Genetic variation in tolerance to high concentrations of soil boron exists in lentil germplasm

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Abstract

Soils with high concentrations of boron are common in the grain belt of south-eastern Australia. The reduction of soil boron concentration is considered impractical and so the viable alternative is to develop boron tolerant crop cultivars.

A glasshouse experiment was conducted with 310 lentil accessions to determine the genetic variation in response to high concentrations of soil boron. The collection of accessions included Australian and overseas cultivars, advanced breeding lines and landraces from diverse geographic origins. Boric acid (54 mg/kg) was added to a soil collected from the southern Mallee of Victoria to artificially create a high boron treatment. No boron was added to the soil for the control treatment. Seeds were pre-germinated and boron toxicity was assessed according to the extent of foliar symptoms four weeks after emergence.

Large variation in tolerance to high concentrations of soil boron was observed. A small number (9%) of accessions were tolerant with the majority being intolerant (70%). The most tolerant accessions generally originated from Ethiopia, Afghanistan and the Middle East. Current Australian cultivars were generally intolerant to high concentrations of soil boron.

The identification of tolerant lentil accessions provides sources of boron tolerance that can be used to develop new boron tolerant varieties that will increase lentil profitability and reliability in southern Australia.

Key Words

boron toxicity, *Lens culinaris*, soil screening

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 (1). 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, varieties are needed that are adapted to the less favourable 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 (2).

In the cropping regions of southern Australia, high concentrations of soil boron has been identified as a possible limitation to crop growth and grain yield. The highest levels of boron in this region have been found to occur at depths between 40 and 100 cm in the soil profile (3, 4). The amelioration of boron toxicity through soil modification is not an economic or practical solution in this region. Hence the breeding of more tolerant cultivars is considered the best approach to minimise yield losses.

Previous research has identified genetic variation in tolerance to high concentrations of boron in cereals (5) and legumes such as medic (*Medicago* spp.) and field pea (*Pisum sativum*)(6). The aim of this study was to evaluate the genetic variability in lentil for response to high concentrations of soil boron and relate the findings to geographic origin of each line.

Methods

Soil preparation

Soil (sandy loam, 5-20 cm layer) was collected from the Birchip (35?98′ S, 142?92′ E) region, (pH = 8.43, B($CaCl_2$) = 0.9 mg/kg, EC = 0.10 dS/m, ESP = 0.01). The soil was sieved, steam pasteurised (for control of weeds and soil borne pathogens) and re-sieved. For the high B treatment, boric acid was dissolved in warm water and applied in solution to 3 kg of air-dry soil and thoroughly mixed. Once dried, the soil-B mix was added to 47 kg of air-dry soil and thoroughly mixed in a concrete mixer, giving a final concentration of 54 mg/kg. To ensure nutrients were non-limiting, a basal nutrient mix was added to the soil while mixing. Large plastic boxes (64 x 40 x 26 cm) which had no free drainage, were filled with the soil, giving a final weight of 50 kg.

Seed preparation and assessment

310 lentil accessions, including Australian and overseas cultivars, advanced breeding lines and landraces, from diverse geographic locations, were screened for boron tolerance. Twelve accessions were randomly assigned a box and were replicated twice. Each box included a reference accession, ILL5883 which was previously reported as moderate boron tolerance (7). To ensure uniform establishment, 16 seeds per accession were pre-germinated for 2 days, and 4 seeds were sown 2 cm deep. The boxes were located in a glasshouse and watered weekly with a uniform amount of reverse osmosis water. Four weeks after planting, individual plants were scored for severity of symptoms on the basis of leaf damage using a 0.0 - 8.0 rating scale (Table 1) (8). Means of individual accessions were compared using standard errors and used to determine the accession’s response to high soil boron.

Table 1. Visual scoring system based on the severity and appearance of foliar symptoms of boron toxicity in lentils

Score	Foliar symptoms
0.0	No apparent symptoms
0.5	Chlorosis on tips of oldest leaves, no marginal necrosis
1.0	Tip necrosis on the oldest set of leaves
1.5	As 1.0, plus chlorosis on tips of second set of leaves
2.0	Tip necrosis on second set of leaves and leaf necrosis on less than or equal to 25% of total leaf area
2.5	As 2.0, plus chlorosis on tips of second set of leaves

3.0	Tip necrosis on third set of leaves and leaf necrosis on 26% to 50% of total leaf area
3.5	As 3.0, plus tip necrosis on fourth set of leaves
4.0	As 3.5 plus complete necrosis of bottom leaves and leaf necrosis on 51 to 75% of total leaf area
4.5	As 4.0, plus tip necrosis on fifth set of leaves
5.0	As 4.5, plus complete necrosis of second set of leaves and leaf necrosis on greater than 76% of total leaf area
5.5	All leaves with marginal necrosis except youngest leaves
6.0	Plant wilted
7.0	Only stem green
8.0	Plant dead

Results

Boron toxicity symptoms were visible on the least tolerant accessions within one week of emergence. Symptoms of boron toxicity were similar to those observed on other pulse species previously studied (6). Symptoms first appeared on the lower (older) leaves and progressed to younger leaves. Tip chlorosis on the leaflets developed and that progressed towards the base of the leaflets, often resulting in total leaf senescence. Genetic variation in expression of symptoms of boron toxicity was observed amongst lentil accessions (Figure 1). The reference accession (ILL5883) was found to have moderate tolerance (mean score of 3.8) which is in accordance with previous literature (7). Most of the current Australian cultivars (eg. Cassab, Digger, Nugget and Northfield) were intolerant to high concentrations of soil boron.

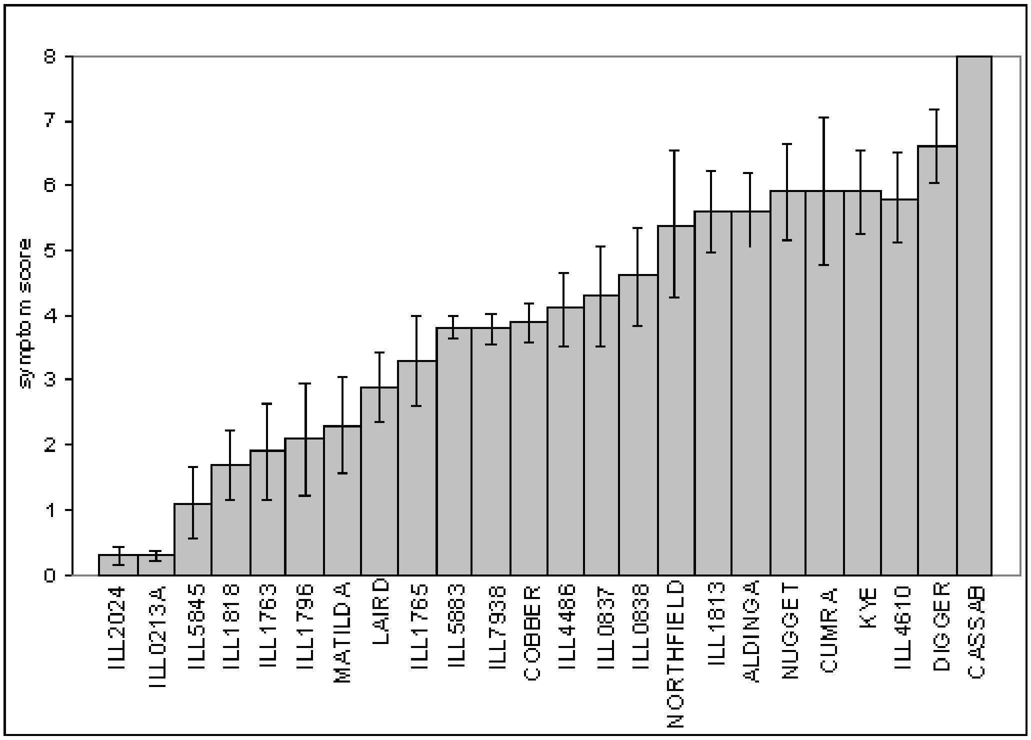

Figure 1. Variation in response of 19 lentil accessions to high concentrations of soil boron. This figure is a sub-sample of the 310 accessions screened.

Table 2. Response of accessions of lentil from regions and individual countries to high concentrations of soil boron.

Origin	Number of lines	Mean score	Range
Middle East	104	5.1	0.3 – 8.0
Afghanistan	16	4.0	0.3 - 6.8
Jordan	3	4.4	3.8 – 5.4
Iran	27	5.2	1.7 - 7.3
Syria	25	5.0	2.6 – 7.3
Turkey	9	6.0	4.0 – 7.3
Asia	66	5.6	3.0 – 7.6

Bangladesh	7	4.8	3.0 – 6.7
India	45	5.6	3.1 – 8.0
Nepal	2	6.1	5.8 – 6.4
Africa	35	5.6	0.3 – 7.6
Ethiopia	30	5.7	0.3 – 7.6
Europe	9	6.2	5.3 – 7.4
North America	8	5.7	2.9 – 6.9
South America	10	5.5	4.0 – 6.4
Unknown	78	5.0	1.1 – 8.0
ICARDA lines	35	4.8	1.1 – 8.0
VIDA Breeding lines	43	5.1	1.6 – 7.3

Matilda, a green lentil, was the most tolerant Australian lentil cultivar tested with a mean score of 2.3. Interestingly, another study (9) found Laird, also a green lentil, to be the most tolerant out of 25 lentil cultivars and breeding lines despite having 49% damage on leaves. In this experiment Laird had a mean score of 2.9 which is less leaf damage than indicated by the previous study. However the most tolerant lentils identified in this study (eg. ILL2024, ILL213A) were red lentils which had greater tolerance than ILL1765, previously reported as tolerant (7).

A small number (9%) of lentil accessions had a mean score below 3.0, while 70% had a score above 5.0 (Table 2). The most tolerant accessions originated from Ethiopia (ILL2024), Afghanistan (ILL213A, ILL1818, ILL1763, ILL1796) and some regions of the Middle East (ILL5845). Accessions from Afghanistan generally showed the greatest tolerance to high soil boron while accessions from Europe had the least tolerance. This is similar to the responses of other crops from different regions to high concentrations of soil boron. Earlier studies on bread wheat (10), barley (11) and peas (8), showed accessions with good boron tolerance also originated from Afghanistan. This suggests crops have evolved with greater boron tolerance when grown on soils with a high concentration of boron. A small number (3) of lentil accessions from the VIDA breeding program were found to be tolerant.

A recent study on boron deficiency in lentil (12) found accessions originating from West Asia were inefficient at extracting boron from the soil. This research also found that lentils originating from boron deficient soils in Nepal are efficient at taking up boron. Although only two lentils from Nepal were included in this study, both were found to be intolerant of high soil boron (Table 2). This follows the suggestion that germplasm relatively tolerant to boron deficiency are also relatively susceptible to boron toxicity and *vice versa* (13).

Previous studies (9) have shown that the ranking of field pea genotypes was the same in terms of a) visual assessment of boron toxicity; b) relative dry matter production; and c) concentrations of boron in shoots. The visual assessment of symptom expression was considered the most appropriate for a breeding program as selection is non-destructive and may be conducted during seedling growth. Tolerance to boron toxicity in lentils at seedling stage has been found to be indicative of tolerance at maturity (7).

Further work is needed to examine whether seedling tolerance persists through to maturity and to determine the genetic and physiological mechanisms underlying this tolerance to boron toxicity.

Conclusion

The identification of tolerant lentil accessions is highly significant as it provides new germplasm for rapid incorporation into the lentil breeding program. Boron tolerant lentil cultivars will greatly assist in improving pulse adaptation, profitability and reliability in southern Australia.

Acknowledgments

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