Boron tolerance of lentil - highlights of a research program

Kristy B. Hobson[1,2], Roger D. Armstrong[1], M. Nicolas[2] , David J. Connor[2] and Michael A. Materne[1]

[1]Primary Industries Research Victoria, Department of Primary Industries-Horsham, PB 260 Horsham, Vic 3401
Email kristy.hobson@dpi.vic.gov.au

[2]The University of Melbourne, School of Agriculture and Food Systems, Parkville, Vic 3010

Abstract

Soils with high concentrations of boron (B) were shown to be prevalent in the north and central Wimmera and southern Mallee region of Victoria. Pulses are generally considered more sensitive than cereals to abiotic stresses, including B toxicity, and the most viable approach is to develop B tolerant crop cultivars. Current Australian cultivars were found to be intolerant of high concentrations of soil B. B tolerance was, however, identified in lentil germplasm and subsequent research revealed that seedling tolerance persisted through to maturity and resulted in yield benefits. Under B toxicity, net photosynthesis is reduced as a result of a reduction in green leaf area, rather than by a decrease in photosynthetic rate of the remaining green leaf area. The incorporation of the boron tolerant genotypes identified in this study into backcrossing programs will allow breeders to produce cultivars that will expand lentil production areas.

Media Summary

Lentil accessions with greater boron tolerance than current cultivars have been identified and are providing invaluable tolerance genes for breeders to expand lentil production areas.

Key Words

boron toxicity, *Lens culinaris*, leaf photosynthetic rate, abiotic stress

Introduction

Pulse crops are an important component of profitable and sustainable cropping systems. The area of land under lentil (*Lens culinaris*) production in Victoria has increased rapidly from 15 000 ha in 1996-1997 to 90 000 ha in 2001-2002 (Anon, 2002). Current Australian cultivars grown in Victoria were selected for release and are now grown predominantly on the grey cracking clay soils of the Wimmera. For lentil production to expand further in Victoria, cultivars are needed that are adapted to the less favourable soil types that dominate the southern Mallee and northern Wimmera regions. However, in these regions pulses are often seen as unreliable due to poor growth and yield, especially in years with low rainfall. This may partly be due to soil abiotic stresses, such as boron toxicity and salinity, as pulse crops are generally considered more sensitive to these constraints than cereal crops (Jayasundara et al*.* 1998). Many farmers use fallow in their cropping systems as an alternative to cereal production in non-viable pulse cropping soils. Fallowing in this region can lead to environmental degradation through wind erosion and along with failed crops can lead to recharge which contributes to dryland salinity (O'Connell et al*.* 1995).

In the cropping regions of southern Australia, high concentrations of soil B have been identified as a possible limitation to crop growth and grain yield. In this region the highest levels of boron have been found to occur at depths between 40 and 100 cm in the soil profile (Cartwright et al*.* 1984; Nuttall et al*.* 2003). The amelioration of B toxicity through soil modification is not an economic or practical solution so the breeding of more tolerant cultivars is considered the best approach to improve grain yield.

This paper highlights some important findings from a research program examining B tolerance in lentil. The findings presented examine the distribution of soil B throughout the southern Mallee, northern and central Wimmera region of Victoria, and the identification and investigation of boron tolerance in lentil germplasm.

Methods

Field survey

A survey of 14 commercial paddocks in the southern Mallee, northern and central Wimmera regions of north-west Victoria was undertaken. A total of 160 individual soil profiles were divided into 6 depth increments 0-10, 10-20, 20-40, 40-60, 60-80 and 80-120 cm. Each increment was analysed for soluble soil B concentration using a hot $CaCl_2$ extracting method (Rayment and Higginson 1992).

Glasshouse experiments

Three studies were conducted:

1. Screening for boron tolerance in lentil germplasm. 310 lentil accessions were grown in a soil containing 54 mg/kg of boron (as boric acid) applied (soluble B (hot $CaCl_2$) = 39 mg/kg). At 28 days after emergence plants were assessed on the severity of foliar symptoms using a 0.0 to 8.0 scale (Hobson et al. 2003), with no visible symptoms rated 0.0 and a dead plant rated 8.0.

2. The effect of high B on 6 lentil accessions varying in tolerance to high soil B. Two tolerant (ILL2024 and ILL213A) two moderately tolerant (ILL5883 and ILL4139 – cv. Laird) and two intolerant (ILL7200 – cv. Cassab and ILL7180 – cv. Nugget) lentil accessions were studied. Two boron rates, 0 and 25 mg of applied B/kg of soil (soluble B (hot $CaCl_2$) = 1.6 and 18.2 mg/kg respectively), were applied with basal nutrients to soil in 20 cm pots. At mid-flowering, gas exchange was measured on the third or fourth-youngest leaf using a LI-COR 6400 portable open gas exchange system (light was provided by a 6400-02 LED light source and photon flux was 600 μmol/m^2/s). After measurement, the part of the leaf inserted into the chamber was removed and total leaf area and green leaf area of this part was recorded to allow calculation of the rate of net photosynthesis per unit leaf area and green leaf area. The final harvest was conducted at physiological maturity when grain yield was recorded.

3. The effect of subsoil B on two lentils varying in tolerance to high soil B. Two lentil accessions, ILL2024 (tolerant) and Cassab (intolerant) were grown in PVC cores (65 cm in length, 15 cm diameter). Two B rates were applied, 0 and 30 mg B/kg of soil to topsoil. Three profiles of B distribution were investigated to simulate ‘natural’ distributions of B in the soil profile: 1) no added B, 2) soil in the 30-60 cm zone spiked with 30 mg B/kg and 3) soil in the 10-50 cm zone spiked with 30 mg B/kg. Basal nutrients without B were applied to the top 10 cm in each treatment. A harvest for grain yield was recorded at physiological maturity.

Results

Field Survey

High concentrations of soil boron are common throughout the southern Mallee, northern Wimmera and central Wimmera region (Table 1). Boron concentration increases with depth in the soil profile. The B concentrations and trends of B distribution reported here are in agreement with Nuttall et al (2003) who examined soils predominantly in the southern Mallee region. These data provide a basis for selection of appropriate soil B concentrations for glasshouse experiments.

Table 1. Mean soluble boron values for 160 profiles in the southern Mallee, northern Wimmera and central Wimmera. Standard deviation is shown in parentheses.

Depth in profile (cm)	B (mg/kg)			
	All profiles (n=160)	Southern Mallee (n=94)	Northern Wimmera (n=36)	Central Wimmera (n=30)
0-10	2.4 (1.71)	2.4 (2.18)	2.3 (0.68)	2.7 (0.07)
10-20	3.2 (2.35)	3.4 (2.96)	3.0 (1.07)	3.0 (0.82)
20-40	7.3 (6.02)	7.8 (7.02)	7.6 (4.28)	5.6 (3.82)
40-60	15.1 (9.05)	14.8 (9.12)	17.0 (8.11)	13.9 (9.80)
60-80	21.1 (9.88)	19.3 (8.12)	22.4 (9.68)	25.6 (13.17)
80-120	23.9 (11.38)	20.8 (7.79)	23.8 (10.83)	33.8 (15.45)

Glasshouse experiments

Large variation in expression of symptoms of B toxicity was observed amongst lentil accessions (Figure 1). Most of the current Australian cultivars (eg. Cassab, Digger, Nugget and Northfield) were intolerant to high concentrations of soil B. The most tolerant lentils identified in this study were ILL2024 and ILL213A, both of which are red lentils that originate from Ethiopia and Afghanistan, respectively. These lentils have greater tolerance than the previously reported tolerant ILL1765 (Yau and Erskine 2000).

Seedling tolerance evident in the first study was shown to persist through to maturity and result in yield benefits (Table 2). There was no significant reduction in grain yield of the tolerant lines (ILL2024 and ILL213A) at the high B rate (B25). Grain yield of the moderately tolerant accessions (ILL5883 and Laird) was significantly reduced compared to the B0 control, but performed better than the intolerant Cassab and Nugget, two widely grown Australian cultivars.

Net photosynthetic rate (P_N) calculated on total leaf area was significantly reduced at the high B rates in all accessions except the tolerant ILL2024 and ILL213A (Table 3). However, when P_N was re-calculated to account for the loss of green leaf area, the reduction in P_N was no longer significant in a number of accessions. This suggests that the reduction of photosythesis is due to a loss of green leaf area caused by leaf chlorosis and necrosis (a symptom of B toxicity) rather than a reduction in photosynthetic rate of the remaining green leaf area. This finding highlights the importance of maintaining maximum green leaf area under high B conditions. If the plant produces or maintains green leaf area at a higher rate than chlorosis progresses, the plant will be able to produce the required photosynthate. This is similar to plant responses to toxic levels of NaCl salt (Munns 2002). Where the foliar symptoms of B toxicity are very severe as was the case with the intolerant Cassab and Nugget at the B25 rate, the photosynthetic rate is so low or non-detectable, that accounting for green leaf area can increase P_N but the reduction remains significant.

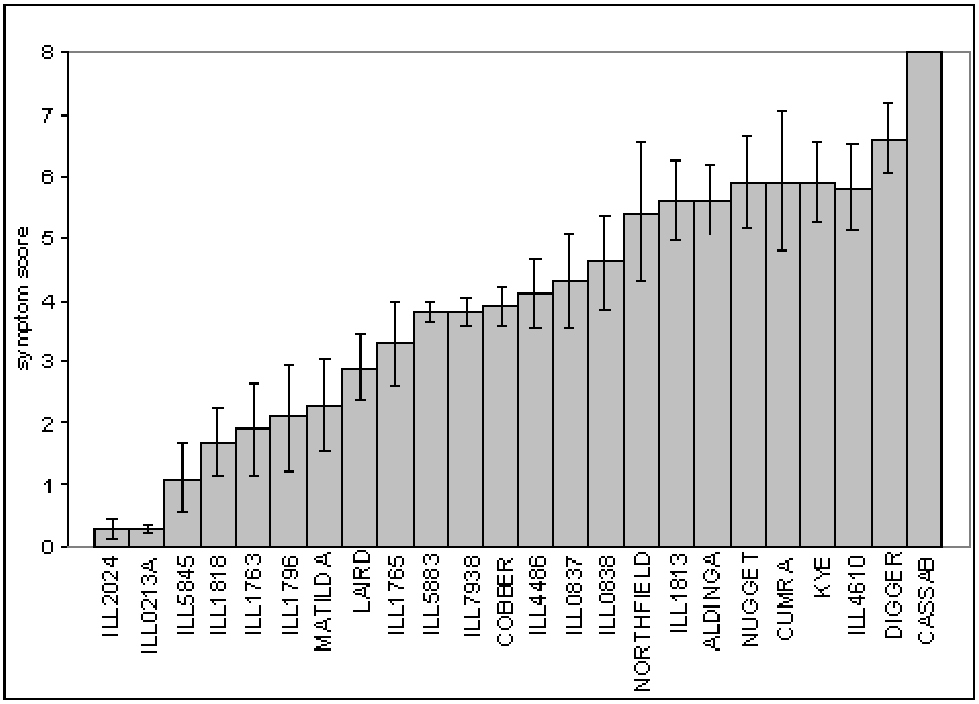

Figure 1. Variation in response of selected lentil accessions to high concentrations of soil boron.

Table 2. Seed weight (g/pot) for six lentils grown at three levels of boron (mg B/kg soil) at three rates of applied B 0, 15 and 25. Statistical analyses were performed on ln-transformed data (mg/pot) presented in parentheses. Significance levels refer to the transformed data. l.s.d = least significant difference for genotype x boron interaction (P<0.001).

	Seed weight (g/pot)		
Genotype	B0	B15	B25
ILL2024	10.4 (9.22)	10.3 (9.23)	5.5 (8.61)
Cassab	11.7 (9.37)	2.0 (7.56)	0.1 (1.46)
ILL213A	16.5 (9.71)		13.3 (9.50)
ILL5883	16.4 (9.69)		4.1 (7.94)
Laird	16.9 (9.73)		1.5 (7.29)
Nugget	11.0 (9.30)		0.41 (5.99)

l.s.d (P<0.001)	(1.21)

When grown in a core resembling ‘natural’ B distribution in a soil profile, no significant reduction in grain yield occurred in any of the B treatments for the tolerant lentil ILL2024 (Table 4). In contrast, the B intolerant Cassab showed significant yield reducions in both treatments where B was added to the soil. The 30 cm zone of B free soil did benefit Cassab and yield was significantly higher than in the treatment where only 10 cm of B free soil existed. These yield responses reveal the benefit that B tolerant lentils could offer growers with high B soils. ILL2024 has been incorporated into a backcrossing program to eliminate some negative traits such as poor disease resistance and unsuitable seed colour associated with this accession.

Table 3. Net photosynthetic rate (µmolCO_2/m^2/s) of the third or fourth youngest leaf of six lentils grown at three levels of boron calculated for total leaf area (including chlorotic/necrotic regions) and green leaf area. l.s.d = least significant difference for interaction (P<0.001).

Genotype	Photosynthetic rate (µmolCO_2/m^2/s)				
	P_N – total area of leaf			P_N – green area of leaf	
	B0	B15	B25	B15	B25
ILL2024	9.8	7.9	9.1	7.9	9.1
Cassab	18.2	9.8	0.0	15.1	0.0
ILL213A	16.8		21.5		21.5
ILL5883	19.4		16.0		21.5
Laird	11.8		7.4		9.5
Nugget	17.5		3.3		5.7
l.s.d		(2.69)		(3.20)	

Table 4. Seed weight (g/core) for two lentils grown at three boron treatments. Statistical analyses were performed on ln-transformed data (mg/core) presented in parentheses. Significance levels refer to the transformed data. LSD = least significant difference for interaction (P<0.001).

Genotype	Seed weight (g/core)
	Boron treatment

	No B applied	B 30-60cm	B 10-60cm
ILL2024	13.2 (9.45)	15.0 (9.59)	13.7 (9.46)
Cassab	11.6 (9.32)	7.4 (8.84)	1.7 (7.32)
l.s.d.		(0.1831)	

Conclusion

B tolerance exists in available lentil germplasm and results in yield benefits under high boron soils. The superior yield of the tolerant lines over the widely grown intolerant cultivars on high B soils shows the benefit of including B tolerant accessions in backcrossing programs. In order to provide robust lentil cultivars, this B tolerance should be pyramided with salt tolerance and disease resistance.

References

Anonymous. 2002. Pulse Australia. http://www.pulseaus.com.au/Marketing%20Information/Statistics.htm

Cartwright B, Zarcinas BA, Mayfield AH (1984) Toxic concentrations of boron in a red-brown earth at Gladstone, South Australia. *Australian Journal of Soil Research* **22**, 261-272.

Hobson KB, Armstrong RD, Connor DJ, Nicolas ME, Materne MA (2003) Genetic variation in response to high soil boron exists in lentil germplasm. In '11th Australian Agronomy Conference'. Geelong, Australia. (Conference Design Pty Ltd)

Jayasundara HPS, Thomson BD, Tang C (1998) Responses of cool season grain legumes to soil abiotic stresses. In 'Advances in Agronomy'. (Ed. DL Sparks) pp. 77-153. (Academic Press: San Diego)

Munns R (2002) Comparative physiology of salt and water stress. *Plant, Cell and Environment* **25**, 239-250.

Nuttall JG, Armstrong RD, Connor DJ, Matassa VJ (2003) Interrelationships between edaphic factors potentially limiting cereal growth on alkaline soils in north-western Victoria. *Australian Journal of Soil Research* **41**, 277-292.

O'Connell M, O'Leary G, Incerti M (1995) Potential groundwater recharge from fallowing in north-west Victoria, Australia. *Agricultural Water Management* **29**, 37-52.

Rayment GE, Higginson FR (1992) 'Australian laboratory handbook of soil and water chemical methods.' (Inkata Press: Melbourne)

Yau SK, Erskine W (2000) Diversity of boron-toxicity tolerance in lentil growth and yield. *Genetic Resources and Crop Evolution* **47**, 55-61.