. in experiment 1, 5 litre buckets were used, whilst in experiments 2a, 2b and 2c, 22 litre tanks were used.

*Lupinus pilosus* and *P. sativum* showed less reduction in the root elongation rate at higher $KHCO_3$ than other species in experiment 1 which is consistent with previous research (Tang, *et al.*, 1996a). Lee and Woolhouse (1969) demonstrated that $HCO_3^-$ inhibited cell elongation and not cell division, whilst Tang, *et al.* (1996b) suggested that the limited root elongation of *L. angustifolius* at high pH is probably related to a failure to acidify the apoplast.

In experiment 1 total root length 28 DAT was also negatively correlated with $KHCO_3$ concentrations. Root growth was generally stimulated from 0.2 to 2 mM $KHCO_3$ concentrations and reduced at concentrations greater than 5 mM. Also at concentrations greater than 5 mM proteoid roots were noted in *L. pilosus*, *L. albus* and *L. atlanticus*. The quantity of proteoid roots increased with increasing $KHCO_3$ concentrations in solution. In previous research in soil systems it has been suggested that proteoid roots are formed as a response to P deficiency which is a major problem in calcareous soils (Gardner, *et al.*, 1982b; Gardner, *et al.*, 1982c). It has been shown that proteoid roots may also improve the uptake of Fe and Mn (Gardner, *et al.*, 1982c). In this research nutrient concentrations in the YEL of all species were often marginal at higher $KHCO_3$ concentrations (> 5 mM) compared with critical levels that have been indicated for *L. albus* or *L. angustifolius*. Fe, Mn and P were all low at higher $KHCO_3$ concentrations, indicating that the production of proteoid roots could be in response to the $HCO_3^-$ induced deficiency of Fe, Mn and/or P.

Consistent with previous studies (Coulombe, *et al.*, 1984b; Chaney, *et al.*, 1992a; Shi, *et al.*, 1993), increasing $HCO_3^-$ in solution induces chlorotic symptoms in intolerant genotypes. At 5 mM $KHCO_3$ in experiment 1 chlorosis symptoms in the YEL resembling Fe deficiency were noted in P22917 (I). The first significant chlorosis in Gungurru, Kiev Mutant and P20954 (MT) was at 7.5 mM, 10 mM and 15 mM $KHCO_3$, respectively. The field pea cultivar showed no chlorosis. These results appear to reflect the relative adaptation of lupins to calcareous soil, in that *L. pilosus* is the most adapted species, whilst *L. angustifolius* and *L. atlanticus* are poorly adapted.

In experiment 2a no chlorosis was noted at 5 mM $KHCO_3$ in any of the *L. pilosus* genotypes except the intolerant line P24036 (I) at 28 DAT. At 10 mM $KHCO_3$ it appeared that only intolerant and moderately intolerant lines could be differentiated, whilst at 15 mM $KHCO_3$ there was a wide range of chlorotic symptoms which reflected the adaptation to a calcareous soil. These $HCO_3^-$ concentrations are similar to that used by Coulombe, *et al.* (1984b), Chaney, *et al.* (1992a) and Chaney, *et al.* (1992b), despite large differences in the basal nutrient concentrations in solution.

On calcareous soils, the major reason for chlorosis in intolerant species/genotypes is $HCO_3^-$ induced Fe deficiency (Coulombe, *et al.*, 1984a; Mengel, *et al.*, 1984). Conversely, the previous chapter suggested that low Mn concentrations or a direct effect of $HCO_3^-$ on chlorophyll production might be the cause for chlorosis. The present study in solution culture showed that increased chlorosis in all lupin species except *L. albus* was related to a reduction in Mn, Zn, P and S and an increase in K concentration. In experiment 1 B and Ca concentrations were reduced at higher $KHCO_3$ concentrations, whilst in experiment 2a increased concentrations of Mg and decreased concentrations of Fe and Cu appear to associated with increased chlorosis. For *L. albus* increased concentrations of Na, K and S in the YEL appear to be associated with increased chlorosis.

Shoot and root dry weights were generally decreased as the concentration of $KHCO_3$ in solution increased. They were also significantly related to chlorosis measurements unlike the previous chapter when plants were grown in a calcareous soil. The reduction in shoot and dry weight is likely to a result of the reduced root elongation rates and increased chlorosis associated with high concentrations of $KHCO_3$ in solution.

*Conditions for solution screening*

The major aim of this chapter was to determine a suitable solution screening method which identified the tolerance of lupins to calcareous soils. Results from this chapter indicate that chlorosis score recorded 21 DAT in a complete nutrient solution with 15 mM $KHCO_3$ will provide good discrimination between tolerant and intolerant genotypes as identified in the preliminary soil screening system.

Although root elongation rate, number of leaves and shoot and root dry weight were inversely related to increased $KHCO_3$ concentrations, there were inconsistent differences between genotypes. The ideal measurement for a rapid solution screen would be root elongation rate as it is non-destructive and can be taken after only a few days growth similar to that used when screening wheat cultivars for tolerance to B toxicity (Campbell, *et al.*, 1998). As indicated in this study differences among genotypes do not correlate with preliminary soil screening, thus root elongation rate is unsuitable for selecting genotypes tolerant of calcareous soils. The only other non-destructive measurement recorded, except chlorosis, was number of leaves. Results again showed very small differences between genotypes, but no relationship with preliminary soil screening results. Both shoot and root dry weight are destructive, hence are unsuitable for a screening system that is to be used with early generation material in a breeding program.

The two methods of analysing chlorosis used in this study were visual chlorosis score and chlorophyll meter readings. There was highly significant correlation between both measurements, therefore chlorosis score was defined as being the most efficient method to select tolerant genotypes, as it is easier and faster to record.

Chlorosis symptoms were generally increased over time and the greatest differentiation among genotypes was at 21 DAT similar to the soil screening system.

The form of $HCO_3^-$ (K or Na) did not appear to affect the ranking of genotypes significantly, thus either form of $HCO_3^-$ could be used. For consistency and as $KHCO_3$ caused a slightly greater discrimination between tolerant and intolerant genotypes $KHCO_3$ was defined as the most suitable form of $HCO_3^-$ for use in the screening method.

This study also examined the effect of FeEDDHA concentrations in nutrient solutions on genotypic variation in response to $HCO_3^-$. Other previous studies with similar screening methods utilised much lower concentrations than was used in the current study. Lower FeEDDHA concentrations in the solution in this study caused very severe chlorosis, making it more difficult to discriminate between genotypes. A reduction in the Fe concentration of the screening system may be suitable if selecting between tolerant and moderately intolerant genotypes.

# CHAPTER 6

## Screening genotypes of *L. pilosus* and *L. atlanticus* for tolerance to calcareous soil.

## 6.1 Introduction

Genotypic variation has been identified in *L. pilosus* for tolerance to calcareous soil, which enabled the development of soil and solution protocols for screening breeding lines and landraces (Chapters 4 and 5). These methods focussed on identifying genotypes tolerant to highly calcareous soils (> 20% $CaCO_3$), as the same genotypes are also likely to perform well on soils that are slightly or moderately calcareous. In this work only a small number of contrasting *L. pilosus* genotypes have been compared. Thus, the experimental work reported in this chapter aims to establish the range of tolerance in *L. pilosus* and *L. atlanticus* relative to other species of lupins.

Identification and incorporation of tolerant genotypes into breeding programs is an essential prerequisite for the release of cultivars adapted to calcareous soils. Solution screening is of particular benefit in early generations as the plants can be screened and then transplanted to soil for recovery and for crossing and seed production.

While the screening systems developed in this research, particularly the solution culture system, have been conducted under easily replicated controlled environmental conditions other factors may need to be considered to enhance the accuracy of the screening. Other research has indicated that seed size and nutritional status can affect the ranking of genotypes in screening for nutrition stress. For example, Khabaz-Saberi *et al.* (1999) has shown that when screening for Mn efficiency in durum wheat all genotypes needed to have a similar Mn content in the seed otherwise apparent efficiency may only be the result of high seed Mn content. Recent research on the tolerance of bread wheat to high levels $HCO_3^-$ in solution has indicated that plants from seed of the same genotype harvested at different sites have different levels of tolerance (C-Y Liu, pers. comm.) suggesting that the $HCO_3^-$ status of the site affects the seedling response.

An understanding of the genetic control of the tolerance would assist in determining the optimum method of incorporating the trait in a breeding program. Tolerance to abiotic stresses is

often controlled by only a few major genes. For example, tolerance to $HCO_3^-$ induced Fe deficiency in soybeans is controlled by a single major gene showing dominance for tolerance together with other minor genes with additive effects (R Chaney pers. comm.). B toxicity in wheat is controlled by at least four major genes (Paull, *et al.*, 1991). When tolerance is controlled by major genes incorporation of the trait into high yielding genotypes can be simply achieved through back-crossing or an $F_2$ progeny method and then screening the progeny. However, if the trait is polygenic, many years of recurrent selection within a population containing the desired trait may need to be undertaken to increase the tolerance to a suitable level (Cowling, *et al.*, 1997).

The major aims of this chapter were to determine the range of intraspecific variation in *L. pilosus* and *L. atlanticus* for tolerance to calcareous soils and to identify whether this tolerance was related with seed size or nutritional status of the seed. Genotypes of *L. angustifolius*, *L. albus*, *L. cosentinii*, and *L. digitatus* were also compared to gauge the relative tolerance of a range of species. A cross between a tolerant and moderately intolerant genotype of *L. pilosus* was screened to indicate the genetic control of tolerance.

## 6.2 Materials and methods

*Genetic Material*

Genotypic screening

The species and genotypes that were screened to identify their tolerance to calcareous soils are summarised in Table 6.1. The work focussed on examining genetic variation in *L. pilosus* and *L. atlanticus* as these species have been identified as having potential to expand the lupin growing areas on to calcareous soils (Buirchell, 1995).

Genetic control of tolerance in *L. pilosus*

The $F_2$ population (120 plants) and 10 plants of the $F_1$ population of the cross and its reciprocal between a tolerant (P23370) and a moderately tolerant (P22932) genotype was screened in solution culture. Both genotypes had been identified from the preliminary soil screening and had shown large differences in chlorosis score in the solution screening system (Chapters 4 and 5).

The solution screening method was not fully developed until 24 months into this 36 month project so the full range of genotypic variation in *L. pilosus* has only recently been identified.

**Table 6.1** The origin, breeding status, flower colour, flowering time, seed weight (g per 100 seed) and rainfall, pH and soil type of collection site plus other relevant information of *L. atlanticus*, *L. pilosus*, *L. albus*, *L. angustifolius*, *L. cosentinii* and *L. digitatus* genotypes tested for response to calcareous soils (Clements and Cowling, 1990; Clements and Cowling, 1991). All genotypes were obtained from the Australian lupin collection at Agriculture Western Australia.

| Species | Genotype | Country origin | Breeding status[1] | Flower colour | Flowering Time (Days) | Seed weight (g 100 seed)[2] | Collection site details | | | Other information[3] |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Rainfall | pH | Soil texture | |
| *L. atlanticus* | AM 2.15 | Australia | mutant | blue | 106 | 28.3 | | | | breeders line |
| | AM 3.18 | Australia | mutant | blue | 101 | 26.0 | | | | breeders line |
| | P22918 | Morocco | landrace | blue | 98 | 35.0 | 375 | 7.5 | loam | |
| | P22919 | Morocco | landrace | blue | 102 | 29.4 | 200 | 7.2 | loam | |
| | P22920 | Morocco | landrace | blue | 122 | 25.1 | 400 | 7.6 | loam | |
| | P22921 | Morocco | landrace | blue | 104 | 35.3 | 490 | 7.6 | loam | |
| | P22924 | Morocco | landrace | blue | 106 | 29.9 | | | | |
| | P22927 | Morocco | landrace | blue | 100 | 30.1 | | | | single plant selections a collection |
| | P22928 | Morocco | landrace | blue | 104 | 33.1 | | | | |
| | P22930 | Morocco | landrace | blue | 120 | 27.2 | 500 | 7.5 | loam | |
| | P22931 | Morocco | landrace | blue | 108 | 29.5 | 300 | 7.8 | loam | |
| | P26943 | Morocco | landrace | blue | 110 | 29.3 | 350 | 7.0 | loam | |
| | P26944 | Morocco | landrace | blue | 109 | 38.4 | 350 | 7.0 | loam | |
| | P26945 | Morocco | landrace | blue | 110 | | 250 | 7.5 | loam | |
| | P26946 | Morocco | landrace | blue | | 31.5 | 250 | 7.5 | sand | |
| | P26947 | Morocco | landrace | blue | | 29.8 | 250 | 7.5 | loam | |
| | P26948 | Morocco | landrace | blue | 118 | 32.3 | | | | |
| | P26950 | Morocco | landrace | blue | 118 | 24.3 | 250 | 7.5 | loam | |
| | P26951 | Morocco | landrace | blue | | 35.1 | 250 | 6.5 | sandy loam | |
| | P26953 | Morocco | landrace | blue | 111 | 29.2 | | | | |
| | P27030 | Morocco | landrace | blue | 114 | 28.9 | | | | |
| | P27032 | Morocco | landrace | blue | 108 | 32.2 | | | | |
| | P27216 | Morocco | landrace | blue | | 27.4 | 350 | 7.5 | loamy sand | |
| | P27217 | Morocco | landrace | blue | | 33.4 | 350 | 6.5 | loamy sand | |
| | P27218 | Morocco | landrace | blue | | 33.9 | 350 | 8.0 | loamy sand | |
| | P27219 | Morocco | landrace | blue | | 32.2 | 400 | 7.7 | loamy sand | |
| | P27222 | Morocco | landrace | blue | | 27.3 | 350 | 8.0 | loamy sand | |
| | P27224 | Morocco | landrace | blue | | 32.0 | 400 | 7.7 | sandy loam | |
| | P27229 | Morocco | landrace | blue | | 36.0 | 480 | 7.7 | sandy loam | |

| Species | Genotype | Country origin | Breeding status | Flower colour | Flowering Time (Days) | Seed weight (g 100 seed) | Collection site details | | | Other information |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Rainfall | pH | Soil texture | |
| *L. atlanticus* | P27230 | Morocco | landrace | blue | | 35.9 | 480 | 7.7 | sandy loam | |
| | P27233 | Morocco | landrace | blue | | 43.0 | 480 | 7.7 | sandy loam | |
| | P27234 | Morocco | landrace | blue | | 42.6 | 500 | 8.7 | sandy loam | |
| | P27236 | Morocco | landrace | blue | | 38.0 | 500 | 7.7 | sandy loam | |
| | P27237 | Morocco | landrace | blue | | 33.9 | 500 | 9.5 | sandy loam | |
| | P27239 | Morocco | landrace | blue | | 29.0 | 500 | 8.0 | sandy loam | |
| | P27240 | Morocco | landrace | blue | | | 500 | 8.0 | sandy loam | |
| | P27242 | Morocco | landrace | blue | | 35.5 | 600 | 9.5 | sandy loam | |
| | P27243 | Morocco | landrace | blue | | 41.9 | 600 | 9.2 | sandy loam | |
| | P27248 | Morocco | landrace | blue | | 41.1 | 520 | 9.0 | sandy loam | |
| | MAR 5133.3 | Morocco | landrace | blue | | 34.3 | | | | |
| | 93E02-4-12 | Australia | mutant | white | | 13.1 | | | | soft seeded breeders line small seed |
| | 93E02-3-12 | Australia | mutant | white | | 15.9 | | | | soft seeded breeders line small seed |
| | 93E02-4-11 | Australia | mutant | white | | 10.6 | | | | soft seeded breeders line small seed |
| | 93E02-1-11 | Australia | mutant | white | | 11.8 | | | | soft seeded breeders line small seed |
| | 93E02-3-11 | Australia | mutant | white | | 8.9 | | | | soft seeded breeders line small seed |
| | 93E02-1-13 | Australia | mutant | white | | 13.1 | | | | soft seeded breeders line small seed |
| *L. pilosus* | P20953 | Australia | mutant | purple | | 59.8 | | | | Early flowering |
| | P20954 | Australia | mutant | orange pink | | 49.0 | | | | Early flowering, naturalized W.A. |
| | P20959 | Australia | mutant | pale pink | | | | | | |
| | P22932 | Denmark | collection | pale pink | 86 | 51.6 | | | | Hula plain, Early flowering |
| | P22937 | Israel | landrace | purple | 84 | 79.4 | | | | |
| | P23029 | Syria | landrace | purple | 107 | 50.0 | 1000 | 6.5 | | Golan Heights |
| | P23030 | Syria | landrace | purple | 101 | 45.7 | 900 | 7.0 | | Golan Heights |
| | P23034 | Greece | landrace | purple | 132 | 51.6 | | | | very late flowering |
| | P23338 | USSR | collection | purple | 80 | 55.7 | | | | |
| | P23339 | USSR | collection | pale pink | 81 | 52.0 | | | | |
| | P23341 | Hungary | collection | pale pink | 82 | 63.6 | | | | |
| | P23345 | Israel | landrace | purple | 101 | 76.2 | | | | Tel Abor-Nida, Golan |
| | P23366 | Israel | landrace | purple | 105 | 51.0 | | | | |
| | P23370 | Israel | landrace | purple | 101 | 73.9 | | | | |
| | P24033 | Greece | landrace | purple | 107 | 51.2 | | 7.4 | loam | Crete, soil reacts with HCl |
| | P24034 | Greece | landrace | purple | 89 | 54.1 | | 7.7 | sand | Kikladhes, soil reacts with HCl |

| Species | Genotype | Country origin | Breeding status | Flower colour | Flowering Time (Days) | Seed weight (g 100 seed) | Collection site details | | | Other information |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Rainfall | pH | Soil texture | |
| *L. pilosus* | P24035 | Greece | landrace | purple | 92 | 54.1 | | 8.0 | silt | Kikladhes, soil reacts with HCl |
| | P24036 | Greece | landrace | purple | 91 | 54.4 | | 6.7 | loam | Kikladhes |
| | P25776 | Germany | collection | white | 82 | 55.6 | | | | |
| | P26883 | Israel | landrace | purple | 87 | 68.3 | | | | |
| | P26886 | Israel | landrace | purple | 100 | 68.7 | | | | |
| | P26888 | Israel | landrace | purple | 102 | 66.5 | | 8.7 | loamy clay | Ha-Golan |
| | P26892 | Greece | landrace | purple | 90 | 61.1 | 360 | 6.5 | sand | Kikladhes |
| | P26894 | Greece | landrace | purple | 105 | 55.7 | 720 | 7.0 | sand | Lesuos |
| | P26896 | Greece | landrace | purple | 94 | 51.3 | 650 | 6.5 | sandy loam | Kikladhes |
| | P26897 | Greece | landrace | purple | 99 | 56.3 | 650 | 6.5 | sand | Kikladhes |
| | P26899 | Greece | landrace | purple | 94 | 55.5 | 550 | 6.5 | sand | Kikladhes |
| | P26901 | Syria | landrace | purple | 103 | 63.6 | | | | North Latakia |
| | P26902 | Turkey | landrace | purple | 108 | 70.0 | 1100 | 7.2 | loam | Turungillai village |
| | P26903 | Turkey | landrace | purple | 117 | 45.0 | 1100 | 7.5 | loam | Hatay, late flowering |
| | P26907 | Egypt | collection | purple | 81 | 55.6 | | | | |
| | P26910 | USSR | collection | pale pink | 84 | 59.2 | | | | |
| | P26912 | Germany | collection | purple | 82 | 60.0 | | | | |
| | P26915 | Hungary | collection | purple | 78 | 57.0 | | | | |
| | P26916 | Hungary | collection | pale pink | 81 | 56.9 | | | | |
| | P26917 | USSR | collection | purple | 81 | 68.5 | | | | |
| | P26920 | Hungary | collection | white | 81 | 54.8 | | | | |
| | P26922 | USSR | collection | purple | 75 | 60.0 | | | | |
| | P26927 | USSR | collection | purple | 81 | 58.4 | | | | |
| | P26930 | Greece | landrace | white | 79 | 67.0 | | | | |
| *L. angustifolius* | Danja | Australia | cultivar | white | 75 | 19.7 | | | | |
| | Yorrel | Australia | cultivar | white | 71 | 18.9 | | | | |
| | Merrit | Australia | cultivar | white | 76 | 17.8 | | | | |
| | Gungurru | Australia | cultivar | white | 76 | 17.1 | | | | |
| *L. albus* | Hamburg | Australia | cultivar | white | | 42.3 | | | | |
| | Kiev Mutant | Australia | cultivar | white | | 38.0 | | | | |
| | P27484 | | | white | | 38.6 | | | | |
| | P27485 | | | white | | 37.0 | | | | |
| | P27486 | | | white | | 38.8 | | | | |

| Species | Genotype | Country origin | Breeding status | Flower colour | Flowering Time (Days) | Seed weight (g 100 seed) | Collection site details | | | Other information |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Rainfall | pH | Soil texture | |
| *L. albus* | P27487 | | | white | | 42.9 | | | | |
| | P27488 | | | white | | 38.3 | | | | |
| | P27797 | | | white | | 48.6 | | | | |
| | P28295 | | | white | | 48.3 | | | | |
| *L. cosentinii* | Erregulla-s | Australia | cultivar | white | | 17.5 | | | | |
| *L. digitatus* | D01 | Egypt | landrace | blue | | 13.6 | | | | |

[1.] Breeding status refers to whether the genotype is a landrace, either collected from the field or obtained from another collection (collection), a mutant developed via mutation breeding techniques or a cultivar.

[2.] Seed weights were recorded before ICP-AES analysis of seed.

[3.] All *L. atlanticus* genotypes were collected in the Atlas mountains (> 500 m altitude) of Morocco.

Since then a diallel cross between other tolerant and intolerant genotypes has been developed, but due to long generation times and the time constraints of this project, these $F_2$ generations have not yet been screened.

*Screening Methods*

Soil screening (Plate 6.1)

The soil screening method (Chapter 4) was conducted in a plastic lined wooden box measuring 146 cm x 70 cm x 28 cm. The soil was a calcareous sandy loam collected from Wangary on the Eyre Peninsula of South Australia (Table 4.1; Wilhelm, *et al.*, 1988). The soil was air-dried, placed into the box and watered to a moisture content of 90% of field capacity (Chapter 4). No nutrients were added to the soil. The soil was mixed and equilibrated for one week before sowing. The water content of the soil was recorded using a moisture meter inserted to 15 cm depth so that the soil could be rewatered to 90% of field capacity.

Solution screening (Plate 6.2)

The solution screening method was conducted in 25 litre plastic tanks (Section 5.3.2). The solutions were as described in table 5.1; the treatment used was 15 mM $KHCO_3$ buffered with 1.5 g $L^{-1}$ $CaCO_3$. Nutrient solutions were changed 14 DAT.

*Plant growth and experimental design*

All seeds for genotypic screening had been harvested from plants grown the previous year in the Urrbrae clay loam under field conditions at Waite Campus of the University of Adelaide to minimize size and nutritional effects associated with seed harvested from plants grown at different sites. For the $F_2$ screening, the $F_1$ generation had been grown in a potting mix under glasshouse conditions.

Prior to germination, seeds were surface sterilized and *L. pilosus* and *L. atlanticus* scarified (section 4.3.2).

Soil screening

Seeds of uniform size (for each genotype) were imbibed on moistened filter paper at 20$^{O}$C for 48 hours. Twelve genotypes per box, in two replicates (five seeds per row 5 cm apart), were sown in a completely randomized design at 1 cm depth and inoculated (Section 4.3.2). Plants were grown

under glasshouse conditions (15 - 30°C) for 21 days and boxes watered to 90% of field capacity every two days.

Solution screening

Seeds were germinated, transferred to tanks and grown for 21 days as described in Chapter 5 (Section 5.3.2).

When determining the genetic control of tolerance, the progeny of the cross was only screened in solution culture. After recording the level of chlorosis, seedlings were carefully removed and transferred to a non-calcareous potting mix. Plants were grown to harvest to collect seed and allow for screening of the $F_3$ families to verify the $F_2$ classifications.

A tolerant and an intolerant control *L. pilosus* genotype was grown in every soil box and solution tank. For most of the trials the tolerant control was P23370 (T) and the intolerant control was P22932 (MI). Due to seed shortage, in some trials the tolerant and intolerant controls were P22937 (T) and P24036 (I), respectively.

*Measurements*

Prior to screening, nutrient concentrations of the seed (Table 6.2) were determined by ICP-AES analysis so that effects of seed nutrition on the tolerance of a genotype to calcareous soil could be observed. Seed weight (g per 100 seed) was also recorded (Table 6.1). Individual seed weights were not determined.

For both screening methods, visual chlorosis score (Table 3.2) was recorded 21 days after sowing (soil screen) or transplanting (solution screen). The tolerance of genotypes as referred to in the text was based on the chlorosis scores in the soil screen, i.e. <0.5: tolerant, 0.5-1.5: moderately tolerant, 1.5-2.5: moderately intolerant, >2.5: intolerant. For comparison chlorophyll meter readings of the YELs were recorded in some of the trials for genotypic differences and for all of the lines in the $F_2$ population.

*Statistical analyses*

For genotypic screening, an analysis of variance (Genstat 5.41) was conducted on the chlorosis score for both the soil and solution screening in *L pilosus* and *L. atlanticus* using individual

plants as pseudoreplication (Section 5.3.2). Individual means were compared by LSD. No analyses were conducted on other species as only a few genotypes were included.

To identify the major factors (i.e. seed weight, seed nutrient concentrations, flowering time or rainfall and pH of the original collection site) affecting chlorosis scores and chlorophyll meter readings of *L. pilosus* and *L. atlanticus*, a multiple linear regression analysis using backward elimination was calculated. Seed nutrient contents were not used as they were derived from seed weight and nutrient concentrations, thus display collinearity.

*Genetic Analysis of $F_2$ generation*

For the $F_2$ generation, the number of genes controlling the response was estimated on the basis of the variance of the segregating population compared to the expected variance of the one and two gene models (Mather and Jinks, 1977). The expected variance was calculated from the variance components of the parents and an estimation of variance in the $F_1$ population ($V_{F_1} = (V_{P_1} + V_{P_2})/2$; where $V_{P_1}$ and $V_{P_2}$ refer to the variance components of the parents). Using the model of Mather and Jinks (1977) assuming that there is no linkage, no epistasis and no dominance:

One gene segregation: $V_{F_2} = 1/2d^2+E$

Two gene segregation: $V_{F_2} = 1/4d^2+E$

where $V_{F_2}$ is the variance of the $F_2$ population, d is the departure from the midpoint (m) of the mean of homozygous genotypes (AA and aa), and E is environmental variance ($E = 1/4\ V_{P_1} + 1/4\ V_{P_2} + 1/2\ V_{F_1}$).

The confidence intervals of observed variances for the $F_2$ population were calculated as follows (Chantachume, 1995):

$(V_O \times df)/\chi^2_a <= \text{Confidence interval} <= (V_O \times df)/\chi^2_b$

where $V_O$ = observed variance of the population, *df* = degrees of freedom of n-1, n = number of plants in the $F_2$ population, and $\chi^2_a$ and $\chi^2_b$ are the lower and upper level chi-squared values at $P = 0.95$, $df = n-1$.

The populations were identified as deviating significantly from the expected variances for single or two gene models when the expected variance was outside the range of the confidence interval of the observed variance.

**Table 6.2** Nutrient concentrations (mg $kg^{-1}$) in seed of *L. pilosus*, *L. atlanticus*, *L. albus*, *L. angustifolius* and *L. digitatus* genotypes harvested from plants grown in the Urrbrae clay loam under field conditions at Waite Campus of the University of Adelaide.

| Genotype | Fe | Mn | B | Cu | Zn | Ca | Mg | Na | K | P | S |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | *L. pilosus* | | | | | | |
| P20953 | 34 | 90 | 11 | 6.9 | 53 | 2200 | 2100 | 560 | 7300 | 3900 | 2500 |
| P20954 | 20 | 190 | 11 | 6.3 | 45 | 2100 | 1900 | 510 | 7800 | 2800 | 2000 |
| P20959 | 21 | 53 | 10 | 6.8 | 51 | 2800 | 2200 | 530 | 7300 | 4500 | 2700 |
| P22932 | 22 | 82 | 12 | 6.7 | 57 | 2600 | 2100 | 540 | 7300 | 3300 | 2200 |
| P22937 | 25 | 95 | 11 | 6.6 | 43 | 1600 | 2000 | 700 | 8400 | 3100 | 2600 |
| P23029 | 18 | 75 | 10 | 9.0 | 34 | 1600 | 1900 | 770 | 7600 | 3600 | 3200 |
| P23030 | 19 | 180 | 10 | 6.7 | 41 | 2100 | 1800 | 630 | 7400 | 3100 | 3500 |
| P23034 | 23 | 110 | 11 | 8.1 | 61 | 2200 | 2200 | 850 | 8200 | 4200 | 2600 |
| P23338 | 24 | 110 | 11 | 7.6 | 53 | 2000 | 2100 | 870 | 7500 | 4000 | 2300 |
| P23339 | 18 | 260 | 10 | 7.0 | 44 | 2100 | 1700 | 500 | 7700 | 2600 | 1900 |
| P23341 | 24 | 170 | 12 | 8.1 | 54 | 2300 | 2000 | 630 | 8600 | 3500 | 2300 |
| P23345 | 23 | 330 | 12 | 8.1 | 42 | 2600 | 1900 | 510 | 8200 | 2800 | 2700 |
| P23366 | 17 | 100 | 11 | 7.6 | 34 | 1900 | 1700 | 620 | 8500 | 3200 | 3300 |
| P23370 | 27 | 100 | 11 | 6.5 | 47 | 1800 | 1800 | 740 | 7800 | 3200 | 2600 |
| P24033 | 30 | 76 | 11 | 6.9 | 48 | 1900 | 1900 | 810 | 8600 | 3400 | 2600 |
| P24034 | 26 | 110 | 13 | 7.5 | 46 | 2200 | 2000 | 730 | 8100 | 3000 | 2500 |
| P24035 | 25 | 100 | 10 | 6.0 | 51 | 2200 | 1900 | 740 | 8100 | 3200 | 2700 |
| P24036 | 23 | 89 | 12 | 7.5 | 46 | 2000 | 1900 | 800 | 7300 | 3000 | 2500 |
| P25776 | 21 | 120 | 11 | 7.3 | 47 | 1900 | 1700 | 660 | 6800 | 3300 | 2200 |
| P26883 | 22 | 97 | 13 | 7.3 | 37 | 1700 | 1700 | 890 | 7600 | 2700 | 2300 |
| P26886 | 21 | 59 | 13 | 8.0 | 42 | 2000 | 1900 | 850 | 7700 | 3000 | 2500 |
| P26888 | 19 | 110 | 13 | 6.7 | 36 | 1900 | 1800 | 750 | 7200 | 2700 | 2500 |
| P26892 | 19 | 180 | 13 | 8.7 | 45 | 2000 | 2000 | 820 | 7700 | 3000 | 2500 |
| P26894 | 23 | 240 | 13 | 7.9 | 51 | 2200 | 1800 | 990 | 7600 | 2800 | 3400 |
| P26896 | 21 | 200 | 11 | 8.2 | 43 | 2500 | 1900 | 820 | 7200 | 2400 | 2400 |
| P26897 | 23 | 310 | 11 | 8.4 | 49 | 2100 | 2000 | 550 | 7300 | 2400 | 2500 |
| P26899 | 25 | 380 | 13 | 8.6 | 65 | 2700 | 2100 | 510 | 7200 | 3000 | 3000 |
| P26901 | 23 | 260 | 14 | 7.4 | 32 | 1800 | 1900 | 840 | 7900 | 2300 | 2600 |
| P26902 | 18 | 170 | 12 | 6.0 | 39 | 2200 | 1800 | 650 | 7900 | 1900 | 2400 |
| P26903 | 21 | 190 | 10 | 6.8 | 40 | 2500 | 2000 | 300 | 6600 | 3100 | 2700 |
| P26907 | 23 | 320 | 12 | 9.1 | 50 | 2100 | 2000 | 520 | 7600 | 2500 | 2100 |
| P26910 | 21 | 310 | 13 | 7.7 | 47 | 2000 | 1800 | 460 | 7400 | 2700 | 2000 |
| P26912 | 21 | 270 | 13 | 7.1 | 42 | 2000 | 1900 | 570 | 7100 | 2400 | 2200 |
| P26915 | 23 | 320 | 13 | 8.4 | 48 | 2200 | 2100 | 540 | 7800 | 2500 | 2000 |
| P26916 | 21 | 150 | 12 | 8.2 | 47 | 1900 | 1900 | 490 | 8100 | 2900 | 2200 |
| P26917 | 18 | 330 | 12 | 6.2 | 41 | 2200 | 2000 | 530 | 7200 | 2800 | 2100 |
| P26920 | 23 | 400 | 13 | 8.1 | 48 | 2400 | 2100 | 540 | 6800 | 2600 | 2200 |
| P26922 | 22 | 290 | 11 | 7.5 | 49 | 2000 | 2000 | 500 | 7100 | 2500 | 1900 |
| P26927 | 20 | 440 | 12 | 8.9 | 47 | 2000 | 1800 | 560 | 7300 | 2300 | 2000 |
| P26930 | 26 | 410 | 14 | 9.9 | 56 | 2200 | 2000 | 760 | 7300 | 2500 | 2300 |
| | | | | | *L. atlanticus* | | | | | | |
| AM 2.15 | 28 | 290 | 10 | 7.0 | 54 | 2300 | 1900 | 270 | 8800 | 2900 | 4000 |
| AM 3.18 | 34 | 290 | 10 | 6.2 | 50 | 1900 | 1800 | 390 | 8700 | 2700 | 4000 |
| P22918 | 35 | 230 | 13 | 7.2 | 59 | 1900 | 1900 | 330 | 9000 | 2900 | 4000 |
| P22919 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| P22920 | 26 | 430 | 12 | 7.1 | 40 | 2300 | 2100 | 72 | 9800 | 3100 | 3600 |
| P22921 | 34 | 240 | 11 | 7.6 | 46 | 2600 | 2000 | 170 | 8900 | 2900 | 4000 |

| Genotype | Fe | Mn | B | Cu | Zn | Ca | Mg | Na | K | P | S |
|---|---|---|---|---|---|---|---|---|---|---|---|
| P22924 | 35 | 300 | 12 | 9.0 | 55 | 2500 | 2200 | 210 | 9600 | 3400 | 4300 |
| P22927 | 45 | 320 | 13 | 8.2 | 58 | 2500 | 2200 | 250 | 11000 | 3700 | 4800 |
| P22928 | 39 | 400 | 15 | 8.6 | 58 | 3300 | 2400 | 330 | 11000 | 3500 | 5100 |
| P22930 | 39 | 290 | 11 | 7.4 | 57 | 2400 | 2200 | 440 | 12000 | 4400 | 4700 |
| P22931 | 38 | 280 | 14 | 7.8 | 54 | 2000 | 2300 | 400 | 11000 | 3200 | 4800 |
| P26943 | 37 | 300 | 15 | 8.0 | 42 | 2200 | 2300 | 280 | 11000 | 3600 | 4500 |
| P26944 | 57 | 310 | 17 | 9.9 | 54 | 2400 | 2400 | 370 | 12000 | 3800 | 4000 |
| P26945 | 26 | 290 | 12 | 7.3 | 54 | 2000 | 1900 | 300 | 11000 | 4100 | 4400 |
| P26946 | 36 | 440 | 13 | 7.8 | 54 | 2500 | 2000 | 210 | 10000 | 3700 | 4300 |
| P26947 | 35 | 670 | 10 | 9.9 | 58 | 3000 | 2700 | 210 | 10000 | 4500 | 5500 |
| P26948 | 36 | 430 | 11 | 7.5 | 49 | 2300 | 2100 | 200 | 11000 | 3600 | 3900 |
| P26950 | 39 | 480 | 11 | 7.9 | 48 | 2200 | 2100 | 230 | 9200 | 3700 | 4600 |
| P26951 | 41 | 320 | 13 | 7.9 | 49 | 2200 | 1800 | 240 | 9800 | 3600 | 4200 |
| P26953 | 28 | 400 | 10 | 8.3 | 50 | 2400 | 2100 | 260 | 10000 | 4100 | 5200 |
| P27030 | 31 | 430 | 12 | 7.6 | 52 | 2600 | 1900 | 210 | 9600 | 3400 | 4600 |
| P27032 | 28 | 440 | 12 | 8.0 | 55 | 2400 | 2100 | 360 | 10000 | 4100 | 4800 |
| P27216 | 25 | 270 | 12 | 7.5 | 48 | 2400 | 2000 | 200 | 10000 | 3200 | 5000 |
| P27217 | 33 | 310 | 14 | 8.5 | 51 | 2100 | 2000 | 320 | 12000 | 4100 | 5000 |
| P27218 | 27 | 310 | 12 | 6.8 | 53 | 1900 | 1800 | 190 | 10000 | 4100 | 4900 |
| P27219 | 51 | 490 | 12 | 7.6 | 64 | 2900 | 2300 | 200 | 11000 | 4100 | 4800 |
| P27222 | 34 | 480 | 12 | 8.5 | 59 | 2800 | 2200 | 220 | 10000 | 4600 | 4800 |
| P27224 | 49 | 330 | 16 | 7.9 | 50 | 2100 | 2200 | 180 | 11000 | 3900 | 4100 |
| P27229 | 51 | 490 | 13 | 8.6 | 55 | 2800 | 2400 | 240 | 11000 | 5300 | 4700 |
| P27230 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| P27233 | 45 | 270 | 11 | 8.7 | 51 | 2400 | 2200 | 98 | 10000 | 3600 | 5000 |
| P27234 | 39 | 330 | 10 | 8.0 | 48 | 2400 | 2400 | 250 | 11000 | 4000 | 4600 |
| P27236 | 45 | 370 | 13 | 9.1 | 60 | 2700 | 2600 | 240 | 11000 | 5300 | 5000 |
| P27237 | 36 | 260 | 10 | 9.9 | 49 | 2300 | 2100 | 68 | 10000 | 4100 | 4100 |
| P27239 | 26 | 190 | 10 | 9.1 | 58 | 1800 | 1900 | 260 | 11000 | 4500 | 5200 |
| P27240 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| P27242 | 32 | 320 | 07 | 9.9 | 48 | 2000 | 2000 | 370 | 9800 | 3400 | 4600 |
| P27243 | 33 | 220 | 08 | 7.8 | 46 | 1800 | 2100 | 360 | 11000 | 3700 | 4600 |
| P27248 | 37 | 230 | 08 | 9.4 | 51 | 1800 | 1900 | 280 | 11000 | 4200 | 4900 |
| MAR 5133.3 | 25 | 380 | 09 | 7.5 | 49 | 2200 | 1900 | 240 | 8400 | 3800 | 3900 |
| | | | | | *L. angustifolius* | | | | | | |
| Danja | 47 | 180 | 17 | 5.6 | 34 | 3200 | 1900 | 230 | 9500 | 4000 | 3600 |
| Yorrel | 48 | 200 | 17 | 6.2 | 37 | 2800 | 2000 | 200 | 11000 | 4200 | 3400 |
| Merrit | 47 | 190 | 18 | 5.0 | 35 | 3400 | 1900 | 200 | 9300 | 3900 | 3100 |
| Gungurru | 43 | 72 | 15 | 4.5 | 36 | 2400 | 1600 | 360 | 8500 | 2600 | 3000 |
| | | | | | *L. digitatus* | | | | | | |
| D01 | 39 | 700 | 17 | 9.4 | 57 | 2200 | 1900 | 51 | 9900 | 4100 | 5200 |
| | | | | | *L. albus* | | | | | | |
| Hamburg | 35 | 1800 | 18 | 8.3 | 58 | 2200 | 1600 | 550 | 11000 | 4600 | 2900 |
| Kiev Mutant | 23 | n.d. | 14 | 7.6 | 45 | 1500 | 1200 | 660 | 9800 | 2800 | 2500 |
| P27484 | 40 | 2600 | 19 | 8.4 | 57 | 2000 | 1500 | 250 | 9200 | 3400 | 2900 |
| P27485 | 33 | 3000 | 16 | 9.0 | 46 | 2300 | 1400 | 260 | 8400 | 3200 | 3900 |
| P27486 | 39 | 2500 | 24 | 9.3 | 56 | 2300 | 1500 | 280 | 8300 | 3600 | 2900 |
| P27487 | 36 | 2100 | 18 | 9.3 | 45 | 1700 | 1400 | 370 | 8300 | 2800 | 3000 |
| P27488 | 44 | n.d. | 26 | 9.1 | 64 | 1500 | 1200 | 380 | 9600 | 3300 | 2900 |
| P27797 | 37 | n.d. | 24 | 8.5 | 50 | 2200 | 1100 | 540 | 8800 | 3300 | 3200 |
| P28294 | 33 | 2000 | 17 | 8.6 | 39 | 1900 | 1100 | 350 | 8000 | 2800 | 2800 |
| P28295 | 34 | 2500 | 22 | 7.4 | 38 | 2400 | 1300 | 590 | 8700 | 2700 | 2600 |

## 6.3 Results

*Genotypic screening*

The ANOVA suggested that there were significant differences among genotypes of *L. pilosus* and *L. atlanticus* ($P < 0.05$) in chlorosis scores in both soil and solution screening systems.

Chlorosis symptoms on the YEL in *L. pilosus* were first noted from 10 to 14 DAS in the soil screening and 7 to 14 DAT in the solution screening. Generally, the genotypes with higher chlorosis scores at 21 DAS displayed symptoms earlier. At 21 DAS chlorosis scores of the YEL ranged from 0.1 to greater than 3.0 in both screening methods (Fig. 6.1; Plate 6.1a and 6.2b). Chlorosis scores from both methods were highly correlated (Fig. 6.2). P23370 (T) and P22937 (T) had the lowest chlorosis scores, while P24035 (I) and P26903 (I) had the highest scores in the soil screening and P24033 (MI), P24034 (I), P24035 (I) and P24036 (I) had the highest scores in the solution screening (Fig. 6.1). Furthermore, when the genotypes were allowed to grow for longer periods in the soil screening trials, most recovered from chlorosis. Generally the genotypes with higher chlorosis scores were slower to recover.

Chlorosis symptoms on the YEL in *L. atlanticus* were first noted from 7 to 12 DAS in the soil and 7 to 14 DAT in the solution screening. Similar to *L. pilosus*, the genotypes with higher chlorosis scores displayed symptoms earlier. At 21 DAS chlorosis scores of the YEL ranged from 0.1 to 4.5 in the soil (Plate 6.1b) and 0.3 to 3.7 in the solution screening, most being greater than 1.5 (Fig. 6.3). However, there was no correlation in chlorosis scores between soil and solution screening methods (Fig. 6.2). The only genotypes with chlorosis scores less than one in the soil screening were P27233 and P27248 (Fig. 6.3). P27233 had the lowest chlorosis score in the solution screening. There was generally less variability among replicates (individual plants) in the soil than the solution screening (standard deviation 0.5-1.0 *c.f.* 0.7 - 2.0). Unlike *L. pilosus*, most genotypes grown in the soil did not recover from chlorosis. By 42 DAS, most genotypes were dead or dying; only P27233 and P27248 recovered.

The genotypes of *L. cosentinii* and *L. digitatus* had chlorosis scores of less than 1 in both soil and solution cultures. In *L. albus* there was little genotypic variation in chlorosis scores with both methods, ranging from 0.5 to 1.5 (Fig. 6.4). Both P27486 and P27797 were the most tolerant genotypes in both methods, whilst P27484 and P27487 were the most intolerant. All *L. angustifolius* genotypes had chlorosis scores greater than 2.5 in both screening methods;

**Figure 6.1** Genotypic variation in the tolerance of *L. pilosus* genotypes to calcareous soils as screened (chlorosis score) in soil and solution culture. Genotypes ranked according to soil screening results. LSD solution = 0.71; soil = 0.40 ($P < 0.05$). Bars indicate standard errors. Tolerant (P23370 and P22937) and intolerant (P22932 and P24036) controls are indicated.

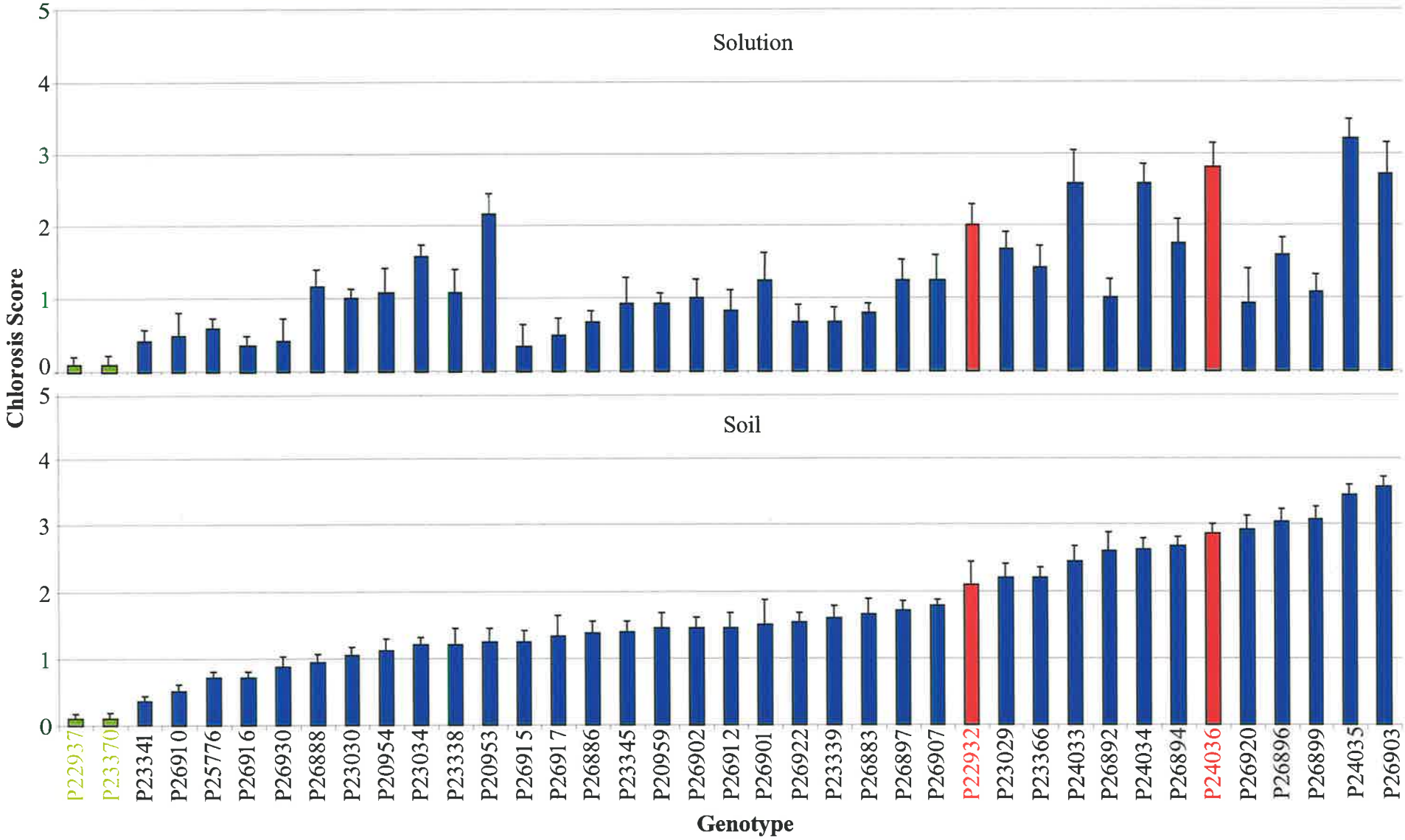


**Plate 6.1** Soil screening box for identifying genotypes of lupins tolerant to calcareous soils.

a. Chlorosis symptoms on *L. pilosus* 21 days after sowing (rows horizontal). Note: on the right hand side in the foreground *L. angustifolius* cv. Gungurru is completely chlorotic and likely to die; a tolerant line of *L. pilosus* is directly behind it.

b. Chlorosis symptoms on *L. atlanticus* genotypes 21 days after sowing (rows vertical). Note: the tolerant *L. pilosus* genotype used as a control is much darker green and shows no chlorosis.

**Plate 6.2** Solution screening container for identifying genotypes of lupins tolerant to calcareous soils.

a. A tank with aeration system on the base.

b. Chlorosis symptoms on genotypes of *L. pilosus* in seedling trays 21 days after transplanting. (left - 0 mM $KHCO_3$, right - 15 mM $KHCO_3$).

**Figure 6.2** Relationship between the chlorosis score in soil and solution screening methods for *L. pilosus* and *L. atlanticus*. Tolerant (P23370 and P22937) and intolerant (P22932 and P24036) controls are indicated.

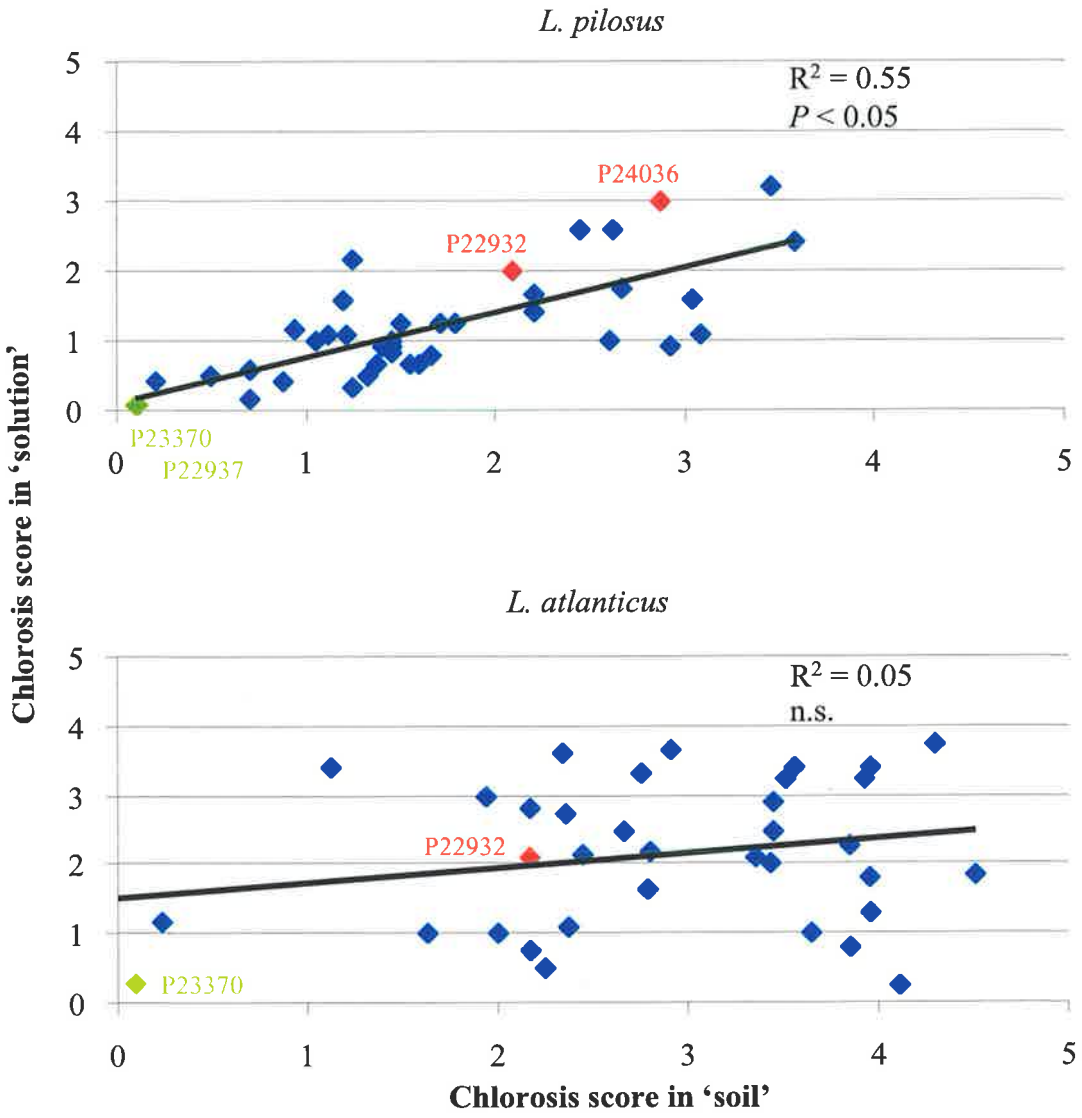


**Figure 6.3** Genotypic variation in the tolerance of *L. atlanticus* genotypes to calcareous soils as screened (chlorosis score) in soil and solution culture. Genotypes ranked according to soil screening results. LSD solution = 0.96; soil = 0.5 ($P < 0.05$). Chlorosis score of the tolerant (P23370) and intolerant (P22932) controls were 0.2 and 2.1, respectively in both screening methods. Bars indicate standard errors, n.d. - not determined.

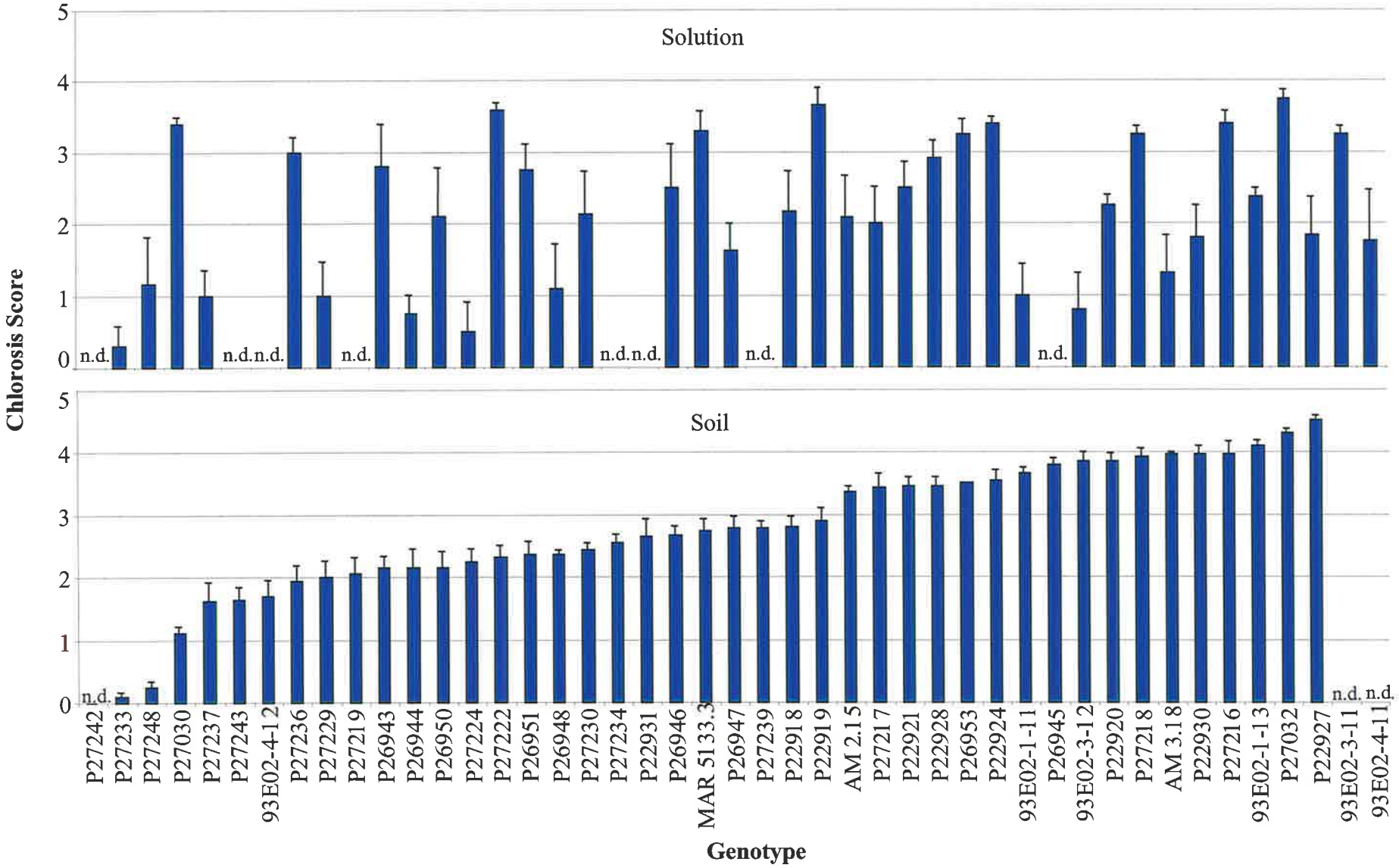
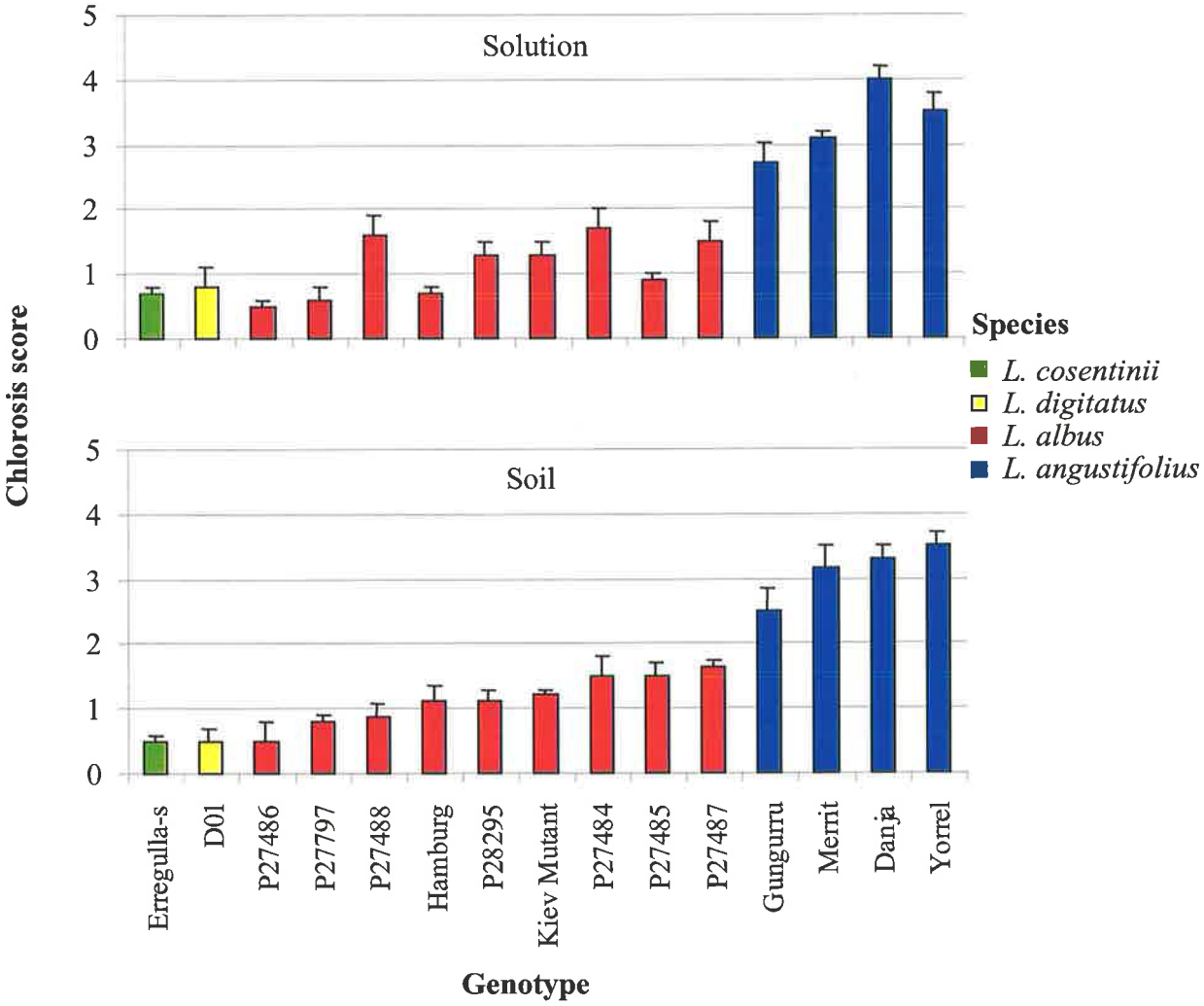


**Figure 6.4** Genotypic variation in the tolerance of *L. cosentinii*, *L. digitatus*, *L. albus* and *L. angustifolius* to calcareous soils as screened in soil and solution culture. Genotypes ranked according to soil screening results.



Gungurru had the lowest chlorosis score (Fig. 6.4). When grown in the soil for periods longer than 21 days, *L. angustifolius* did not recover, whilst in *L. albus*, *L. cosentinii* and *L. digitatus* symptoms remained constant up to 42 DAS.

Chlorophyll meter readings were also recorded 21 DAS or DAT in many of the screening trials for *L. pilosus* and *L. atlanticus*. There was a good agreement between chlorophyll meter readings and chlorosis score for all species (Fig. 6.5).

Correlation coefficients between chlorosis scores in the soil and solution screening methods and seed weight and nutrient concentrations and contents in the seed for *L. pilosus* and *L. atlanticus* are indicated in Table 6.3. Chlorosis score showed a highly significant negative correlation with seed weight in both *L. pilosus* and *L. atlanticus*. For *L. atlanticus*, some seed nutrient concentrations and contents were also correlated with chlorosis scores, particularly notable was the negative correlation with Fe. Generally, all nutrient contents for both species showed negative relationships with chlorosis score, although many were not significant (Table 6.3).

The multiple linear regression analysis indicated that the major factors affecting chlorosis score of *L. pilosus* in the soil screening were seed weight ($P < 0.01$) and Ca ($P < 0.01$), Na ($P < 0.05$) and Zn ($P < 0.05$) concentrations in the seed, accounting for a total of 48% of the variance. For the chlorophyll meter readings only seed weight ($P < 0.01$) was significant, accounting for 63% of the variance. In the solution screening the major factors affecting chlorosis score were seed weight and Ca, Fe, Mn and Zn concentrations ($P < 0.01$) accounting for a total of 57% of the variance. Similarly for chlorophyll meter readings, seed weight and Ca, Fe, Zn and Na concentrations ($P < 0.01$), rather than Mn, accounted for 57% of the variance. In *L. atlanticus* there were no significant factors for chlorosis score in the soil screening and there were an insufficient number of chlorophyll meter readings to allow calculation of the multiple linear regression. In the solution screening the major factors affecting chlorosis score were B, Ca, Fe, K, Mn, Na and P ($P < 0.05$) together accounting for 71% of the variance, whilst for chlorophyll meter readings B, Ca, Fe, Mg and P ($P < 0.05$) accounted for 60% of the variance.

**Figure 6.5** Relationship between chlorosis score and chlorophyll meter readings on the YEL 21 DAS or DAT in soil and solution screening for *L. pilosus* and *L. atlanticus*.

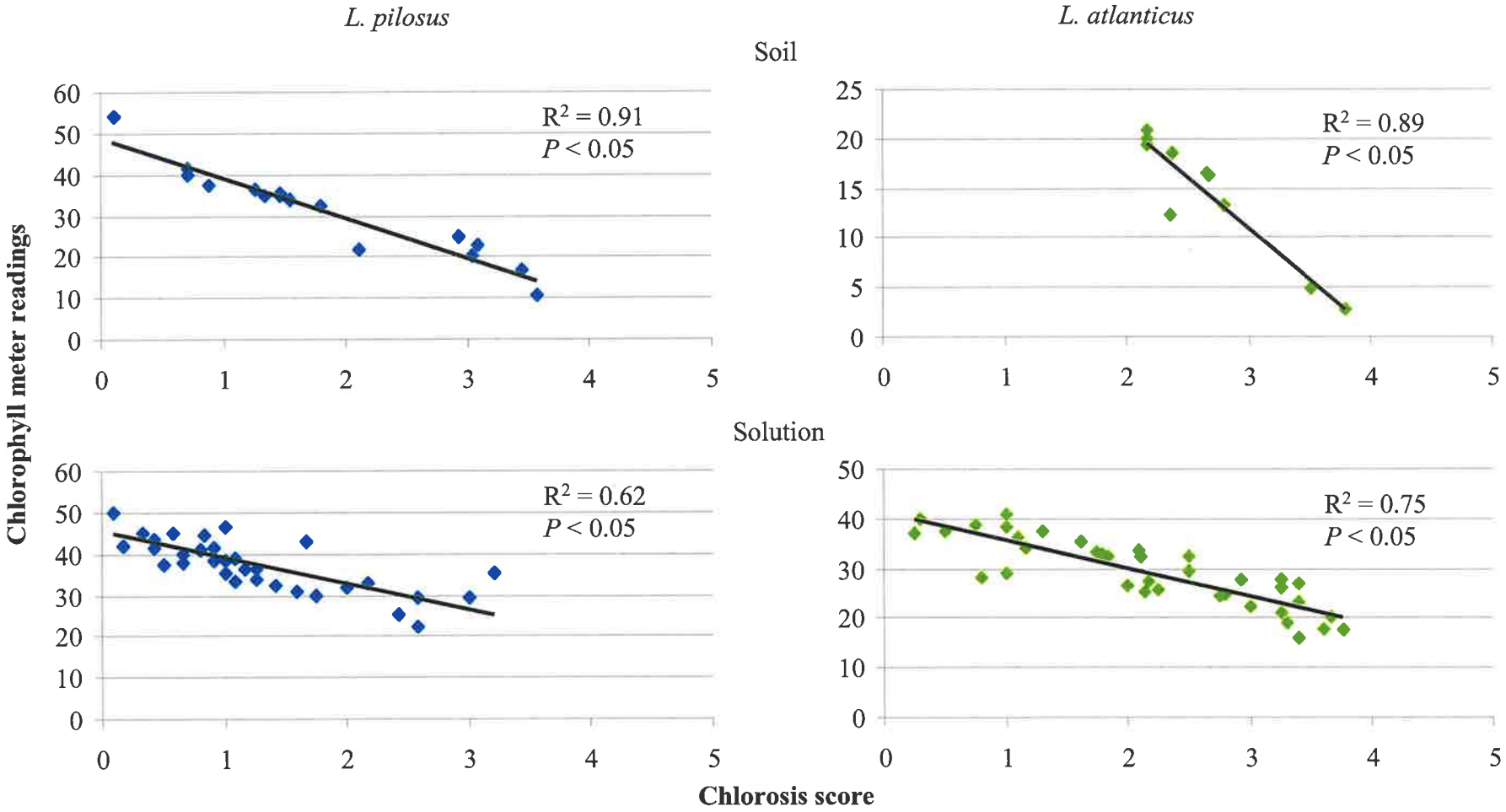


**Table 6.3** Correlation coefficients between chlorosis score in soil and solution screening methods for *L. pilosus* and *L. atlanticus* and seed weight, seed nutrient concentration and seed nutrient content.

| | *L. pilosus* | | *L. atlanticus* | |
|---|---|---|---|---|
| | **Soil** | **Solution** | **Soil** | **Solution** |
| **Solution** | 0.74** | | 0.41** | |
| **Seed weight** | -0.53** | -0.53** | -0.54** | -0.37* |
| | *Nutrient concentrations* | | | |
| **Fe** | -0.01 | 0.29 | -0.34* | -0.63** |
| **Mn** | 0.05 | -0.35* | 0.03 | 0.15 |
| **B** | -0.09 | -0.26 | 0.28 | -0.07 |
| **Cu** | 0.08 | -0.18 | -0.51** | -0.25 |
| **Zn** | 0.03 | 0.07 | 0.11 | 0.05 |
| **Ca** | 0.37* | 0.18 | 0.09 | 0.21 |
| **Mg** | 0.12 | 0.10 | -0.10 | -0.18 |
| **Na** | 0.06 | 0.19 | 0.15 | 0.09 |
| **K** | -0.23 | 0.01 | -0.06 | -0.32* |
| **P** | -0.07 | 0.22 | -0.16 | -0.04 |
| **S** | 0.30 | 0.33* | -0.07 | 0.20 |
| | *Nutrient contents* | | | |
| **Fe** | -0.28 | -0.10 | -0.50** | -0.61** |
| **Mn** | -0.06 | -0.39* | -0.25 | -0.01 |
| **B** | -0.30 | -0.35* | -0.16 | -0.26 |
| **Cu** | -0.23 | -0.35* | -0.60** | -0.39* |
| **Zn** | -0.25 | -0.21 | -0.40** | -0.25 |
| **Ca** | -0.07 | -0.15 | -0.32* | -0.12 |
| **Mg** | -0.27 | -0.26 | -0.44** | -0.38* |
| **Na** | -0.16 | -0.08 | -0.14 | -0.01 |
| **K** | -0.36* | -0.28 | -0.45** | -0.44** |
| **P** | -0.32* | -0.09 | -0.46** | -0.23 |
| **S** | -0.10 | -0.06 | -0.46** | -0.17 |

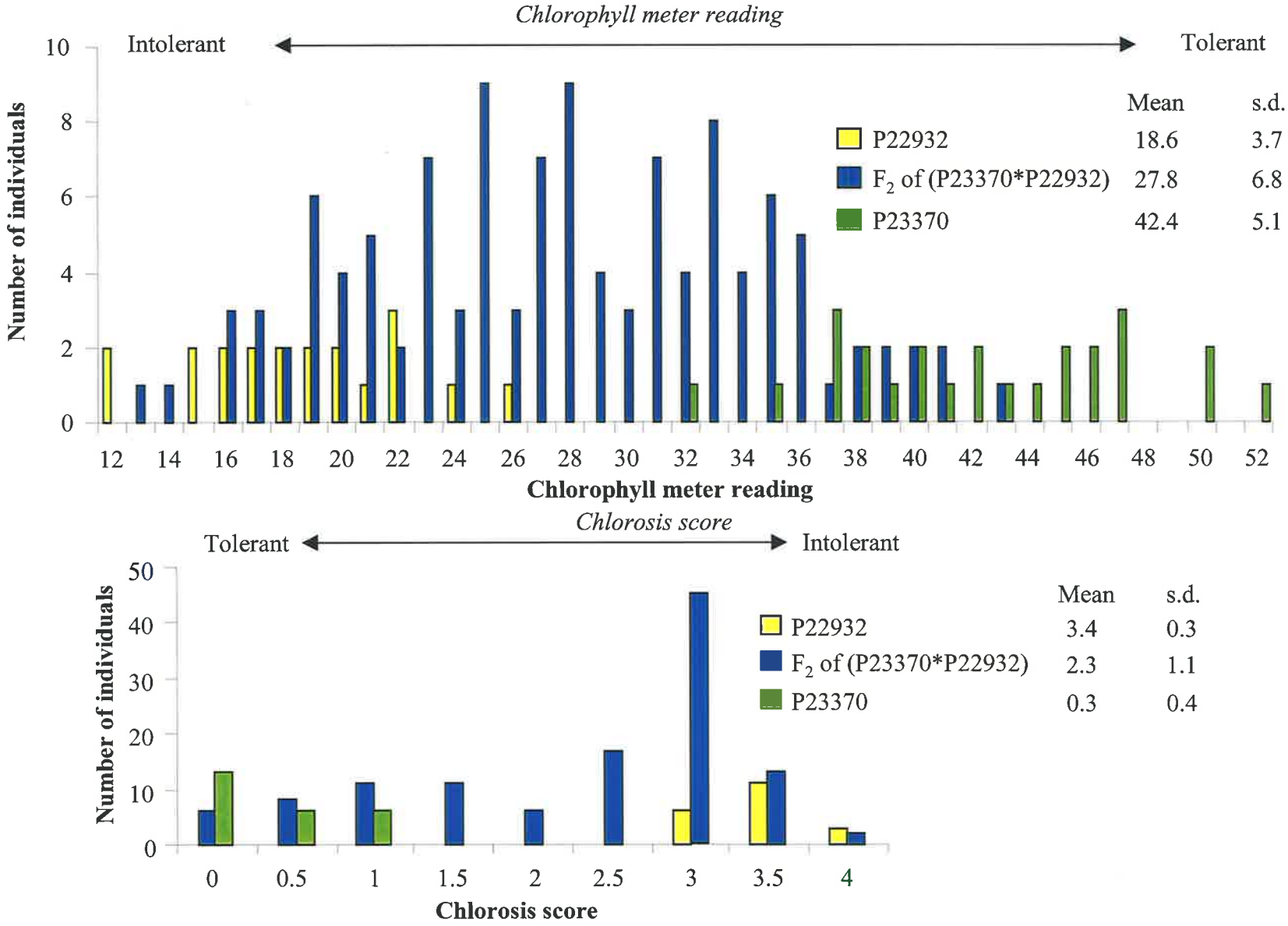
* $P < 0.05$, ** $P < 0.01$

*Genetic control of tolerance in L. pilosus*

In the cross between a tolerant and a moderately intolerant genotype only 10 plants of the $F_1$ population were screened due to a limited seed supply. All $F_1$ plants had chlorosis scores between 0.8 and 1.5, which was intermediate between the two parents (P23370 (T) - 0.2; P22932 (MI) - 2.2).

Frequency distributions of both chlorosis score and chlorophyll meter reading in the $F_2$ population were continuous with no clear-cut points for distinguishing between the tolerant and intolerant categories (Fig. 6.6). There appeared to be a greater proportion of the $F_2$ population showing symptoms similar to the intolerant parent rather than the tolerant parent (Fig. 6.6).

**Figure 6.6** Chlorophyll meter readings and chlorosis score 21 DAT of $F_2$ seedlings and parents grown in solution culture with 15 mM $KHCO_3$.



The number of genes conferring tolerance to calcareous soil was estimated, based on the comparison of the observed variance with those of expected variance, assuming no linkage or epistasis. Comparison of expected with observed variances indicated that when chlorophyll meter readings were used the population appeared to be segregating for two genes (Table 6.4) as the expected variance for two genes was similar to the observed variance and within its confidence interval. However, when chlorosis score was used the population appeared to be segregating for one gene (Table 6.4).

**Table 6.4** Comparisons between expected and observed variances of the $F_2$ population tested in the solution screening system. See materials and methods for description of calculations.

| | Estimated parameters | | | Observed variance | | | | Expected variance | |
|---|---|---|---|---|---|---|---|---|---|
| **Chlorosis measurement** | **d** | **m** | **E** | **$V_{P_1}$ (P23370)** | **$V_{P_2}$ (P22932)** | **$V_{F_2}$ (P23370* P22932)** | **Confidence interval** | **1 gene** | **2 genes** |
| Chlorophyll meter | 11.9 | 30.5 | 20.0 | 26.2 | 13.7 | 45.8 | 36.1 – 60.5 | 101.6 | 55.5 |
| Chlorosis score | 1.5 | 1.9 | 0.1 | 0.2 | 0.1 | 1.1 | 0.9 – 1.5 | 1.3 | 0.7 |

## 6.4 Discussion

*Genotypic variation*

There was interspecific and intraspecific variation of lupins in response to a calcareous soil and high $HCO_3^-$ in solution. The solution screening method was correlated with soil screening for all species except *L. atlanticus*, indicating that the solution method is suitable for breeding programs. Solution screening is preferable because early generation breeding material can be screened and transplanted to soil for growth through to maturity or further crossing.

Similar to Chapters 4 and 5 and previous research (Buirchell and Cowling, 1989; Tang, *et al.*, 1993; Tang, *et al.*, 1996a) *L. pilosus* had the most tolerant material and *L. angustifolius* and *L. atlanticus* genotypes were generally moderately intolerant to intolerant of calcareous soils and high $HCO_3^-$. *Lupinus albus*, *L. cosentinii* and *L. digitatus* were moderately tolerant of calcareous soils and of high $HCO_3^-$.

Similar to other crops (Coulombe, *et al.*, 1984b; Chaney, *et al.*, 1992a) chlorosis measurements of *L. pilosus* indicated a wide range of tolerance in this species ranging from tolerant (P23370 and P22937) to intolerant (P26903 and P24035). Thus, there is potential to develop cultivars of *L. pilosus* that will grow on highly calcareous soil. This range of tolerance was expected as wild types and landraces had been collected from a wide range of soil types varying from acidic soils to alkaline or calcareous (Clements and Cowling, 1990). However, there was no relationship of the tolerance in soil and solution screening with the collection site soil pH among genotypes for which it was available. For example, the genotypes P24034 (I), P24035 (I), and P24036 (I) were reported to be collected from a calcareous, high pH soil (Table 6.1; Clements and Cowling, 1990), but in this screening all these appeared to be either moderately intolerant or intolerant. When comparing countries of origin it was noted that both of the tolerant genotypes came from Israel, whilst a majority of the intolerant genotypes were collected from Greece. Other moderately tolerant material came from Syria and Turkey. All these countries have large areas of calcareous soils hence more tolerant to moderately tolerant material is likely to exist as landraces or in the untested material of the Australian lupin collection.

Correlation coefficients with seed size and nutrient concentrations and contents indicated that the chlorotic symptoms on the YEL of smaller seeded genotypes were generally more severe than large seeded genotypes. This was also confirmed by the multiple linear regression indicating that seed weight was particularly important in *L. pilosus*, but less so in *L. atlanticus*. There was also a negative trend of chlorosis score with the nutrient contents. The multiple linear regression also indicated that many of the nutrients related to chlorosis; the most common in both species being Ca. In *L. pilosus* Zn, Na and Fe also appear important, whilst in *L. atlanticus* there were a range of nutrients involved. This indicates that the overall nutrient concentrations or content of the seed is important in the development of chlorosis which is in agreement with previous research (Khabaz-Saberi, 1999). Conversely, as the seeds were harvested from the same site it would be expected that the ranking of genotypes would be representative of the actual tolerance as there is likely to be natural variation in seed size and nutrition among genotypes.

In *L. atlanticus*, most genotypes were moderately intolerant to intolerant and there was no relationship between the two screening methods. The only genotype classed as tolerant in both screening methods was P27233. These results indicate that the potential for breeding *L. atlanticus* for highly calcareous soils (> 20% $CaCO_3$) is limited and further research is required to confirm the tolerance of P27233. However, genotypes may tolerate lower $CaCO_3$

concentrations than used in the soil screen of this study. Field research has indicated that some genotypes of *L. atlanticus* will grow and yield significantly more than *L. angustifolius* at sites where the soil is calcareous, but $CaCO_3$ contents are less than 10% (Egan and Hawthorne, 1994). Thus the screening systems employed here may be too severe for *L. atlanticus*. The difference in ranking among genotypes between soil and solution screening systems could be related to soil factors other than $HCO_3^-$ which was used in the solution screen in this study.

The more tolerant genotypes of *L. atlanticus* had larger seed sizes, similar to *L. pilosus*, and, in particular, higher seed Fe contents. This indicates that Fe deficiency may be a more important factor in *L. atlanticus* than *L. pilosus*. Thus, the method developed by Chaney, *et al.* (1992a) which focuses particularly on tolerance to Fe deficiency, may be more suitable for screening *L. atlanticus*. The results again suggest that seed with lower nutrient contents have less tolerance to the calcareous soil.

*L. albus* and *L. angustifolius* had a limited range of genotypic variation. As expected from previous research (C Hyughe, pers. comm.), there were no tolerant genotypes of *L. albus*, although the genotypes ranged between moderately tolerant and moderately intolerant. In *L. angustifolius* the most tolerant cultivar was Gungurru which was moderately intolerant, whilst Danja and Yorrel were intolerant.

The only species to recover from chlorosis when grown for longer than 21 days was *L. pilosus*, which further supports the assertion that *L. pilosus* is the most tolerant species. Possible reasons for this recovery were discussed in Chapter 4. Although there was a recovery from chlorosis, it is still important to breed for improved tolerance to early chlorosis, as this is still likely to result in a yield reduction.

Overall, for highly calcareous soils (> 10% $CaCO_3$) *L. pilosus* is the only species with suitable tolerance, whilst in calcareous soils with $CaCO_3$ contents less than 10%, improved varieties may be developed from *L. pilosus* and possibly *L. atlanticus* and *L. albus*. However, further research is required to confirm screening results under field conditions.

*Genetic control of tolerance in L. pilosus*

Results from the cross between a tolerant (P23370) and a moderately intolerant (P22932) genotype suggested that one or two partially recessive genes were likely to control tolerance to

calcareous soils. There were likely to also be other genes with minor effects related to the tolerance. As the $F_1$ population was intermediate between the two parents it can be suggested that the dominance of the intolerance gene was incomplete. The reciprocal cross was not analysed separately, so it is unknown whether there was a maternal effect.

The results of this work show an inverse response to genetic studies with other nutrient toxicities. For example, the genes controlling tolerance to B toxicity in bread wheat (Paull, *et al.*, 1991) or $HCO_3^-$ induced Fe deficiency in soybean (R Chaney, pers. comm.) were classified as dominant whilst in this study the tolerance was partially recessive. The recessiveness of the tolerance may be beneficial in breeding programs as tolerant material selected from early generations are more likely to contain the homozygous recessive alleles (cal, cal) and would therefore not segregate. When the gene is dominant some of the selected material is likely to be heterozygous, thus the population will segregate in the next generation and further selection will be needed.

Overall, as genetic control appeared to be under a relatively simple mechanism and there was no transgressive segregation, therefore genes conferring tolerance could be easily incorporated into higher yielding genotypes. Further work is required to confirm these findings, hence a diallel cross between two tolerant and intolerant genotypes has been conducted and inbred populations are being developed.

# CHAPTER 7

## Field assessment of the genotypic variation in *L. pilosus* for tolerance to calcareous soils

## 7.1 Introduction

In the previous chapters screening methods have been defined and genotypic variation identified in *L. pilosus* and *L. atlanticus* for tolerance to calcareous soil. Since screening procedures are aimed at predicting field responses, it is imperative to validate the rankings of genotypes with grain yields under field conditions.

To establish those parallels it is essential to grow genotypes which differ widely in their response to a calcareous soil. Thus genotypes of *L. pilosus* were compared in these field studies because this species had shown the largest range of variation for tolerance to the calcareous soil (Chapter 6). As most of the *L. pilosus* genotypes were wild types or landraces, they also differed widely in many other characteristics which affect grain yield, including flowering time, seed weight, resistance to diseases and tolerance to nutrient toxicities or deficiencies. To overcome these problems the relative yields of genotypes grown on a calcareous soil were compared with those on a structurally and chemically similar non-calcareous soil.

Throughout many of the cropping zones of South Australia, small outcrops of calcareous soil can occur in an otherwise non-calcareous paddock. These soils are similar in most aspects, but differ in $CaCO_3$ content. Hence relative yields from trials grown in the calcareous patch and in adjacent non-calcareous soil, with most other factors including crop rotation and rainfall the same can give a measure of the tolerance to calcareous soil.

Environmental conditions and soils vary greatly from one site to another, thus it is important to confirm the tolerance through similar comparisons with responses under a variety of conditions at a range of sites. In particular, these should include a range of $CaCO_3$ levels in the soils.

In this chapter six field trials were conducted, restricted in replication by the quantity of seed available. One replicated trial compared *L. pilosus* genotypes, which displayed a range of tolerance in both soil and solution screening, on adjacent calcareous and non-calcareous soils.

Five other unreplicated field trials on calcareous soils compared a range of *L. pilosus* genotypes to gauge their adaptation to a range of environments.

## 7.2 Materials and methods

*Genetic material*

The genotypes of *L. pilosus* grown in each of the field trials were chosen to represent the range of genotypic variation classified in the soil screening procedures (Table 7.1). Geographical origins of the genotypes and collection details are listed in Table 6.1. The limited supplies of seed dictated the genotypes which could be grown at individual field sites. All the seed grown in field trials was harvested from plants grown on the Urrbrae clay loam at the University of Adelaide, Waite Campus during 1997 to minimise confounding effects associated with seed source.

**Table 7.1** Sites at which genotypes of *L. pilosus* were grown, their tolerance to a calcareous soil as classified by the soil screening procedure (Chapter 6) and sowing and harvest dates for the six field trials.

| | | Site | | | | | |
|---|---|---|---|---|---|---|---|
| | **Tolerance** | **Tarlee** | **Minnipa** | **Cungena** | **Yandra** | **Yeelanna** | **Coobowie** |
| P22937 | T | X | X | X | X | X | X |
| P23370 | T | X | X | X | X | | |
| P20954 | MT | X | X | X | X | X | X |
| P23030 | MT | | X | X | X | | X |
| P26886 | MT | X | | | | | |
| P22932 | MI | X | X | X | X | X | X |
| P26892 | MI | X | | | | | |
| P24036 | I | | X | X | X | | X |
| Sowing date | | 07-05-99 | 11-05-99 | 11-05-99 | 11-05-99 | 12-05-99 | 26-05-99 |
| Harvest date | | 18-11-99 | 20-10-99 | 20-10-99 | 20-10-99 | 20-11-99 | 17-11-99 |

*Field Sites*

The location of the six field trials in South Australia during 1998 are shown in Plate 7.1.

Tarlee

The Tarlee site was chosen as variation in $CaCO_3$ content across the paddock allowed the trial to be grown on structurally and chemically similar adjacent soils differing in $CaCO_3$ content.

**Plate 7.1** Map of South Australia indicating field trial sites for testing *L. pilosus* genotypes differing in tolerance to calcareous soils. T - Tarlee, Co - Coobowie, Ye - Yeelanna, M - Minnipa, Cu - Cungena, Ya - Yandra

Cu

M

Ya

Ye

Co

SOUTH AUSTRALIA
SHOWING
LAND UTILIZATION
REFERENCE
Department of Lands
May 1983
SCALE 1:2 000 000

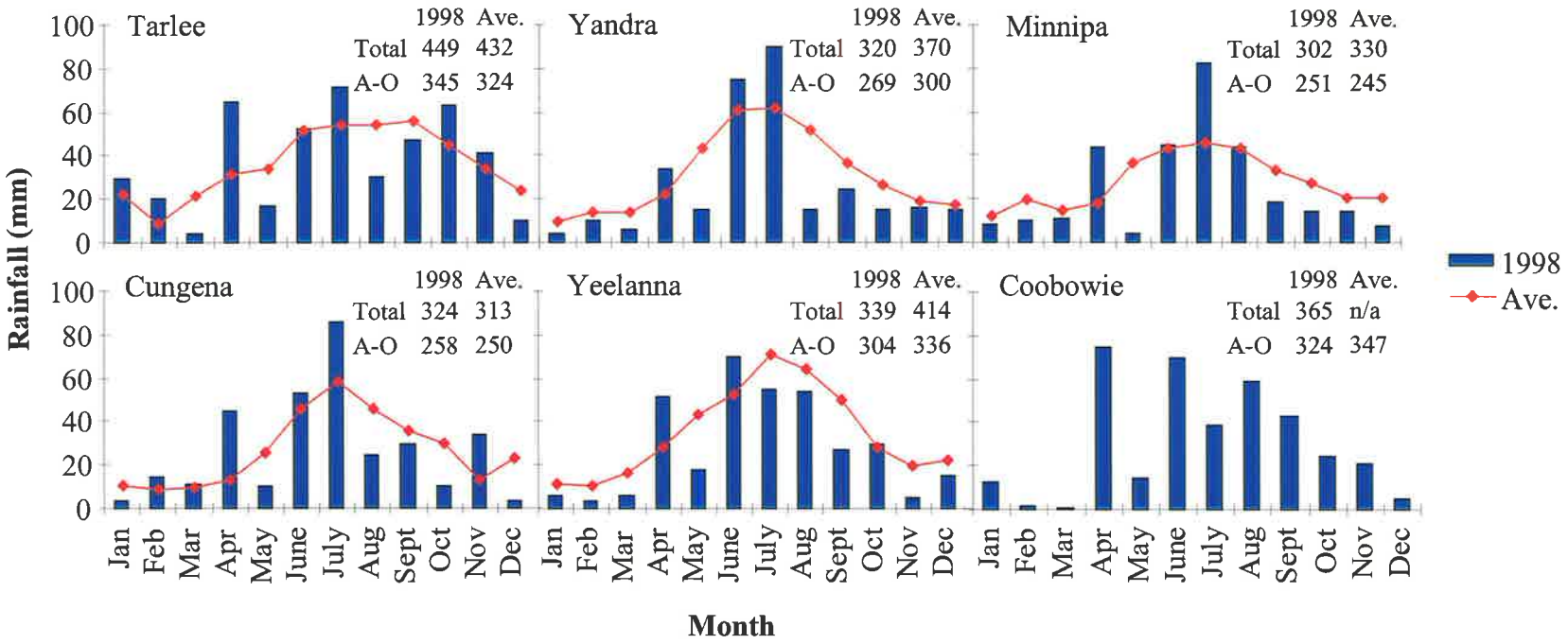
Tarlee is located in the mid-north of South Australia. The trial was conducted on Mr J Rhodes property in a paddock being cropped with narrow-leafed lupins (sown 4th of May 1998). In a section of the paddock there was a small patch of calcareous soil 30 to 40 metres in diameter in which narrow-leafed lupins had previously shown chlorosis and stunted growth. Prior to sowing and at harvest soil samples were taken at 0-10 cm and 10-20 cm in the calcareous patch and in the surrounding non-calcareous soil. Samples were air-dried and analysed (Section 3.2.3) for pH, electrical conductivity, sodium absorption ratio, total $CaCO_3$, texture, organic carbon, field capacity and wilting point (Table 7.2). The soil was not fertilized prior to sowing as the property had a history of high rates of fertilizer applications.

The large quantities of seed handled for these experiments made the method of scarification in earlier chapters relatively inefficient. An alternative method developed by Horn and Hill (1974) involved soaking the seed in concentrated $H_2SO_4$ for 4-7 hours. This method was undesirable as $H_2SO_4$ is very corrosive and evidence indicated that the method can significantly reduce germination of the seed in rough-seeded lupins (J P Egan, pers. comm.). Another scarification method, involving soaking seed in liquid nitrogen for a very short period of time to crack the seed coat (Peacock and Hummer, 1996), had not been previously used in lupins and was tested in a preliminary experiment. Seeds from several genotypes were placed in liquid nitrogen for various periods of time between 1 and 10 seconds and then allowed to geminate. Results showed that this method did not significantly improve the percentage gemination compared with a control in which the seed had been untreated (10% - 50% gemination). As the germination percentages for seed scarified with a scalpel was 85% to 100%, scarification with a scalpel was adopted despite the work involved.

Five replicate plots (1.5 m * 1 m) with six rows (20 cm apart) were marked out in the calcareous patch of soil and duplicated the adjacent non-calcareous soil. Twenty five to thirty scarified seeds inoculated with Group G inoculant (Section 3.2.4) of each of the six genotypes were sown at 3 cm depth into the rows arranged randomly in each of the 10 plots (Table 7.1). The plots were watered at seeding to ensure that a high percentage emergence would be achieved. Plots were inspected at least once monthly and weeded by hand as required. No pesticides were sprayed throughout the duration of the experiment. Rainfall was recorded by the farmer (Fig. 7.1).

Number of seedlings established, visual chlorosis score and chlorophyll meter readings were recorded 53 days after sowing (20th June). Ten YEL samples were taken from each genotype in

**Figure 7.1** Total monthly, annual (Total) and growing season (Apr-Oct) precipitation (mm) during 1998 and long term averages for the field trial sites at Talee, Yandra, Minnipa, Cungena, Yeelanna and Coobowie. n.a. not available (No long term monthly averages were available for Coobowie).



each plot, washed in 0.1 *M* HCl for 30 seconds and rinsed in RO water for 60 seconds to remove any surface contamination, and dried in an oven at 80°C for 48 hours. The nutrient concentrations were determined by ICP-AES analysis. Throughout the rest of the experiment, prior to harvest, chlorotic symptoms, time to flowering and podding and growth were visually monitored. Plants were harvested 195 days after sowing (Table 7.1), when the lowest pods on the main stem of the earliest maturing genotypes were dry and almost ready to shatter. The number of plants, height from the ground to the base of the first pod on the main stem and number of pods per plant were recorded. The plants were then cut 10 cm above the soil surface and bulked for each genotype within a plot and placed in open weave plastic bags to allow air drying without excessive humidity promoting the growth of fungus. The bags were placed in a glasshouse and plants allowed to dry so that pods would shatter. After drying, the plants were threshed with a Kingaroy® belt thresher. The total number of seeds were recorded and seed weight and grain yield per plant calculated. One hundred plants from the surrounding narrow-leafed lupin crop were also harvested and the seed yield recorded.

All measurements were statistically analysed using analysis of variance (Genstat 5.41). Relative number of seedlings established, chlorophyll meter readings, number of plants, height, number of pods per plant, number of seeds, seed weight and grain yield of plants grown in the calcareous soil compared with the non-calcareous soil was calculated. Correlation coefficients were calculated using both actual and relative measurements on the calcareous soil and presented separately. Results for chlorosis score of the genotypes from soil and solution screening methods and chlorophyll meter reading from the solution screening method were included in the correlation analyses.

Minnipa, Cungena, Yandra, Yeelanna, Coobowie

These trials were a preliminary investigation aimed at identifying the adaptation of genotypes differing in tolerance on calcareous soil to a range of calcareous soils in a number of environments in the field.

All trial sites were in South Australia; Minnipa, Cungena and Yandra are all located on the northern Eyre Peninsula, while Yeelanna is on the southern Eyre Peninsula and Coobowie is on the Yorke Peninsula (Plate 7.1). Both the Minnipa and Cungena trials were grown on trials sites leased or owned by the Minnipa Research Centre (Plate 7.1). Yandra and Coobowie were on farmers' properties (I Morgan and P Farrow, respectively), where the surrounding crops were

**Table 7.2** Texture, pH, field capacity, wilting point, organic carbon content, $CaCO_3$ content, electrical conductivity (EC) and sodium absorption ratio (SAR) of the calcareous and non-calcareous soils, sampled at 0-10 cm and 10-20 cm depth prior to sowing and prior to harvest, on which the field trial was conducted at Tarlee and soils sampled at 0-10 cm and 0-20 cm depth prior to sowing, on which the field trials were conducted at Coobowie, Yeelanna, Minnipa, Yandra and Cungena.

| Site | Soil | Depth of sample | Texture | | | pH | Field capacity (%) | Wilting point (%) | Organic carbon (%) | $CaCO_3$ content (%) | EC (µS/cm) | SAR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Sand | Silt | Clay | | | | | | | |
| | | | | | | *Prior to sowing* | | | | | | |
| Tarlee | non-calcareous | 0-10 cm | 83 | 5 | 12 | 7.3 | 16.8 | 6.2 | 1.6 | 0.0 | 200 | 0.74 |
| | non-calcareous | 10-20 cm | 73 | 8 | 19 | 6.7 | 18.9 | 7.2 | 1.5 | 0.0 | 200 | 0.64 |
| | calcareous | 0-10 cm | 80 | 3 | 17 | 8.0 | 25.4 | 9.6 | 2.0 | 2.1 | 315 | 0.87 |
| | calcareous | 10-20 cm | 78 | 8 | 14 | 8.4 | 19.3 | 8.8 | 1.6 | 12.2 | 210 | 0.84 |
| | | | | | | *Prior to harvest* | | | | | | |
| | non-calcareous | 0-10 cm | 83 | 3 | 14 | 7.2 | 16.6 | 4.5 | 1.6 | 0.1 | 185 | 0.25 |
| | non-calcareous | 10-20 cm | 80 | 4 | 16 | 7.6 | 17.2 | 6.8 | 0.8 | 0.0 | 125 | 1.98 |
| | calcareous | 0-10 cm | 80 | 1 | 19 | 8.2 | 19.1 | 10.2 | 1.8 | 3.9 | 190 | 2.18 |
| | calcareous | 10-20 cm | 63 | 8 | 29 | 8.5 | 20.8 | 12.8 | 0.9 | 15.1 | 160 | 1.37 |
| | | | | | | *Other Sites* | | | | | | |
| Coobowie | | 0-10 cm | 53 | 18 | 29 | 8.5 | 30.7 | 18.2 | 2.6 | 15.1 | 205 | 0.55 |
| | | 10-20 cm | 55 | 10 | 35 | 8.7 | 38.9 | 22.1 | 2.1 | 18.2 | 175 | 0.49 |
| Yeelanna | | 0-10 cm | 48 | 13 | 39 | 8.7 | 26.2 | 14.4 | 1.7 | 7.3 | 130 | 0.40 |
| | | 10-20 cm | 38 | 15 | 47 | 8.8 | 31.0 | 18.7 | 0.8 | 22.6 | 130 | 0.49 |
| Minnipa | | 0-10 cm | 75 | 10 | 15 | 8.6 | 21.4 | 7.9 | 1.6 | 4.4 | 145 | 0.47 |
| | | 10-20 cm | 65 | 13 | 22 | 9.1 | 22.0 | 10.0 | 0.8 | 8.5 | 90 | 2.83 |
| Yandra | | 0-10 cm | 78 | 8 | 14 | 8.7 | 20.7 | 10.9 | 1.0 | 55.7 | 150 | 0.82 |
| | | 10-20 cm | 68 | 10 | 22 | 9.1 | 25.7 | 16.6 | 1.2 | 63.6 | 80 | 0.54 |
| Cungena | | 0-10 cm | 14 | 4 | 82 | 8.7 | 17.9 | 7.9 | 1.7 | 46.7 | 377 | 0.93 |
| | | 10-20 cm | 80 | 5 | 15 | 9.1 | 22.3 | 12.1 | 1.6 | 50.4 | 75 | 0.44 |

wheat and chickpeas, respectively. The trial at Yeelanna was on a farmer's property (M Wilksch) at a field day (Yeelanna Focus Fields) site alongside a crop of faba beans. Prior to sowing, soil samples of all sites were taken at 0-10 cm and 10-20 cm depth. Samples were air-dried and analysed (Section 3.2.3) for pH, electrical conductivity, sodium absorption ratio, total $CaCO_3$, organic carbon, texture, field capacity and wilting point (Table 7.2). Fertilizer was not applied prior to sowing.

Scarified seeds (Section 3.2.4) were inoculated with Group G inoculant and three rows of each genotype (25-30 seed per row), except P23370 where there was insufficient seeds, were hand sown at 3 cm depth at Minnipa, Cungena and Yandra. In these trials only 1 row of 25-30 seed of P23370 was sown. At Yeelanna genotypes were sown in two rows of 20 seeds and at Coobowie genotypes were sown in two rows of 35-40 seeds (Table 7.1).

The rows measured 1.5 m in length and the spacing between individual rows was 20 cm. After sowing, the plots were watered once to ensure a high percentage emergence. Sites were inspected bimonthly and weeded by hand if required. No pesticides were applied. Monthly rainfall was recorded by the Minnipa Research Centre or by the farmers (Fig. 7.1).

Seedling establishment, visual chlorosis score and chlorophyll meter readings were recorded approximately eight weeks after sowing. Throughout the rest of the experiment chlorosis, time to flowering and podding and general growth was visually monitored. The experiments were harvested 162 - 193 DAS (Table 7.1). The height from the ground to the base of the first pod on the main stem and number of pods per plant were visually estimated. Other harvest procedures were as recorded for the Tarlee site. The grain yield, number of seed and individual seed weight were recorded. No statistical analyses could be performed as there was no replication. No correlations were conducted among sites, however the relative ranking of genotypes at each site was compared in the text.

## 7.3 Results

*Weather conditions*

Tarlee

After high rainfall in April, May was relatively dry (Fig. 7.1). June and July were about average, whilst there were long periods through August with little rainfall resulting in moisture stress. The farmer reported that temperatures were generally about average.

Minnipa, Cungena and Yandra

After a very high rainfall in April, May was extremely dry (Fig. 7.1). June and July were generally on average or slightly above, whilst the August to October rainfall, when plants were flowering and podding, was low resulting in moisture stress. The farmer and research staff also reported that temperatures appeared above average during this period, further exacerbating the moisture stress.

Yeelanna

Yeelanna also had low rainfall in May (Fig. 7.1). July, August and September rainfall was slightly below average, but due to the more mild temperatures (M Wilksch, pers. comm.), compared with the Northern Eyre Peninsula sites, flowering was later and plants were able to mature over a longer period of time.

Coobowie

Rainfall was high in April followed by dry conditions in May. In other months rainfall was about average, although the farmer also reported that late September and October were dry compared with previous years, despite no confirming data. In addition, the farmer reported that temperatures appeared above average during September and October.

*Field Trials*

Tarlee

*Seed emergence, chlorosis, and estimated flowering time*

Initial emergence had occurred in all plots by 11 DAS and by 26 DAS most seedlings had emerged. In some plots emergence was reduced and further investigation indicated that a fungal rot was present. No chlorosis was evident 26 DAS.

By 53 DAS there were significant differences among genotypes ($P < 0.05$) in the total number of plants emerged and a significant interaction between soil type and genotype ($P < 0.05$) for chlorosis score, and chlorophyll meter readings (Table 7.3). Generally fewer seeds of P22937, P23370 and P26886 had established than for the other genotypes (Table 7.3; Plate 7.2a). There were no significant difference between the calcareous and non-calcareous soils (Table 7.3).

On the calcareous soil, the genotypes that had been identified as tolerant (P22937 and P23370) in the soil and solutions screening (Chapter 6) showed significantly less chlorosis (lower chlorosis score and higher chlorophyll meter readings; Table 7.3; Plate 7.2a). The narrow-leafed lupin plants surrounding the plots on the calcareous soil were fully chlorotic. On the non-calcareous soils there was no visual chlorosis, although P23937 (T) and P23370 (T) had higher chlorophyll meter readings. All genotypes showed a significant reduction in chlorophyll meter readings between the non-calcareous and calcareous soil; the decrease was least for P22937 (T) and P23370 (T) and highest for P26892 (MI) (Table 7.3). Over the rest of the growing season the chlorotic symptoms generally decreased, so that by flowering, plants showed no chlorosis (Plate 7.3).

Nutrient concentrations in the YEL of plants sampled 53 DAS are summarised in Table 7.4a. Statistical analyses indicated that there was a significant two-way interaction between genotype and soil for Mg concentrations ($P < 0.05$), whilst the main effect of genotype was significant for Fe, B, Zn, Ca, Mg, K, P and S ($P < 0.05$) and the main effect of soil was significant for Fe, Mn, Cu, Zn, Ca, K, P and S ($P < 0.05$).

Generally plants grown in the calcareous soil had lower concentrations of Fe, Mn, Cu, Zn, P and S, and higher concentrations of Ca and K (Table 7.4a). When comparing among genotypes the main nutrients of interest are those that display differences relating to the tolerance of genotypes. Generally the relative concentrations of Fe and Mn were higher and B, K and Na lower in the tolerant genotypes (P22937 and P23370) than the moderately intolerant genotypes (P22932 and P26892; Table 7.4a).

The concentrations of Fe in the YEL of the surrounding crop of narrow-leafed lupins was lower than *L. pilosus* on the non-calcareous soil and higher than *L. pilosus* on the calcareous soil (Table 7.4b). Concentrations of Mn, Cu, Zn and S were generally similar to *L. pilosus*, whilst

**Plate 7.2** Chlorosis symptoms approximately eight weeks after sowing on *L. pilosus* genotypes grown on calcareous soils at two field sites.

a. Tarlee

Genotypes (L to R): P26892 (moderately intolerant), P22932 (moderately intolerant), P22937 (tolerant), P23370 (tolerant), P26886 (moderately tolerant), P20954 (moderately tolerant). Note: chlorosis on the YEL of *L. angustifolius* surrounding the plot).

b. Yandra

Genotypes (L to R): P23370 (tolerant; only a single row - the other genotypes were in plots of three rows), P23030 (moderately tolerant), P24036 (intolerant), P20954 (moderately tolerant), P22937 (tolerant), P22932 (moderately intolerant).

**Plate 7.3** *Lupinus pilosus* genotypes recovered from chlorosis and flowering (123 DAS; 7th September, 1999) at Tarlee.
(L to R): P26886 (moderately tolerant), P22932 (moderately intolerant), P23370 (tolerant), P22937 (tolerant), P20954 (moderately tolerant), P26892 (moderately intolerant).

concentrations of B, Ca, Mg, K and P were increased and Na decreased compared with *L. pilosus* on both the calcareous and non-calcareous soils (Table 7.4b).

Plants had begun to flower by 105 DAS (20$^{th}$ August) and plants on the calcareous soil generally flowered 2 to 5 days later than those on the non-calcareous soil (Table 7.3). The ranking of genotypes was similar on both soils, i.e. P22937 (T) flowered earliest followed by P20954 (MT) and P22932 (MI), then P26886 (MT) and P26892 (MI) and finally P23370 (T) which was 12 to 13 days later flowering than P22937 (Table 7.3). Also it was notable that although P22937 (T) was the first to flower, it appeared to be very slow to set lateral branches. The first pods were visible approximately two weeks after flowering had begun for each genotype and all genotypes appeared to pod normally. The first genotype to senesce was P22937 (T).

*Harvest - yield components*

At harvest there were significant differences among genotypes ($P < 0.05$) for the number of plants per plot, height, seed weight, number of seed per pod, number pods per plant and grain yield per plant (Table 7.3).

The number of plants per plot at harvest was slightly less than the number of seedlings established 53 DAS, although the ranking of genotypes was similar (Table 7.3). Generally there were fewer plants of P22937 (T), P23370 (T) and P26886 (MT) than other genotypes and there were no significant differences in number of plants between the calcareous and non-calcareous soil.

Although analyses indicated no significant differences in height, visually among genotypes P22937 (T) and P23370 (T) appeared the tallest and P26892 (MI) the shortest (Table 7.3). Also all genotypes grown on the non-calcareous soil appeared taller than those on the calcareous soil.

There were large differences in seed weight among genotypes, but no difference between the calcareous and non-calcareous soils (Table 7.3). P23370 (T) and P22937 (T) produced the largest seed whilst P20954 (MT) and P22932 (MI) were smallest (0.25 g lighter; Table 7.3).

The number of seed per pod were lowest for P26892 (MI) in both soils and highest for P23370 (T) in the non-calcareous soil and P20954 (MT) in the calcareous soil (Table 7.3). The relative number of seeds per pod in the calcareous soil was high for P20954 (MT) and low for P22937

**Table 7.3** Seedlings established per plot, chlorophyll meter readings and chlorosis score 53 days after sowing and estimated flowering time and number of plants per plot, height, seed weight, number of seed per pod, number of seed per plant, number of pods per plant and yield per plant at harvest of the six *L. pilosus* genotypes grown in the field trial at Tarlee during 1998 on a non-calcareous (NC) compared with a calcareous (C) soil. Number in parenthesis indicates the percentage of that in the non-calcareous plots. n.s. - not significant; n.d. - not determined

| | | Pre Harvest | | | | | | | | Harvest | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Genotype** | **Tolerance** | **Chlorosis score** | | **Seedlings establised plot$^{-1}$** | | **Chorophyll meter reading** | | **Estimated flowering time (DAS)** | | **Plants plot$^{-1}$** | | **Height (cm)** | |
| | | **NC** | **C** | **NC** | **C** | **NC** | **C** | **NC** | **C** | **NC** | **C** | **NC** | **C** |
| P22937 | T | 0.0 | 0.4 | 16.2 | 13.8(85) | 57.0 | 51.4(90) | 108 | 111 | 14.4 | 11.4(79) | 54.2 | 47.2(87) |
| P23370 | T | 0.0 | 0.4 | 14.6 | 14.6(100) | 58.0 | 51.8(89) | 121 | 123 | 12.2 | 14.6(120) | 53.4 | 49.4(93) |
| P20954 | MT | 0.0 | 1.4 | 21.4 | 21.6(101) | 53.2 | 45.2(85) | 110 | 112 | 17.4 | 17.8(102) | 46.4 | 46.8(101) |
| P26886 | MT | 0.0 | 1.0 | 19.2 | 16.2(84) | 55.6 | 43.2(78) | 114 | 118 | 18.2 | 14.4(79) | 51.6 | 49.8(97) |
| P22932 | MI | 0.0 | 1.2 | 22.4 | 23.0(103) | 53.2 | 42.4(80) | 110 | 112 | 21.0 | 21.4(102) | 49.4 | 43.4(88) |
| P26892 | MI | 0.0 | 1.8 | 22.0 | 19.8(90) | 54.4 | 37.2(68) | 115 | 118 | 17.0 | 17.0(100) | 46.6 | 45.2(97) |
| LSD * $P < 0.05$, ** $P < 0.01$ | | | | | | | | | | | | | |
| Genotype x Soil | | 0.3** | | n.s. | | 4.1** | | n.d. | | n.s. | | n.s. | |
| Genotype | | 0.2** | | 3.2** | | 2.9** | | n.d. | | 3.6** | | n.s. | |
| Soil | | 0.1** | | n.s. | | 1.7** | | n.d. | | n.s. | | n.s. | |

| | | Harvest | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Genotype** | **Tolerance** | **Seed weight (g seed$^{-1}$)** | | **Seed pod$^{-1}$** | | **Seed plant$^{-1}$** | | **Pods plant$^{-1}$** | | **Yield plant$^{-1}$ (g)** | |
| | | **NC** | **C** | **NC** | **C** | **NC** | **C** | **NC** | **C** | **NC** | **C** |
| P22937 | T | 0.71 | 0.70(98) | 2.5 | 2.1(85) | 13.1 | 12.8(97) | 5.1 | 5.1(99) | 9.3 | 8.8(95) |
| P23370 | T | 0.68 | 0.72(107) | 2.7 | 2.3(83) | 11.9 | 12.6(106) | 4.3 | 5.6(130) | 8.3 | 9.1(110) |
| P20954 | MT | 0.45 | 0.45(101) | 2.1 | 3.0(144) | 15.4 | 18.8(122) | 7.2 | 7.1(98) | 7.0 | 8.5(122) |
| P26886 | MT | 0.63 | 0.65(104) | 2.5 | 2.8(108) | 13.4 | 13.8(103) | 3.8 | 5.0(129) | 8.5 | 9.0(107) |
| P22932 | MI | 0.46 | 0.46(99) | 2.4 | 2.4(99) | 17.0 | 11.6(68) | 7.1 | 4.8(68) | 7.9 | 5.2(67) |
| P26892 | MI | 0.52 | 0.52(98) | 2.0 | 2.0(101) | 10.6 | 9.1(86) | 5.2 | 4.5(87) | 5.6 | 4.7(84) |
| LSD * $P < 0.05$, ** $P < 0.01$ | | | | | | | | | | | |
| Genotype x Soil | | n.s. | | n.s. | | n.s. | | n.s. | | n.s. | |
| Genotype | | 0.04** | | 0.31 | | n.s. | | 1.6* | | 2.8* | |
| Soil | | n.s. | | n.s. | | n.s. | | n.s. | | n.s. | |

**Table 7.4** (a) Concentrations of Fe, Mn, B, Cu, Zn, Ca, Mg, Na, K, P, S in the YEL 53 days after sowing of the six *L. pilosus* genotypes grown in the field trial at Tarlee during 1998 on a non-calcareous (NC) compared with a calcareous (C) soil. Number in parenthesis indicates the percentage of that in the non-calcareous plots. (b) Nutrient concentrations in the surrounding crop of *L. angustifolius* on the calcareous and non-calcareous soil. n.s. - not significant.

(a)

| | | Fe | | Mn | | B | | Cu | | Zn | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Genotype** | **Tolerance** | **NC** | **C** | **NC** | **C** | **NC** | **C** | **NC** | **C** | **NC** | **C** |
| P22937 | T | 153 | 123(80) | 48 | 27(55) | 16 | 15(94) | 4.8 | 3.5(74) | 35 | 29(82) |
| P23370 | T | 129 | 108(84) | 43 | 27(63) | 21 | 20(94) | 5.9 | 3.2(54) | 37 | 34(92) |
| P20954 | MT | 150 | 105(70) | 52 | 24(47) | 21 | 19(90) | 5.2 | 3.5(68) | 46 | 39(84) |
| P26886 | MT | 162 | 100(61) | 47 | 29(61) | 22 | 22(100) | 6.1 | 3.7(61) | 41 | 39(97) |
| P22932 | MI | 134 | 102(76) | 57 | 24(42) | 22 | 22(101) | 5.5 | 4.1(74) | 37 | 36(97) |
| P26892 | MI | 111 | 81(73) | 51 | 21(41) | 22 | 25(111) | 5.0 | 3.4(68) | 45 | 35(77) |
| LSD * $P < 0.05$, ** $P < 0.01$ | | | | | | | | | | | |
| Genotype x Soil | | n.s. | | n.s. | | n.s. | | n.s. | | n.s. | |
| Genotype | | 25* | | n.s. | | 1.7** | | n.s. | | 4.6** | |
| Soil | | 14** | | 4.2** | | n.s. | | 0.8** | | 2.6** | |

| | | Ca | | Mg | | Na | | K | | P | | S | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Genotype** | **Tolerance** | **NC** | **C** | **NC** | **C** | **NC** | **C** | **NC** | **C** | **NC** | **C** | **NC** | **C** |
| P22937 | T | 10920 | 13540(124) | 2500 | 2540(102) | 2120 | 1750(83) | 12240 | 12200(100) | 4460 | 3660(82) | 2960 | 2660(90) |
| P23370 | T | 8940 | 11320(127) | 2320 | 2364(102) | 2714 | 2246(83) | 11900 | 13300(112) | 4380 | 3900(89) | 2920 | 2820(97) |
| P20954 | MT | 12715 | 12700(100) | 2940 | 2678(91) | 2200 | 2396(109) | 12200 | 13620(112) | 4820 | 3960(82) | 2600 | 2520(97) |
| P26886 | MT | 10440 | 15960(153) | 2320 | 2900(125) | 2610 | 2824(108) | 12080 | 13380(111) | 4540 | 3740(82) | 3000 | 2760(92) |
| P22932 | MI | 15240 | 17000(112) | 3240 | 3160(98) | 2424 | 2096(86) | 11580 | 13880(120) | 4460 | 3840(86) | 2740 | 2540(93) |
| P26892 | MI | 13680 | 15860(116) | 2540 | 2780(109) | 2044 | 2042(100) | 13840 | 17000(123) | 5020 | 4400(88) | 2760 | 2540(92) |
| LSD * $P < 0.05$, ** $P < 0.01$ | | | | | | | | | | | | | |
| Genotype x Soil | | n.s. | | 349** | | n.s. | | n.s. | | n.s. | | n.s. | |
| Genotype | | 2381** | | 247** | | n.s. | | 1197** | | 402* | | 123** | |
| Soil | | 1374** | | n.s. | | n.s. | | 691** | | 232** | | 71** | |

(b)

| **Soil** | **Fe** | **Mn** | **B** | **Cu** | **Zn** | **Ca** | **Mg** | **Na** | **K** | **P** | **S** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| non-calcareous | 112 | 74 | 37 | 6.4 | 33 | 19600 | 6100 | 350 | 17200 | 6050 | 3100 |
| calcareous | 164(146) | 22(29) | 45(119) | 1.9(30) | 32(98) | 22000(112) | 5950(98) | 1160(331) | 24400(141) | 5200(86) | 3150(102) |

(T) and P23370 (T). The number of seeds per plant showed a different trend to the number of seeds per pod. Despite the analyses showing no significant differences, P26892 (MI) appeared to produce fewer seeds per plant than the other genotypes (Table 7.3). For all genotypes there was little difference between the calcareous and non-calcareous soil, except P20954 (MT) which had a greater number and P22932 (MI) which had fewer seed per plant. The number of pods per plant showed a different trend among genotypes. On the non-calcareous soil P20954 (MT) and P22932 (MI) produced the greatest number of pods per plant; P20954 (MT) also produced the greatest number of pods on the calcareous soil (Table 7.3). The number of pods per plant was increased for P23370 (T) and P26886 (MT) and decreased for P22932 (MI) on the calcareous soil compared with the non-calcareous soil.

P22937 (T) had the highest grain yield in the non-calcareous soils, whilst P23370 (T) was highest in the calcareous soil. P26892 (MI) had the lowest yield in both soils (Table 7.3). Generally the grain yields of the *L. pilosus* genotypes grown on both the calcareous and non-calcareous soil in this study were comparable or greater than the surrounding crop of *L. angustifolius* (7.0 g plant $^{-1}$) on the non-calcareous soil. All *L. angustifolius* plants on the calcareous soil were dead (Table 7.3).

When relative measurements were compared, the number of seed per plant, pods per plant and grain yield all showed similar trends in that the moderately intolerant lines (P22932 and P26892) had the greatest reduction on the calcareous soil relative to the non-calcareous soil (Table 7.3). All other genotypes performed similar in both soils or showed increases in the calcareous soil.

Chlorosis score obtained from soil and solution cultures gave a negative relationship with actual and relative chlorophyll meter readings and actual grain yield per plant and a positive relationship with chlorosis score in the field (Table 7.5). Chlorophyll meter readings from the solution screening were negatively correlated with actual seedling emergence and the number of plants per plot and positively correlated with actual chlorophyll meter readings and seed weight in the field. Actual Fe concentration in the YEL was negatively correlated with number of seed per pod, number seed per plant and number of pods per plant (Table 7.5a). Relative Fe concentration was positively correlated with grain yield (Table 7.5b). Data for other nutrients correlations has not been shown as they were not significant.

Minnipa, Cungena, Yandra, Yeelanna, Coobowie

For all genotypes there was good seedling emergence at all field sites, except P22937 (T) at Cungena when only approximately 50% of the seed emerged (Table 7.6; percentage emergence not shown as the exact number of seed planted was not recorded).

All genotypes showed chlorosis eight weeks after sowing. P24036 (I) showed the most severe chlorosis at all sites at which it was grown. This was particularly notable at Yandra and Cungena; plants were severely chlorotic and did not survive until harvest (Table 7.6). However, rankings of genotypes were different among sites. At Minnipa, Yandra and Coobowie P22937 (T), P23370 (T) and P23030 (MT) generally showed the least chlorosis in the YEL, followed by P20954 (MT), and P22932 (MI) showing moderate chlorosis (Table 7.6). At Cungena all genotypes, except P24036 (I), showed moderate chlorosis, while at Yeelanna all genotypes showed slight chlorosis (Table 7.6).

The flowering times were later on the northern Eyre Peninsula than other sites. At all sites P22937 (T) and P22932 (MI) flowered earliest and P23370 (T) and P23030 (MT) flowered latest (Table 7.6). After flowering during pod set and maturation, plants appeared stressed at Minnipa, Yandra and Coobowie due to lack of moisture.

At all sites except Cungena plants survived until harvest. Harvest measurements were not recorded at Cungena as most plants had died and any pods that had been set were eaten by *Heliothus* sp. caterpillars. The height of most plants ranged from 30 to 40 cm, P22937 (T) was commonly tallest and P23030 (MT) shortest although at Yandra P24036 (I) was severely stunted. The development of lateral branches was poor at all sites except Yeelanna, where both P20954 (MT) and P22932 (MI) grew many lateral branches.

At all sites except Yeelanna estimated number pods per plant, number of seed per plant, and grain yield were low. This was particularly evident at Yandra where plants produced only 1-2 pods and seed per plant resulting in estimated grain yields of less than 0.22 g plant$^{-1}$. The highest yielding genotypes at Minnipa and Yandra was P22932 (MI) and at Coobowie was P24036 (I). P23030 (MT) and P24036 (I) were lowest yielding at Minnipa, P24036 (I) and P23370 (T) produced almost no seed at Yandra and P22932 (MI) and P20954 (MT) were lowest yielding at Coobowie. At Yeelanna grain yields were 2 to 6 g plant$^{-1}$ greater than all other sites. P22932 (MI) had a particularly high yield at this site.

**Figure 7.5** (a) Correlation coefficients between chlorosis scores from soil (sCS) and solution methods (snCS), chlorophyll meter reading in solution screening methods (snCM) (Chapter 6) and seedlings established per plot (Em), chlorophyll meter readings (CM) and chlorosis score (CS) 8 weeks after sowing and number of plants per plot (nPl) and height (H), seed weight (SW), number of seed per pod (nS/P), number of seed per plant (nS/Pl), number of pods per plant (nP/Pl), grain yield per plant (GY/Pl) and Fe concentrations at harvest of the six *L. pilosus* genotypes grown in the field trial at Tarlee during 1998 on a calcareous soil. (b) Correlation coefficients for relative measurements on the calcareous soil. Other nutrients are not shown as there were no significant correlations.

(a) Actual data from the calcareous soil

| | sCS | snCS | snCM | Em | CM | CS | nPl | H | SW | nS/Po | nS/Pl | nP/Pl | GY/Pl |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Em** | 0.75 | 0.76 | -0.92** | | | | | | | | | | |
| **CM** | -0.98** | -0.92** | 0.81* | -0.69 | | | | | | | | | |
| **CS** | 0.90* | 0.89* | -0.77 | 0.79 | -0.93** | | | | | | | | |
| **nPl** | 0.70 | 0.71 | -0.88* | 0.94** | -0.60 | 0.65 | | | | | | | |
| **H** | -0.64 | -0.77 | 0.67 | -0.77 | 0.52 | -0.56 | -0.72 | | | | | | |
| **SW** | -0.73 | -0.76 | 0.87* | -0.98** | 0.69 | -0.84* | -0.86* | 0.76 | | | | | |
| **nS/Po** | -0.10 | -0.27 | -0.28 | 0.27 | 0.03 | 0.11 | 0.18 | 0.31 | -0.28 | | | | |
| **nS/Pl** | -0.40 | -0.46 | 0.02 | 0.12 | 0.33 | -0.08 | -0.02 | 0.31 | -0.18 | 0.88* | | | |
| **nP/Pl** | -0.38 | -0.35 | 0.07 | 0.19 | 0.33 | -0.01 | 0.07 | 0.21 | -0.25 | 0.73 | 0.93** | | |
| **GY/Pl** | -0.87* | -0.96** | 0.69 | -0.69 | 0.78 | -0.72 | -0.69 | 0.86* | 0.66 | 0.47 | 0.61 | 0.49 | |
| **Fe** | -0.53 | -0.61 | 0.09 | 0.00 | 0.48 | -0.30 | -0.10 | 0.36 | -0.03 | 0.85* | 0.96** | 0.83* | 0.71 |

(b) Relative data (calcareous soil / non-calcareous soil x 100)

| | sCS | snCS | snCM | Em | CM | CS | nPl | H | SW | nS/Po | nS/Pl | nP/Pl | GY/Pl |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Em** | 0.10 | 0.21 | -0.39 | | | | | | | | | | |
| **CM** | -0.93** | -0.87* | 0.64 | 0.17 | | | | | | | | | |
| **CS** | 0.90* | 0.89* | -0.77 | 0.17 | -0.85* | | | | | | | | |
| **nPl** | -0.01 | 0.15 | 0.00 | 0.79 | 0.09 | 0.06 | | | | | | | |
| **H** | 0.28 | 0.20 | -0.21 | 0.05 | -0.38 | 0.59 | 0.14 | | | | | | |
| **SW** | -0.42 | -0.53 | 0.33 | 0.17 | 0.31 | -0.41 | 0.44 | 0.27 | | | | | |
| **nS/Po** | 0.28 | 0.20 | -0.50 | 0.31 | -0.17 | 0.59 | -0.01 | 0.78 | -0.04 | | | | |
| **nS/Pl** | -0.56 | -0.59 | 0.48 | -0.07 | 0.44 | -0.20 | 0.04 | 0.63 | 0.48 | 0.50 | | | |
| **nP/Pl** | -0.59 | -0.72 | 0.63 | -0.37 | 0.34 | -0.52 | -0.02 | 0.28 | 0.83* | -0.11 | 0.66 | | |
| **GY/Pl** | -0.57 | -0.62 | 0.48 | -0.07 | 0.43 | -0.23 | 0.07 | 0.63 | 0.58 | 0.46 | 0.99** | 0.74 | |
| **Fe** | -0.24 | -0.39 | 0.29 | -0.34 | 0.02 | 0.00 | -0.13 | 0.77 | 0.58 | 0.45 | 0.84* | 0.79 | 0.87* |

* $P < 0.05$, ** $P < 0.01$

**Table 7.6** Seedlings established per plot, chlorosis score and chlorophyll meter readings eight weeks after sowing, estimated flowering time (days after sowing) and height, estimated number of pods per plant, seed weight, grain yield per plant and number of seed per plant at harvest of the *L. pilosus* genotypes grown in the field trial at Minnipa, Cungena, Yandra, Yeelanna and Coobowie during 1998 on calcareous soils. n/a - not available due to the small volume of seed harvested; n.d. - not determined.

| Genotype | Tolerance | Seedlings establised plot$^{-1}$ | Chlorosis score | Chorophyll meter reading | Estimated flowering time (DAS) | Height (cm) | Estimated pods plant$^{-1}$ | Seed plant$^{-1}$ | Grain yield plant$^{-1}$ (g) | Seed weight (g seed$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | *Minnipa - northern Eyre Peninsula* | | | | | | |
| P22937 | T | 64 | 0-0.5 | n.d.[1] | 112 | 35-45 | 3-6 | 3.0 | 1.3 | 0.43 |
| P23370 | T | 20 | 0-0.5 | n.d. | 118 | 32-33 | 4-6 | 3.5 | 1.0 | 0.30 |
| P20954 | MT | 64 | 1 | n.d. | 112 | 34-40 | 5-7 | 4.7 | 1.3 | 0.29 |
| P23030 | MT | 70 | 0-0.5 | n.d. | 117 | 25-30 | 4-6 | 3.3 | 0.6 | 0.18 |
| P22932 | MI | 78 | 0.5-1 | n.d. | 109 | 35-42 | 5-9 | 4.0 | 1.6 | 0.39 |
| P24036 | I | 65 | 1.5-2 | n.d. | 114 | 30-38 | 3-5 | 3.0 | 0.8 | 0.27 |
| | | | | *Cungena - northern Eyre Peninsula* | | | | | | |
| P22937 | T | 39 | 1.5 | 44 | n.d. | n.d. | | | | |
| P23370 | T | 24 | 0.5-1 | 50 | n.d. | n.d. | | | | |
| P20954 | MT | 73 | 1-1.5 | 45 | n.d. | n.d. | NO SEED HARVESTED[2] | | | |
| P23030 | MT | 73 | 0.5-1 | 48 | n.d. | n.d. | | | | |
| P22932 | MI | 68 | 1.5-2 | 42 | n.d. | n.d. | | | | |
| P24036 | I | 72 | 3 | 36 | n.d. | n.d. | | | | |
| | | | | *Yandra - northern Eyre Peninsula* | | | | | | |
| P22937 | T | 71 | 0.5-1 | 50 | 119 | 50 | 1-2 | 0.51 | 0.13 | 0.26 |
| P23370 | T | 25 | 0.5-1 | 51 | 135 | 51 | 1-2 | 0.08 | 0.00 | n/a |
| P20954 | MT | 71 | 1.5 | 43 | 118 | 43 | 1-2 | 0.68 | 0.11 | 0.16 |
| P23030 | MT | 76 | 0.5-1 | 52 | 130 | 52 | 1-2 | 1.01 | 0.18 | 0.17 |
| P22932 | MI | 78 | 1.5-2 | 45 | 114 | 45 | 1-2 | 1.01 | 0.22 | 0.21 |
| P24036 | I | 76 | 4 | 10 | 122 | 10 | 1-2 | 0.05 | 0.01 | n/a |
| | | | | *Yeelanna* - southern Eyre Peninsula | | | | | | |
| P22937 | T | 34 | 0-0.5 | 50 | 105 | 30-40 | 4-7 | 8.27 | 5.32 | 0.64 |
| P20954 | MT | 26 | 0-0.5 | 47 | 108 | 30-40 | 7-15 | 19.53 | 8.50 | 0.44 |
| P22932 | MI | 33 | 0-0.5 | 50 | 113 | 30-40 | 15-20 | 31.67 | 14.42 | 0.46 |
| | | | | Coobowie - Yorke Peninsula | | | | | | |
| P22937 | T | 58 | 1 | 37 | 100 | 35-39 | 1-3 | 2.51 | 1.47 | 0.59 |
| P23030 | MT | 62 | 0-0.5 | 49 | 112 | 27-32 | 2-5 | 3.00 | 1.31 | 0.43 |
| P20954 | MT | 63 | 2.5 | 22 | 107 | 32-35 | 2-4 | 2.07 | 0.82 | 0.40 |
| P22932 | MI | 60 | 2 | 27 | 104 | 30-34 | 2-4 | 1.76 | 0.67 | 0.38 |
| P24036 | I | 66 | 2.5 | 17 | 107 | 27-33 | 2-6 | 3.56 | 1.84 | 0.52 |

[1] No chlorophyll meter readings were recorded at Minnipa because of a heavy dew on the leaves

[2] Most plants of all genotypes at Cungena had died by harvest. On those plants that were able to set pods, there was a heavy infestation of *Helitothus* preventing seed set.

## 7.4 Discussion

*Tarlee*

The field trial at Tarlee confirmed results from soil and solution screening procedures that genotypes of *L. pilosus*, classified as tolerant or moderately tolerant, show less chlorosis and had higher actual and relative yields than intolerant genotypes when grown on a calcareous soil under field conditions. Despite the $CaCO_3$ content of the field soil being much lower than the soil used for screening (5% compared with 50%), genotypes still developed significant $HCO_3^-$ induced chlorosis in the YEL. Theoretically the concentration of $HCO_3^-$ in soil solution at Tarlee would be lower than in the screening system as the $CaCO_3$ and the moisture contents were less. Thus it may have been expected that all genotypes would show less chlorosis. However, the tolerant genotypes developed more chlorosis and the moderately intolerant genotypes less chlorosis than was observed in the screening procedures (see Chapter 6). There were no clear reasons for the differences although it may be related to temperature differences between the glasshouse and the field. Under field conditions the temperatures were significantly lower than in the glasshouse, thus plant growth and symptom development was much slower. Similar to previous chapters all genotypes recovered from chlorosis symptoms. Reasons for this recovery have been discussed in Chapters 3 and 4.

It was notable that, unlike *L. pilosus* genotypes, the crop of narrow-leafed lupins growing in the calcareous area did not recover from chlorosis and all plants had died by flowering and pod setting stages. In previous research the alkalinity (or pH) of the soil has been suggested to be the main cause of symptoms resembling Fe deficiency in *L. angustifolius* (Tang, *et al.*, 1993; Tang, *et al.*, 1995b). In this experiment however, even the non-calcareous soil had an alkaline pH (up to 7.6), yet narrow-leafed lupins growing on this soil showed no chlorosis. Thus the presence of $CaCO_3$ in the soil *per se* and not the alkalinity is likely to be the major cause of chlorosis and yield reductions observed in narrow-leafed lupins on calcareous soil. These results also suggest that a simple measure of pH may not be adequate in determining whether the land is suitable for narrow-leafed lupin cultivation; it is much more important to measure the $CaCO_3$ content, both on the surface and at depth.

The chlorotic symptoms observed in the calcareous soil are related to $HCO_3^-$ induced nutrient deficiencies or a direct toxicity of $HCO_3^-$ (Boxma, 1972; Coulombe, *et al.*, 1984b; Chapters 3, 4 and 5). In this experiment, chlorosis was related to lower Fe and Mn concentrations in the YEL

of plants growing on the calcareous soil compared with non-calcareous soil which supports findings in previous chapters and other research (Jessen, *et al.*, 1986; White and Robson, 1989b). However, only Mn appeared to be deficient on the calcareous soil, whilst Fe concentrations still appear adequate (Table 3.3; Reuter and Robinson, 1997). Also the concentrations of Mn in narrow-leafed lupins on the calcareous soil were deficient, whilst Fe concentrations were adequate. Other research has indicated that Mn deficiency is a major limitation for plant growth on calcareous soils and screening methods to identify Mn efficient genotypes of barley and wheat use a calcareous soil (Marcar and Graham, 1987; Huang, *et al.*, 1994; Khabaz-Saberi, 1999). Field trials to identify Mn efficiency in wheat cultivars have been conducted on a calcareous soil on the Yorke Peninsula of South Australia (Bansal, *et al.*, 1992).

Absolute nutrient concentrations, particularly for micronutrients, however are unlikely to provide good differentiation between tolerant and intolerant genotypes as there is likely to be intraspecific variation in nutrient concentrations required to maintain adequate growth (i.e. a tolerant genotype may have lower concentrations of Fe or Mn than a intolerant genotype, yet show less chlorosis and better growth and yields). Hence comparison of relative nutrient concentrations may provide a better indication of the limiting nutrients. These indicate that the more tolerant genotypes, in terms of chlorosis, are generally better able to maintain Fe and Mn concentrations in the calcareous soil compared with the non-calcareous soil than the less tolerant genotypes. It was also notable that in the moderately intolerant genotypes the relative B and K concentrations were slightly higher than more tolerant genotypes. However, higher relative B and K concentration may be a result of lower concentrations Fe and Mn, i.e. the plant is attempting to compensate for the deficiencies.

The results of chlorosis scores from soil and solution screening showed a significant negative relationship with grain yield under field conditions at Tarlee. The early chlorosis symptoms in the field also tended to be correlated negatively with grain yield. Previous research on soybeans had also indicated an analogous correlation between field results and soil and solution screening results, similar to those developed in previous chapters, that use chlorosis score as the identifier of tolerance. R Chaney (pers. comm.) estimated that that the incorporation of tolerance into already high yielding cultivars resulted in a grain yield increases of 5-20% on calcareous soils. Thus the incorporation of tolerance into more agronomically suitable genotypes in the breeding program, should be an important objective in the future.

It was notable that the tolerant genotypes had the larger seeds, 0.2 to 0.25 g heavier than the moderately intolerant genotypes. This may partly explain their tolerance as they are likely to have a higher seed nutrient content, enabling them to overcome the early chlorosis. If larger seeds, greater number of pods and seed per pod could be incorporated into a genotype with improved tolerance, then even higher grain yields would be expected. However in practice, it may be desirable to target an intermediate seed size and higher number of pods per plant in a tolerant genotype as seed weight, number of pods and number of seed per pod are likely to have a negative relationship.

Although a crop may show improved tolerance to calcareous soil its grain yields may be inherently low, thus being unsuitable for economic production. In this trial all moderately tolerant to tolerant genotypes had grain yields equivalent to or greater than the surrounding narrow-leafed lupin crop. For instance, P22937 had estimated grain yields approximately 1.8 to 2.3 g plant$^{-1}$ greater than the surrounding narrow-leafed crop. This indicates that *L. pilosus* has potential for growth on non-calcareous soils. In many areas of South Australia farmers will not grow lupins due to large areas of calcareous soil within a paddock, despite most of the paddock being non-calcareous. As *L. pilosus* shows good yield even on the non-calcareous soil, it will be a suitable alternative when calcareous outcrops of soil in a paddock are a problem.

*Minnipa, Cungena, Yandra, Yeelanna, Coobowie*

The other field trials in this chapter were conducted to observe the growth of *L. pilosus* on calcareous soil differing in $CaCO_3$ and in a range of environments, particularly focussing on the lower rainfall and warmer areas of South Australia. All results need to be treated with caution as genotypes were undomesticated and all trials not replicated. Also at Cungena there was likely to be another major soil toxicity similar to that which was suggested in Chapter 3. Although plants display chlorosis on this soil, they also show stunted growth and some curling of leaves, indicative of herbicide toxicity.

At all sites, except Cungena, plants generally showed good early growth and only slight to moderate chlorosis, indicating that *L. pilosus* will grow on these calcareous soils. However, due to a long vegetative stage, and slow pod setting and maturing coupled with a susceptibility to flower and pod shedding on dry hot days the grain yields were low across the northern Eyre Peninsula and at Coobowie.

Also unlike the trial at Tarlee, grain yield results did not show a good correlation with screening results in Chapter 6. The poor correlations are likely to be due to a large genotype x environment effect. For example, the major yield limitation on the northern Eyre Peninsula and at Coobowie was likely to be dry spring conditions. Also on the northern Eyre Peninsula the higher average temperatures were likely to adversely effect grain yield. Thus genotypes tolerant to drought and high temperatures were likely to have highest yields on the northern Eyre Peninsula, whilst at Coobowie temperatures were more mild and only those genotypes with tolerance to drought were likely to have a yield advantage. Results among sites, however, were inconsistent indicating that there are many environmental factors operating at each site. From the limited data P22932, despite being moderately intolerant of calcareous soil, appeared to have the best adaptation to the northern and southern Eyre Peninsula. P24036, an intolerant genotype, had the highest yield at Coobowie, even though it showed moderate chlorosis in early growth. The incorporation of tolerance into these genotypes is likely to further enhance their yield advantage.

Grain yield at Yeelanna was particularly notable and indicated that in this environment *L. pilosus* has a high yield potential. Grain yields of 2-3 t $ha^{-1}$ would be competitive with other legumes.

*Conclusion*

These field trials demonstrated that *L. pilosus* has potential for growth on calcareous soils, particularly in higher rainfall areas. In low rainfall zones the grain yields were generally low and may not be economically viable. However, results from these experiments need to be treated with caution, as all genotypes were undomesticated. It is only going to be possible to estimate their true yield potential when the first domesticated lines are released from the breeding program.

Chlorotic symptoms early in growth in the field may not be the most reliable indicator of actual grain yield as there are many other genotype x environment effects, particularly, drought and high temperature.

Screening systems using chlorosis score 28 DAS as the indicator of tolerance will identify more tolerant genotypes, but the genes controlling tolerance need to be incorporated into an agronomically suitable background. In particular, tolerance needs to be incorporated into domesticated breeding lines, so that larger scale replicated field analysis can be undertaken.

# CHAPTER 8

## General discussion

Several crop species, including soybean, chickpea and grapevines (Froehlich and Fehr, 1981; Mengel, *et al.*, 1984; Chaney, *et al.*, 1992b) have been investigated for tolerance to calcareous soils and in most cases a wide range of genetic variation has been found. Therefore, it could be expected that both inter- and intraspecific variation for tolerance would exist in lupins, especially since many of the landraces and wild types of most species have been collected from the circum-Mediterranean where calcareous soils are prominent (Gladstones, 1974). This study showed that different species differ in their tolerance to calcareous soils (Chapter 6). There was also intraspecific variation for tolerance in *L. pilosus*, *L. atlanticus* and *L. albus*, but not in *L. angustifolius* (Chapter 6).

Intolerant species and genotypes of lupins grown in the calcareous soils in this study developed chlorosis in the younger leaves and displayed stunted growth similar to other intolerant crops (e.g. soybean, chickpea and apple) (Ao, *et al.*, 1987; Fairbanks, *et al.*, 1987; Chaney, *et al.*, 1992b). Generally *L. angustifolius* genotypes showed the most severe chlorosis, followed by *L. atlanticus* and *L. albus*, while *L. pilosus* genotypes were least chlorotic (Chapter 6). Collection site data (Clements and Cowling, 1990; Table 6.1) showed that most wild types and landraces of *L. angustifolius* had been collected from neutral to acid soils, while *L. albus* has been commonly collected from neutral soils and *L. pilosus* from neutral to alkaline / calcareous soils. Although, most *L. atlanticus* genotypes have also been collected from alkaline soils, in this study most were identified as moderately intolerant to intolerant of calcareous soils (Chapter 6). A possible explanation for this observation is that not all alkaline soils are calcareous, thus the accessions may have been collected from non-calcareous soils. Alternatively, the concentrations of active $CaCO_3$ in soils used in this study could be much higher than those at the collection sites, thus inducing more severe symptoms.

In a breeding program it is important to be able to identify and utilise intraspecific variation for traits that will improve the adaptation of a crop to the environments in which it will be grown. Therefore in this study, soil and solution screening methods were developed that could discriminate the tolerance of genotypes to a calcareous soil (Chapters 4, 5 and 6). The solution

screening was specifically developed to improve efficiency (*c.f.* soil screening) and provide a selection method for early generation breeding material, as lines could be transplanted to pots after testing for growth to harvest.

Chlorosis scores in the soil and solution screening were correlated for all species, except *L. atlanticus*. To improve this correlation for *L. atlanticus*, the $HCO_3^-$ concentration used in solution screening could be lowered to reduce the severity of symptoms, increasing the range between tolerant and intolerant, and concentrations of nutrients adjusted to provide an improved growth environment. Alternatively, to reduce the severity of the soil screening, a calcareous soil could be used that contains a lower content of $CaCO_3$ than the Wangary soil (50% $CaCO_3$). In addition, the major limiting factor in calcareous soils for *L. atlanticus* may not be directly related to $HCO_3^-$. Nutrient deficiencies, such as Fe, Mn, Zn and P, could occur, thus experiments using increasing rates of individual nutrients could be conducted to determine the actual deficient nutrient or combination of nutrients. Screening methods could therefore be developed for each nutrient rather than just using $HCO_3^-$.

In soil and solution screening for *L. pilosus*, a large range of genotypic variation for tolerance to calcareous soils was observed from tolerant (no chlorosis) to intolerant (highly chlorotic) (Chapter 6). Collection site data showed that the tolerant to moderately tolerant material were from Israel, Syria and Turkey, while a majority of the intolerant genotypes were collected from Greece. Additional tolerant to moderately tolerant genotypes are likely to exist in these countries as they have large areas of calcareous soils, thus warranting further collection expeditions. Further screening of untested genotypes in the Australian lupin collection is also likely to identify other tolerant genotypes.

Most genotypes of *L. atlanticus* were moderately intolerant to intolerant, while genotypes of *L. albus* were moderately tolerant to moderately intolerant. No intraspecific variation was observed in *L. angustifolius*, which was expected as all cultivars tested had been identified as intolerant in previous field research (Egan, *et al.*, 1992-93; Buirchell pers. comm.). However, some variation could be expected in *L. albus* and *L. angustifolius* if the complete collections were screened as wild types and landraces have been collected in countries from which tolerant genotypes of *L. pilosus* have been collected (Clements and Cowling, 1990). In *L. atlanticus*, potential tolerant lines have been identified (Chapter 6), but need to be confirmed in further research once screening methods for *L. atlanticus* have been improved.

The screening procedures have identified suitable tolerant parental genotypes within *L. pilosus* (P23370 and P22937) for use in a breeding program. As the genetics of tolerance appeared to be relatively simply inherited (one or two partially recessive genes; Chapter 6), backcrossing could be used to incorporate the trait into genotypes that are relatively well adapted to the southern Australian environment, but are intolerant of calcareous soils. Screening of the $F_2$ generation and selected tolerant $F_3$ families from these backcrossing populations by the solution method would enable the elimination of most of the intolerant material from the breeding population. With new techniques being developed in genomic research, in the future it may be possible to isolate the gene(s) conferring tolerance to calcareous soils from another crop species and insert them into the domesticated and adapted lupin species, such as *L. angustifolius* and *L. albus*.

High chlorosis scores recorded 21 days after sowing in *L. pilosus* in the soil and solution screening methods were correlated with decreased relative grain yields of plants grown in the field on a calcareous soil compared with a non-calcareous soil (Chapter 7). However, actual grain yields on the calcareous soil were generally not closely correlated with chlorosis rankings in the screening methods. The poor correlation could be due to the diverse genetic background of the genotypes, thus adaptation to the environment, apart from tolerance to calcareous soils, is likely to differ. For example, the moderately intolerant genotype, P22932, was early flowering, which was likely to be a major factor determining the yield potential at the lower rainfall sites used in this study. Also, even genotypes that have been classified as moderately tolerant in screening, recover from chlorosis in later growth, thus overall effects of moderate chlorosis on the grain yield may be slight. Development of isogenic lines that differ in tolerance to calcareous soil could be used to determine the actual increase in grain yield in *L. pilosus* following the incorporation of genes that confer tolerance to calcareous soils. In soybeans, the incorporation of genes conferring tolerance into intolerant high yielding cultivars resulted in grain yield increases of 5 -100% on these soils (W Fehr, pers. comm.).

The $CaCO_3$ content of the soil can vary both spatially and at depth (i.e. both calcareous and non-calcareous soils can occur within one paddock). Thus, to be viable in a cropping rotation, a tolerant *L. pilosus* cultivar that will grow on calcareous soils must also be able to produce grain yields similar to an intolerant species, such as *L. angustifolius*, when grown on non-calcareous soils. In this study, tolerant *L. pilosus* genotypes showed equivalent or improved growth and up

to a 35% grain yield increase compared with *L. angustifolius* cultivars in non-calcareous soils (Chapter 3 and 7).

*L. pilosus* also appeared to be more tolerant of toxic concentrations of B and sodicity than *L. angustifolius*, both of which can be detrimental to plant growth in the southern Australian cereal growing zone (Chapter 3; Cartwright, *et al.*, 1986; Rengasamy and Olsson, 1991). Results in this study confirmed previous research (Brand, 1995) showing that *L. pilosus* grown on B toxic subsoils was able to maintain lower concentrations of B in the shoots than *L. angustifolius*. *L. pilosus* produced more dry matter than *L. angustifolius* when grown in a sodic subsoil (Chapter 3).

The chlorosis and stunted growth of plants grown in calcareous soils has commonly been attributed to $HCO_3^-$-induced Fe deficiency. However, in the soil experiments conducted in this study on *L. pilosus*, plants were not deficient in Fe on the basis of the nutrient analyses, despite displaying significant symptoms resembling Fe chlorosis (Chapter 3 and 4). Also, Fe fertilization only partly alleviated symptoms, despite increasing active and total Fe concentrations (Chapter 4). Kaur, *et al.* (1984) suggested that the Fe may be inactivated inside the leaf in response to calcareous soils, so that theoretically total concentrations may be adequate, but the active Fe may be deficient. This study showed that in *L. pilosus* there were no differences in active Fe concentrations between tolerant, moderately tolerant and moderately intolerant genotypes (Chapter 4). Also preliminary experiments showed that the Fe reducing and proton excretion capacities of tolerant and intolerant genotypes were similar suggesting that plants were not showing a significant response to Fe deficiency (Appendix 1).

Mn deficiency appeared to be contributing to the chlorosis in *L. pilosus*, as in all experiments on calcareous soils the concentrations of Mn in the shoots were low compared with those on the non-calcareous soils. However, Mn concentrations did not correlate with the difference in chlorosis scores among the tolerant, moderately tolerant and moderately intolerant *L. pilosus* genotypes used in Chapter 4, indicating that Mn is unlikely to be a major factor.

Ca concentrations were higher in plants grown on calcareous than non-calcareous soils, but were not toxic to growth (Tang, *et al.*, 1995a; Reuter and Robinson, 1997). In addition, a tolerant genotype (P23370) was able to maintain lower concentrations in the YEL than a moderately intolerant genotype (P22932), but this response may be related to a dilution effect as the tolerant

genotype also showed more vigorous growth (Chapter 4). Other nutrients which were expected to be deficient on calcareous soils, such as Zn, Cu and P, showed no relationships with chlorosis measurements.

Since $HCO_3^-$ induced nutrient deficiencies or toxicities, individually or combined, were unlikely to be the complete cause of chlorosis, it is hypothesized that $HCO_3^-$ was having a direct effect on chlorophyll synthesis in intolerant genotypes, thereby inducing chlorosis (Chapter 4). In this study high $HCO_3^-$ concentrations were related to chlorotic symptoms and poor growth. For example, increased $CaCO_3$ concentrations and moisture contents in a calcareous soil which are likely to increase the $HCO_3^-$ concentration in soil solution caused an increase in the severity of chlorosis and a reduction in growth (Chapter 4). Solution culture experiments also showed that chlorosis symptoms increased and root and shoot growth decreased proportionally with the $HCO_3^-$ concentration (Chapter 5).

Various mechanisms of tolerance to calcareous soils or $HCO_3^-$-induced chlorosis have been proposed and discussed (Chapters 3 and 4; Chaney, *et al.*, 1992a; Marschner, 1995). This study suggests that the most probable mechanism is related to an ability to exclude the $HCO_3^-$ or efficiently sequester it once inside the root, preventing increases in internal pH or transport of $HCO_3^-$ to the shoot (Chapter 4).

Production of proteoid roots, which enhance the uptake of P and to a lesser extent Zn and Mn (Gardner, *et al.*, 1981; Dinkelaker, *et al.*, 1989), is a possible alternative mechanism of tolerance. However, there were no differences in the number of proteoid roots produced by tolerant and intolerant genotypes of *L. pilosus*, despite the quantity of these roots increasing proportionally to increasing $HCO_3^-$ concentrations in solution culture in both *L. pilosus* and *L. albus* (Chapters 4 and 5). Hence, their role in the chlorosis response is unclear.

In tolerant genotypes of *L. pilosus* the mechanism of tolerance appeared to function from germination, whereas in moderately tolerant to moderately intolerant genotypes which show a recovery from chlorosis (Chapter 3 and 4), the mechanism appeared to be 'switched on' in response to the soil environment. This indicates that there could be two separate mechanisms that control tolerance to calcareous soils. The specific physiological differences between tolerant and intolerant genotypes and mechanisms of tolerance need to be determined by experiments that

measure changes in root exudation, proton excretion and pH in the root and shoots in a calcareous soil over time.

*Conclusion*

Development of rough-seeded lupins for tolerance to calcareous soils could significantly expand the area sown to lupins in southern Australia and contribute to farm profitability and sustainability. This study showed that *L. pilosus* is generally more tolerant to calcareous soils than other lupin species and that intraspecific variation for tolerance exists, particularly in *L. pilosus*. Thus, selection of tolerant parental breeding material, and through the application of efficient screening procedures on segregating populations, the incorporation of genes conferring tolerance into higher yielding intolerant genotypes could be a major objective of the breeding program aimed at developing the rough-seeded lupins for calcareous soils.

# APPENDIX 1

## Preliminary studies on proton excretion and Fe and Mn reduction in the rhizosphere zones of roots of genotypes of *L. pilosus* tolerant or moderately intolerant of calcareous soil

### A1.1 Introduction

The physiological basis for the genotypic variation in *L. pilosus* for tolerance to calcareous soils is unknown. High concentrations of $HCO_3^-$ in soil solution induces chlorosis in calcareous soils by inhibiting absorption by the roots and translocation of Fe in the plant (Coulombe, *et al.*, 1984a). Deficiency of other nutrients also affects plant growth on calcareous soils. Results in Chapters 4 and 5 suggested that in *L. pilosus* the $HCO_3^-$ may also induce Mn deficiency or have a direct effect on chlorophyll synthesis. Therefore the tolerant genotypes are likely to have a mechanism which reduces or overcomes the toxic effects of $HCO_3^-$.

Possible mechanisms of tolerance to calcareous soils were outlined in Chapter 3. Most mechanisms focus on the plants ability to alter the rhizosphere conditions, either by excreting protons and/or reducing agents that improve the availability of nutrients. The Fe and Mn reduction and pH in the rhizosphere can be estimated by embedding roots in agar containing indicators (Marschner, *et al.*, 1982).

This experiment examined the proton excretion and Fe and Mn reduction capacity of the roots of a tolerant and an intolerant genotype using agar techniques to determine the physiological basis for the differences in the chlorosis observed in a calcareous soil.

The experiment is presented as an appendix as results were only subjective

### A1.2 Materials and methods

Plants of a tolerant (P23370) and an intolerant (P24036) genotype of *L. pilosus* were grown in both the soil and solution screening systems outlined in Chapter 6 and harvested at 7, 14 and 21 DAS. Visual chlorosis symptoms were monitored to confirm previous screening results. For comparison, plants were also grown in a control solution system without $HCO_3^-$. At each harvest

two intact plants were carefully removed and roots washed in RO water to remove contamination associated with the soil or solution. The roots were then embedded in agar as outlined below.

*Agar preparation*

All methods have been modified from those outlined in Marschner, *et al.* (1982). The final agar concentration in all solutions was 1%.

To measure pH changes in the rhizosphere, bromocresol purple (pH 7.0 - purple; pH 6.0 - red; pH 4.5 - yellow) was mixed with the liquid agar to a final concentration of 0.006% and the pH of the solution was adjusted to 6.5 with NaOH or $H_2SO_4$. To enable estimation of pH, approximately 10 mls of the agar was poured into each of 10 plastic containers and pH adjusted to give a range between 4 and 8.5 (see plate A1.1).

To demonstrate the Fe reducing process at the root surface, nutrients at the same concentrations as indicated in the solution screening experiments (Table 5.1) and BPDS (Bathophenanthroline Disulfonic Acid) (300 μM) were added to the agar. BPDS is a chelator for $Fe^{II}$, forming a red coloured $Fe^{II}$ complex.

To demonstrate the Mn reducing process at the root surface nutrients as described above without the Fe were added to the liquid agar. The nutrient solution containing agar was then boiled and $KMnO_4$ dissolved with a final concentration of 1 mM. The solution was kept at $50^{O}C$ for 2 hours and pH adjusted to 6.0 with NaOH.

The agar solutions for all treatments were kept fluid at $40^{O}C$ in a water bath until embedding of plant root.

*Embedding roots in agar*

Duplicate plants of each of the genotypes were used for each agar treatment. The roots were carefully laid out in perspex boxes (100 mm x 400 mm x 10 mm) and liquid agar ($40^{O}C$) poured over the roots until fully covered. The containers were covered with black plastic to exclude light and placed in a growth room (light intensity - 550 to 650 μmol $s^{-1}$).

*Measurements*

Changes in colour of the agar were recorded over a 24 hour period. For rhizosphere pH, the colour of the agar changing from purple to yellow indicated acidification. For the Fe reducing capacity, intensification of the red colour was seen if the roots were producing the reducing agent. For Mn reduction, the colour of the agar changed from a brown colour to clear if roots were reducing Mn.

## A1.3 Results

*Plant Growth and Chlorosis*

The growth responses were similar in the calcareous soil and in the solution system containing 15 mM $HCO_3^-$. As observed in Chapters 5 and 6, P24036 showed significant chlorosis at 14 and 21 DAS, whilst P23370 showed slight chlorosis at 21 DAS. There was no difference between genotypes at 7 DAS. In terms of root growth, both genotypes had visually similar root systems and had produced proteoid roots by 14 DAS. Plants grown in 0 mM $HCO_3^-$ showed no chlorosis and more vigorous shoot growth than those in 15 mM $HCO_3^-$. Root systems of plants in 0 mM $HCO_3^-$ were also much larger and had no proteoid roots compared with 15 mM $HCO_3^-$.

*Rhizosphere pH*

Roots of both P23370 and P24036 which had been grown in the soil culture showed no response when embedded in the agar with bromocresol purple as an indicator at any harvest.

Roots of both P23370 and P24036 which had been grown solution culture at 0 mM and 15 mM $HCO_3^-$ showed similar responses when embedded in agar with bromocresol purple as an indicator at each harvest. Generally, 30 minutes after embedding there was an acidification around the root tips in plants from the 0 mM $HCO_3^-$ solution. The acidification around the root tips of plants that had been grown at 15 mM $HCO_3^-$ occurred one hour later. Twenty four hours after embedding the roots there was a strong acidification around the entire root system of plants of both genotypes which had been grown at 0 and 15 mM $HCO_3^-$ (Plate A1.1).

*Fe and Mn reduction*

The Fe reducing processes of both genotypes were similar and only occurred in the plants which had been grown in the soil system. The colour changes were most visible 14 and 21 DAS, after proteoid roots had been formed. The first changes in colour were noted in P23370 around the

proteoid roots. Plate A1.2 shows the intense red colour 24 hours after embedding roots, particularly prevalent around the proteoid roots and around the taproot.

With the Mn reducing agar there were no responses at any harvest in either genotype which had been grown in either soil or solution culture.

## A1.4 Discussion

This preliminary investigation indicates that further research is warranted into the $H^+$ excretion and Fe or Mn reducing systems of lupins. All results show no clear differences between the tolerant and intolerant genotypes for all measurements recorded. However, plants seemed to be able to acidify their rhizosphere, which was likely to increase the availability of many nutrients for uptake, and reduce $Fe^{III}$ which increases $Fe^{II}$ uptake. The lack of difference between genotypes may indicate that the mechanism of tolerance is not related to either increased $H^+$ excretion or Fe reduction; rather there may be a mechanism which either excludes $HCO_3^-$ from the root or sequesters it once inside the root. It also supports the hypothesis that $HCO_3^-$ directly induces chlorosis in the leaves of intolerant plants.

Alternatively, to gauge the actual differences between genotypes it may be necessary to develop or utilise methods that can measure actual pH or concentrations of the reducing agent. For example with pH a microelectrode can be used to estimate the pH in the rhizosphere in any section of the root system (Marschner, *et al.*, 1982).

**Plate A1.1** Acidification (yellow) of the bromocresol purple agar by roots of *L. pilosus* P24036 grown in solution culture at 15 mM $KHCO_3$ (left) and 0 mM $KHCO_3$ (right) 14 days after transplanting. Containers on left side indicate change in colour of agar due to pH (top - pH 4, bottom - pH 8.5).

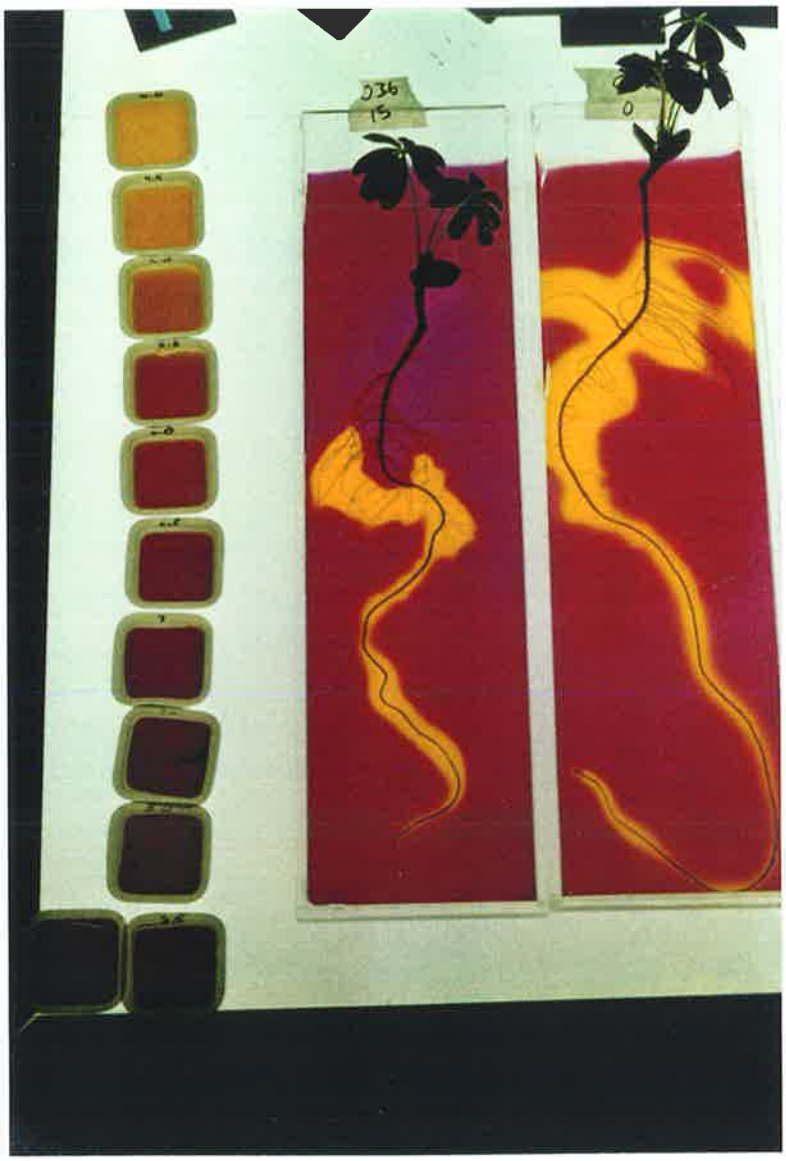
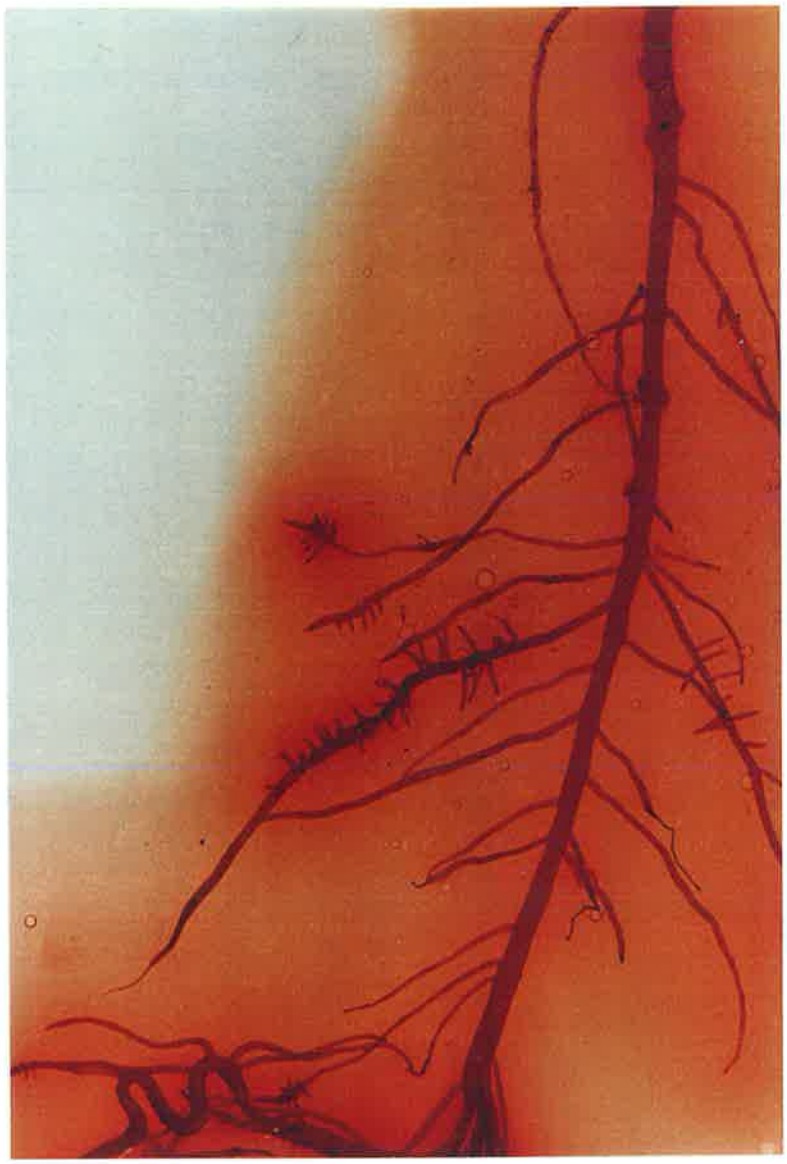


**Plate A1.2** Darker red colour around taproot and particularly proteoid roots of *L. pilosus* P23370 embedded in BPDS agar due to Fe reduction 21 days after sowing in the calcareous Wangary soil.



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