Genetics of Boron Tolerance in Durum Wheat

by

Sansanee Jamjod

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Abstract

Genetic studies of tolerance of durum wheat (*Triticum turgidum* L. var *durum*) to high concentrations of boron (B) were undertaken in order to identify genetic variation in response to B, the mode of gene action, number of genes and chromosomal locations of genes controlling tolerance to B.

Sources of B tolerance and the range of response of durum wheat to high concentrations of B were investigated by using filter paper soaked in solutions with high B concentrations and soil screening methods. Tolerant genotypes were identified among landrace lines, originating from Asian countries, but not in the agronomically adapted varieties. Significant differences between tolerant and sensitive genotypes were recorded for expression of symptoms of toxicity, dry matter production and shoot B concentrations when plants were grown in soils containing high concentrations of B. All parameters measured in high B soil showed high correlations with the root length of seedlings in high B filter paper. It was concluded that the root length of seedlings grown under high B conditions was an appropriate criterion for determining B tolerance in durum wheat.

Major genes were identified from the evaluation of F_2 and F_3 segregating populations derived from seven parents which represented four levels of tolerance. Single gene segregation resulted between:-

- tolerant x moderately tolerant (AUS 10105 (T) and AUS 10110 (T) x AUS 10344 (MT) and AUS 14010 (MT)) lines,
- moderately tolerant x moderately sensitive (AUS 10344 (MT) x Yallaroi (MS), AUS 14010 (MT) x AUS 10348 (MS) and Yallaroi (MS)) lines,
- moderately sensitive x sensitive (AUS 10348 (MS) x AUS 13244 (S)) lines.

Transgressive segregation resulted between:-

- moderately tolerant (AUS 10344) x moderately tolerant (AUS 14010) lines,
- moderately sensitive (AUS 10348) x moderately sensitive (Yallaroi) lines,
- moderately tolerant (AUS 10344) x moderately sensitive (AUS 10348) lines.

No segregation was observed in the cross between:-

- tolerant (AUS 10105) x tolerant (AUS 10110) lines.

Tolerance to B in durum wheat was controlled by three independent loci, which act additively, designated *Bot1*, *Bot2* and *Bot3* with the tolerance character being expressed as a partially dominant trait, depending on the concentration of applied B. Genotypes in respect to B tolerance were proposed as:-

- tolerant (T): *Bot1 Bot2 Bot3* (AUS 10110, AUS 10105),
- moderately tolerant (MT): *Bot1 bot2 Bot3* (AUS 10344) and *bot1 Bot2 Bot3* (AUS 14010),
- moderately sensitive (MS): *bot1 Bot2 bot3* (AUS 10348) and *bot1 bot2 Bot3* (Yallaroi), and
- sensitive (S): *bot1 bot2 bot3* (AUS 13244).

The chromosomal locations of genes controlling B tolerance were studied by aneuploid analysis using Langdon D-genome disomic substitution lines. It was found that *Bot1* was located on chromosome 7B of AUS 10344 (MT), *Bot2* on 7B of AUS 14010 (MT) and both genes on 7B of AUS 10110 (T). Linkage analysis using restriction fragment length polymorphisms indicated that *Bot2* was likely to be located on the distal region of the long arm of chromosome 7B. *Bot1* and *Bot2* segregated independently of each other.

The results from this thesis demonstrated the range and sources of genetic variation for response to B. Tolerance to high concentrations of B is under simple genetic control as observed in bread wheat. High levels of tolerance can be transferred into sensitive commercial varieties via backcrossing and selection can be performed during seedling growth at early generations. These findings are being adopt in a durum wheat breeding program at the Waite Institute in South Australia.

Declaration

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

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

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Chapter 1

Introduction

Boron (B) toxicity in Australia has been reported in a number of crop species and regions with early reports in citrus and vines grown in Mildura, north-western Victoria (Penman and McAlpin, 1949; Sauer, 1958). B toxicity in South Australia was first recognized in barley in 1983 (Cartwright et al., 1984), and subsequently in bread wheat (Paull, 1985; Cartwright et al., 1986), peas (Materne, 1989) and durum wheat (Brooks, 1991). In these crops, a yield reduction was associated with high concentrations of B in soils and plant shoots or grains. Soils usually high in concentrations of B include red brown earths, calcareous earths and heavy grey clays of the northern Mt Lofty Ranges and southern Flinder Ranges of upper Eyre Peninsula and upper Yorke Peninsula, parts of the Murray Mallee, and the Wimmera soils in South Australia and Victoria (Cartwright et al., 1986). B toxicity has also been reported to be widespread in the cereal belt of Western Australia (Khan et al., 1985) following the release of Stirling, the sensitive barley variety.

As soil amelioration appears not to be feasible, the use of tolerant varieties is the best option to overcome the problem of B toxicity (Rathjen et al., 1987). Genetics of tolerance to B was extensively investigated in bread wheat (Paull, 1990; Chantachume, 1995), barley (Jenkin, 1993) and peas (Bagheri, 1994). Inheritance of B tolerance in these species appears to be controlled by major genes and a breeding method such as backcrossing can be adopted to transfer tolerance to well adapted sensitive varieties. Successful breeding for B tolerance has occurred in bread wheat where B tolerant varieties were produced by backcrossing between the moderately tolerant varieties, Halberd and Dagger, as the donor parents and intolerant varieties as the recurrent parents. Three B tolerant varieties have been released followed the first identification of

B toxicity in South Australia in 1983. These varieties are BT-Schomburgk (1992), Barunga (1993) and Frame (1994) (Campbell et al., 1994).

Durum wheat is an important crop in the Mediterranean basin and West Asia, the plains of North Dakota (USA), Saskatchewan (Canada), USSR, and central India (Breth, 1975; Bozzini, 1988). About 55% of the world's durum is produced in West Asia and North Africa (Srivastana, 1984). The average annual production of durum wheat in the world is 5.1% of that of bread wheat (International Wheat Council, 1986).

In Australia, durum wheat is a minor crop, compared to bread wheat. The areas of production were previously mainly in the regions of northern New South Wales and southern Queensland, where the average current grain production per year is 80,000t (Hare, 1994b). Large scale production of durum wheat in South Australia was initiated in 1991 with the importation of the variety Yallaroi from New South Wales. Production of approximately 30,000t was reported in 1992 by the Durum Wheat Growers Association (Sharpe, 1993) and it is expected that production will increase to 100,000t by 1998 (Brooks, 1994).

Durum wheat has received less attention than bread wheat from wheat breeders and only six varieties have been released in Australia since 1956 (Table 1). Only the three most recently released varieties are currently grown in major areas of NSW and Queensland. The durum wheat varieties currently available lack adaptation to many regions of South Australia (Brooks et al., 1994) and yields have been poor relative to bread wheat and barley. Yallaroi is the only variety which is currently recommended in South Australia. Yallaroi is resistance to cereal cyst nematode (*Heterodera avenae* Woll.), stem rust (*Puccinia graminis* f. sp. *tritici*), leaf rust (*Puccinia recondita* f. sp. *tritici*) and stripe rust (*Puccinia striiformis*) but susceptible to black point (*Alternaria alternata*) and crown rot (*Fusarium graminearum* Group 1), compared to bread wheat varieties (Hare, 1989; Rathjen and Brooks, 1994).

The areas of southern Australia which have the greatest potential to grow durum wheat with high protein concentrations are mostly duplex and sodic soils (Rathjen, 1994) and durum lines have been found to produce only half the grain of bread wheat in some field trials in these areas. In these soils Yallaroi showed poor adaptation to imbalances of micronutrients, and in particular to B toxicity. For example, trial results from 1993 showed that the yield of Yallaroi was about 50-60% of Spear, a moderately B tolerant bread wheat, when grown at Two Wells, Rudall and Kimba (Brooks, 1994), areas where high concentrations of B is a major factor (Hollomby et al., 1994). Therefore, there is an urgent need for B tolerant varieties to be developed for cultivation in these areas. Currently, most of the durum in South Australia is produced on the red-brown earths and related soils in high rainfall areas, where B toxicity is a minor factor.

Table 2.2 Name, pedigree and year of released of Australian durum wheat varieties.

Name	Pedigree	Year of release	Reference
Dural	Aleppo/Palestine	1956	Matheson (1959)
Duramba	(Mex Dwarf durum (S23-1)/3*Dural)	1971	Matheson and Wark (1972)
Durati	(Zenati Bouteille/Wells//Mex Dwarf)	1977	Bird et al. (1977)
Kamilaroi	(Durati sib/Leeds)	1983	Hare (1983)
Yallaroi	(Guillemot seln. No.3/Kamilaroi sib)	1988	Hare (1989)
Wollaroi	(TAM1B-17/Kamilaroi sib/Rokel Sel./ Kamilaroi sib)	1993	Hare (1994a)

The main sources of breeding materials to date for southern Australia are:-

1) the Australian durum breeding program located at Tamworth, NSW,

2) the CIMMYT (Mexico) program where varieties are selected under irrigation,

3) the programs on the Great Plains in North Dakota USA and

4) introduced varieties, lines or accessions from Italy, Turkey and ICARDA, Syria.

Only the materials from Tamworth and some from CIMMYT were comparable or superior to bread wheat in some areas but generally these materials were poorly suited to most conditions in southern Australia (Rathjen and Brooks, 1994).

Since there was a lack of adaptation of commercial varieties and breeding materials for South Australian conditions, the broad objectives of the improvement program at the Waite Institute are to increase yield, and improve pest and disease resistance and grain quality. The stability of yield under specific conditions, such as performance under a deficiency or excess of mineral elements, is also under investigation in other research programs (Rathjen, 1994; Hollamby et al., 1994).

There are three broad phases undertaken when commencing breeding for a new trait:- First, assessment of the available range and identifying potential sources of the characteristic. Second, elucidation of information on the mode of inheritance of the character. Third, actual selection of the progeny of crosses designed to transfer the optimal expression of the desired characteristic into locally adapted varieties. The project reported here aims to provide information necessary for all three phases, in order to facilitate breeding durum wheat for tolerance to high concentrations of B.

A series of experiments was carried out in this thesis. First, genetic variation for B tolerance in durum wheat was investigated and the range of response to B in durum wheat was compared to bread wheat. Genotypes with contrasting levels of tolerance were selected to investigate in more detail their response to B (Chapter 3). The genetic control of tolerance was investigated in the F_1, F_2 and F_3 generations derived from seven genotypes ranging from sensitive to tolerant (Chapter 4) in order to identify the mode of inheritance and number of genes controlling tolerance. The identification of the chromosomal location of these genes was investigated using aneuploid analysis (Chapter 5). The specific location of B tolerance genes on chromosomes was studied by linkage analysis using molecular markers (Chapter 6). The results of individual chapters are discussed separately while the overall results and conclusions are presented in a general discussion (Chapter 7).

Chapter 2

Review of literature

2.1 B toxicity in soil and plants

2.1.1 Role of B in plants

Boron (B) is an essential trace element for plant growth. With respect to its role in plant nutrition, B is still the least understood of all the mineral nutrients. Most information available has arisen from studies of the physiological effects when B was withheld or resupplied after deficiency (Marschner, 1986). B is not known to be a constituent of any enzyme, nor is it able to control enzymatic processes by changing its valency like several cations (Bergmann, 1983).

Although the biochemical role of B is not well understood, it has been shown to be associated with a number of functions. This review does not cover in detail of the possible roles of B in plants, rather, only to indicate the relevant literature which has reviewed the role of B, including,

1) cell elongation, cell division and nucleic acid metabolism (e.g. Chapman and Jackson, 1974; Neales, 1960; Whittington, 1959)

2) carbohydrate and protein metabolism (e.g. Dugger and Palmer, 1980)

3) tissue differentiation, auxin and phenol metabolism (e.g. Jarvis et al., 1983; Lewis, 1980)

4) membrane permeability (e.g. Dugger, 1983; Tanada, 1978; Pollard et al., 1977)

5) cell wall biosynthesis (e.g. Brown and Hu, 1993, Dugger, 1983; Tanaka, 1967)

6) pollen germination and pollen tube growth (e.g. Cheng and McComb, 1992; Vaughan, 1977)

2.1.2 B in soil

B is universally distributed in soils (Gupta et al., 1985). The total B content of normal soils is in the range of 2 to 100 ppm (Swaine, 1955). According to Berger and Truog (1945), B in soils may be divided into three categories, namely, total B, acid soluble B and water soluble B. B which is available to plants is distributed in the soil between the B in the soil solution and that adsorbed to soil particles (Gupta et al., 1985; Keren and Bingham, 1985). Soils provide an adequate supply of B if they contain between 0.5 and 5 ppm of water soluble B (Gupta, 1979).

The total B in soils originates from the soil minerals, and varies according to the B content of parental rock materials. Sedimentary rocks of marine origin have a higher amount of total B than igneous rocks (Norrish, 1975). The lowest values are found in either soils derived from acid igneous rock or from fresh water sedimentary deposits of coarse-texture and in soils low in organic acid. Higher values are found in soils derived from marine shales, loess, and alluvium with all being essentially fine-textured deposits (Fleming, 1980). According to Kebata-Pendias and Pendias (1984), the lowest amounts of B were found in sandy and loamy soils of Poland and New Zealand, whereas the highest concentrations were found in lateritic soils of India, solonchaks of the U.S.S.R. and calcareous soils of Israel.

Boron is unique among the essential nutrients because it is the only element that is present in soil solutions as a non-ionized molecule ($B(OH)_3$) over a pH range suitable for plant growth (Oertli and Grgurevic, 1975). Because of its non-ionic nature, once B is released from soil minerals it can be readily leached (Gupta, 1979) and deficiency commonly occurs in plants. Conversely, where there is limited leaching from the profile, B accumulates in the lower horizontal.

2.1.3 Cause of B toxicity

In general, both total and water-soluble B can be high in arid and semi-arid areas in which B has accumulated naturally and leaching is limited. In these areas, B in the subsoil often exceeds that in surface soils (Gupta et al., 1985). B in these soils exists as sodium and calcium salts and is usually found at toxic levels in saline and sodic soils (Hutchinson and Viets, 1969).

High concentrations of B may occur in ground water and the use of irrigation water that contains high B results in B toxicity symptoms in crops (Gupta et al., 1985). The critical B concentration in irrigation water was between 0.3 and 1.0 mg L^{-1} for sensitive crops (Wilcox, 1960) and between 3 and 6 mg L^{-1} for wheat and peas (Chauhan and Powar, 1978).

B toxicity may also occur from over-fertilization with B fertilizers when correcting B deficiency. Studies on B fertilization have shown that B applications can be toxic to some plants at concentrations only slightly above the optimum for others (Gupta et al., 1985). An additional source of high concentration of B is from industrial pollution. For example, the contamination of land by fly ash can lead to a B toxicity problem (Adriano et al., 1980).

B toxicity has been reported for a number of crops and regions of the world and examples are listed in Table 2.1.

2.1.4 B toxicity in South Australian soils

The agricultural zone of South Australia with an annual rainfall greater than 250 mm, accounts for 17% of the area of the State. The soils over most of the region are generally alkaline, have poor fertility and problems of sodicity, but are rarely saline (Reuter et al., 1988). Alkaline-sodic soil are characterized by a high pH (>8) and the sodium adsorption ratio (SAR) of the clay is greater than 15 (Marschner, 1986). Sodicity is also defined as an exchangeable sodium percentage (ESP) greater than 6

Table 2.1 Examples of countries and crops in which B toxicity has been reported.

Country	Region	Crop	Reference
U.S.A.	California	Fruit trees	Eaton (1935)
	Minnesota	Barley	Christensen (1934)
India	Agra	Wheat	Chauhan and Power (1978)
	Punjab	Lentil, Barley, Oats	Chauhan and Asthana (1981)
Iraq	-	-	Khudairi (1961)
Pakistan	Lahore	Wheat	Shiek and Khanum (1976)
Peru		Cotton, Alfalfa	Fox (1968)
Philippines	IRRI	Rice	Ponnamperuma et al. (1979)
Syria	ICARDA	Wheat, Barley	ICARDA Annual Report (1993)
		Durum wheat	Yau et al. (1995)
Tunisia		Wheat, Barley	ICARDA Annual Report (1993)
Turkey		Wheat, Barley	ICARDA Annual Report (1993)
Australia	South Australia	Wheat, Barley	Cartwright et al. (1984; 1986)
		Durum wheat	Brooks (1991)
	Victoria	Citrus	Penman and McAlpin (1949)
		Grapes	Sauer (1958)
		Wheat, Barley	Ralph (1992)
	Western Australia	Barley	Riley (1987)

(Northcote and Skene, 1972). In these soils, poor physical conditions and correspondingly poor soil aeration are the major constraints for crop production and are often correlated with Na and B toxicity (Marschner, 1986).

Symptoms of B toxicity in cereals grown in South Australia was first recognized in 1983 (Cartwright et al., 1984). Further studies of bread wheat and barley crops confirmed that B toxicity symptoms in these crops were caused by high concentrations of B in soils (Cartwright et. al., 1986; Rathjen et al., 1987; Paull, 1990; Holloway and Alston, 1992). Many soil types in the cereal belts contain concentrations of extractable B more than 20 mg kg^{-1}, and up to 100 mg kg^{-1} in the subsoils . These soils include red-brown earths, calcareous earths and heavy grey clays in the upper Eyre Peninsula, upper Yorke Peninsula and parts of the Murray Mallee (Cartwright et al., 1986). Holloway and Alston (1992) examined the response of wheat to high concentrations of B and Na in alkaline sodic soils of South Australia and concluded that B toxicity had a greater effect on plant growth and yield than Na toxicity.

2.1.5 Requirement, uptake and translocation of B in plants

The concentration of B required for plant growth varies between and within plant species. Generally, monocotyledonous plants have a lower requirement for B than dicotyledons (Fleming, 1980). Gupta (1979) identified interspecific variation in the sufficiency range of B in plant tissues, over a number of crops. For example, the range is about 5-10 mgB kg^{-1} in wheat, 20-30 mgB kg^{-1} in tobacco and more than 100 mgB kg^{-1} in sugar beet. For most plants, the range between deficient and toxic levels of B is narrow and therefore the ratio of toxic to adequate concentrations is narrower than for any other element (Reisenauer et al., 1973).

B uptake by plants is correlated with the concentration of boric acid ($B(OH)_3$) in soil solution (Keren and Bingham, 1985) and adsorption of B by plants has been reported to be a non-metabolic process (Nable, 1988; Brown and Hu, 1993). From a results of a study with a high concentration of B was supplied to cotton, Oertli and Richardson

(1970) suggested that B is passively absorbed and translocated with the transpiration stream.

B is mainly translocated in the xylem but is quite immobile when deposited in the extremities of plants (Nable et al., 1990a; Oertli and Richardson, 1970). A gradient in B concentration occurs within a plant, and within individual leaves, and is related to accumulation with age and accumulation at the end of the transpiration stream. For example, more B was found to accumulate in leaves than in pods and seeds of rape (Marschner, 1986). Variation of B distribution within a leaf was also observed (Oertli and Kohl, 1961) and when an excessive level of B was supplied, most B accumulated at leaf tips, or along the leaf margin. The symptoms of B toxicity coincided with the highest concentrations of B.

2.1.6 Response of plants to B toxicity

Symptoms of B toxicity

Because B is translocated in the xylem, B deficiency symptoms appear at the growing point of the plants, the stem tips, root tips, new leaves and flower buds (Gupta et al., 1985). In contrast, toxicity symptoms typically show first on older leaves. Generally, the symptoms of B toxicity for most plants are similar, consisting primarily of chlorosis and subsequently necrosis of the tip and margins of leaves. Symptoms initially develop, and are most severe on the oldest leaves (Eaton, 1944; Oertli and Kohl, 1961; Gupta, 1979).

B toxicity symptoms of barley have been described by a number of authors (Christensen, 1934; Gupta, 1971; Cartwright et al., 1984; Jenkin, 1993) and contrast in apparent with wheat. The symptom in barley is characterized by development of chlorosis followed by necrosis and the formation of brown necrotic spots or lesions within the affected tissue at the tips, margins and toward the leaf base. Cartwright et al. (1986) observed variation in size, form and density of the brown lesions in different barley genotypes. In wheat and oats, symptoms of B toxicity are similar to barley but

the necrotic spots do not develop (Gupta, 1971; Paull, 1990). Gupta (1971) reported a light browning developing from the leaf tips in wheat while light-yellow bleached leaf tips were observed in oats. Lucerne, clover and chickpea showed scorching on the margin of leaflets, whereas in lupin and faba beans the tips of leaflets become blackened (Reuter et al., 1988).

Root growth

The biochemical role of B is still unclear, therefore, the role of B in root growth has been deduced indirectly from physiological experiments. Most studies have been conducted with B-deficient plants.

Root growth of a number of species has been shown to be restricted by B deficiency. Root elongation of intact squash decreased to zero after a period of 24 h of B deficiency and the cessation of total root elongation was caused by a failure of cell division in meristematic cells. A continuous supply of B is not essential for cell elongation but is required for the maintenance of meristematic activity (Cohen and Lepper, 1977).

Root growth of alfalfa and oats was markedly depressed at concentrations of B in soil more than 10 ppm (Morvedt and Osborn, 1965). In a study of the root growth of maize, field bean, broad bean, flax and pea seeds, radicle growth was dependent upon an external supply of B. At the B free treatment, radicle growth rate was reduced and at the higher B treatment of 50 ppm, restriction of the growth of the radicle was also observed (Neales, 1960). In a study of mungbean cuttings, Jarvis et al. (1983) showed that the optimum B concentration for seedling growth were between 0.1-5 $\mu g\ ml^{-1}$ boric acid, but high concentrations of B had inhibitory effects on subsequent root growth.

In cereals and peas, recent research has shown that B toxicity often reduces root growth (Paull, 1985; Nable, 1988; Holloway and Alston, 1992; Chantachume, 1995) but it is not know whether this is through an effect on cell division, cell elongation, or both.

Shoot growth and yield

Increasing levels of B may result in reduction of growth and development of plants. For example, in a glasshouse experiment, high concentrations of B resulted in a reduction in plant vigour, plant height, number of tillers per plant, a delay in plant development and reduced dry matter yield and grain yield of wheat (Paull et al., 1990). In barley, shoot growth was reduced and leaf senescence was increased (Riley, 1987). Reduction in grain yield due to the effects of B toxicity on field crops have been reported as 17% in barley (Cartwright et al., 1986), 20-40% in barley and wheat (ICARDA Annual Report, 1993) and 10-20% in rice (Cayton, 1985).

A number of authors have reported the critical levels of B that affected plant growth or yield. The critical concentration of B in shoots or leaves varied widely depending on sampling age, plant parts and environmental conditions (Reuter, 1986). For example, toxicity symptoms in field grown barley appear when the B concentration in whole shoots exceeds 30 mgB kg^{-1}, and yield is affected at concentrations above 70 mgB kg^{-1} (Cartwright et al., 1986). Kluge and Podlesak (1985) found that the grain yield of barley was reduced when the B concentration in shoots sampled at Feekes stage 7-8 reached 120-130 mgB kg^{-1} and symptoms of B toxicity were expressed at 60-80 mgB kg^{-1}. On the other hand, Gupta and McLeod (1981) found that there was no yield reduction of barley over the range of 50-200 mgB kg^{-1} in boot stage tissue. It was suggested that the use of leaf or shoot analysis to diagnose B toxicity in wheat and barley were less reliable than B concentration in grain due to the fact that B can be leached from leaves and the effect that accumulation of very high concentrations of B in the leaf tips has on the mean B concentration of the whole plants (Nable et al., 1990b). Oertli and Kohl (1961) also identified that B was not evenly distributed within the plant leaf. For example, the B concentration in the margins is usually four or five times than in the leaf blade alone. The uneven distribution of B may result in highly variable results if the relative leaf weight and ratio between the margin and leaf blade does not remain constant.

2.2 Plant tolerance of excess B and genetics of tolerance

2.2.1 Mechanism of tolerance

There are three mechanisms by which plants might tolerate high concentrations of mineral nutritions, namely, avoidance, exclusion and internal tolerance (Rathjen et al., 1987; Taylor, 1987). Avoidance of a toxic concentration of a mineral might arise from differences in the morphology of the root systems of plants, while mechanism of excluding excess mineral nutrients from the symplasm include the excretion of protective organic compounds or alteration of the chemical properties of the rhizosphere, thus affecting the availability of the nutrient (Marschner, 1986). Mechanisms of internal tolerance proposed include deposition in cell compartments, formation of metabolically inactive complexes and altered enzyme systems (Rathjen et al., 1987).

Although B affects both roots and shoots of plants, more B usually accumulates in the shoots than roots (Eaton, 1944). Wheeler and Power (1995) measured the effects of varying solution concentrations of Mn, Zn, Cu, B, Fe, gallium (Ga) and lanthanum (La) on concentrations, uptake and toxicity in wheat. Only B concentrations were higher in the shoots than roots. Nable (1988) tested barley and wheat genotypes in solution culture containing B concentrations ranging from 15 to 5000 μM and found that internal shoot B concentrations were always greater than those in roots and the difference between shoot and root accumulation of B increased with the level of B supplied.

Experiments examining genotypic variation in response to high concentrations of B have been conducted in soils and solution culture for barley, wheat, annual medics and peas (Nable, 1988; Paull et al., 1988b; 1992, Nable et al., 1990a; Bagheri et al., 1992; Jenkin, 1993). In all species examined, genotypic differences in tolerance to high levels of B were found in terms of symptom expression and plant growth. High concentrations of B in the growth medium resulted in an increase in tissue B

concentrations, more severe symptoms of B toxicity and depressed plant growth. Tolerant genotypes accumulated considerably less B than did sensitive genotypes, in both roots and shoots. Nable (1988) showed that there was no variation between tolerant and sensitive barley genotypes in terms of the relative distribution of B between leaves and roots and none of the genotypes expressed internal tolerance. It was concluded that tolerance to high concentrations of B was attributable to differences in the rate of B accumulation in leaves which was controlled by an exclusion mechanism that affected uptake by the root system (Nable et al., 1990a). The tolerant genotypes showed an ability to restrict B accumulation. Huang and Graham (1990) reported that the difference in tolerance to B between wheat genotypes reported by Nable (1988) was also expressed at the cellular level. They proposed that the different levels of tolerance to high concentrations of B might be associated with differences in permeability of the cell membrane to B.

2.2.2 Genetic variation in tolerance to B

When breeding crops for adaptation to an environmental stress, it is important to identify the variation for the range of response. The screening method should be simple, rapid, reliable and able to reflect the crop situation in order to provide a tool for breeders to select plants of the desire genotypes.

Screening method

There are several methods for screening cereals for response to B. At the Waite Institute, all methods used are based on the correlation between the response under controlled conditions and B accumulation in the grain under field conditions (Cartwright et al., 1984; Paull, 1990). Two systems have been widely adopted, namely growing plants in a high B soil or solution culture in a glasshouse and growing seedlings in filter papers soaked in a high B solution. In the glasshouse screening, seedlings are sown in a large box containing soil with B added up to a concentration of 100-150 mg kg^{-1}. Plants are rated for tolerance to B by comparing their growth to that of the standard

genotypes at 4 to 6 weeks after sowing with respect to vigor, symptom expression and the development of tillers (Moody et al., 1988). Jenkin (1993) tested barley in a hydrophonic system with solutions containing high concentrations of B and used the same criteria as Moody et al. (1988) to rate plants for B tolerance. Chantachume et al. (1995) developed a filter paper method to assess B tolerance in bread wheat. Seedlings were sown in a sheet of filter paper soaked with B solution and root growth was measured after 12 days. Tolerant genotypes produced significantly longer roots than sensitive genotypes and the rankings were consistent with those obtained by Moody et al. (1988) in high B soil.

Extent of genetic variation and source of tolerance

Genetic variation in response to high concentrations of B has been reported for a number of crops. These included barley (Cartwright et al., 1984), bread wheat (Paull et al., 1988b), rice (Ponnamperuma, et al., 1979), peas (Materne, 1989; Bagheri et al., 1992) and annual medics (Paull et al., 1992b).

Genetic variation for tolerance to B was identified among Australian varieties of all crops and the adoption of tolerant varieties by farmers has been notable in regions where high concentration of B predominate (Paull et al., 1992a). For example, the most tolerant Australian varieties of bread wheat (Federation, Insignia, Heron, Halberd, Spear and Dagger), peas (Early Dun, Dundale and Alma) and medics (Cyprus) were found to be widely grown in the areas of south-eastern Australia (Rathjen and Pederson, 1986; Paull et al., 1992b) where high concentrations of B in soils were reported (Cartwright and Hirsch, 1986).

Further sources of tolerance to B have been identified from overseas germplasm and some of these genotypes expressed a higher level of tolerance to B than the most tolerant Australian varieties. Moody et al. (1988) found that 7% of the 1500 wheat lines examined were more tolerant than Halberd, the most tolerant Australian variety, and germplasm originating from Afghanistan, India and Japan were predominantly

tolerant while those from USA, Canada, Egypt and Europe were mostly sensitive. Bagheri et al. (1994) screened 617 pea accessions and identified 4% of accessions originating from Afghanistan, India, China and South America as being more tolerant to B than Alma, the most tolerant Australian variety.

2.2.3 Genetic control of B tolerance

An understanding of the genetic control of a response to a factor, such as an imbalance of a mineral nutrient, is of importance in planning plant breeding programs to produce crops for use in areas where such factor limits crop production. An understanding of the genetics can be applied in a number of aspects, for example, identifying sources of tolerance and the evolution processes leading to changes in adaptation, understanding the physiological and biochemical basis of the mechanism of tolerance and the application to the molecular genetics of tolerance, as well as devising efficient population strategies and screening techniques (Macnair, 1993).

B deficiency

Genetic control of variation in response to B deficiency has been studied for a number of species. In tomato and celery, susceptibility to B deficiency was reported to be controlled by a single recessive gene (Pope and Munger, 1953; Wall and Andrus, 1962). However, Kelly and Gabelman (1960) found that the genetic control of tolerance to low B in table beets was complex. Genetic analysis by the diallel method suggested that efficiency in B uptake in sunflower was controlled by additive or additive epistasis gene action (Blamey et al., 1984). In wheat, tolerance to B deficiency was expressed as a quantitative character and largely controlled by additive gene action (Jamjod et al., 1993).

B toxicity

The genetic control of tolerance to B has been extensively studied for bread wheat (Paull et al, 1992a; Chantachume, 1995), barley (Jenkin, 1993) and peas (Bagheri,

1994). For all species, the measurement was based on the response of parents, F_1, F_2 and F_3 populations derived from intercrosses between parents having contrasting levels of tolerance to B. Generally, response to B was found to be controlled by several major genes. The response of F_1 hybrids was intermediate to the parents and B tolerance was controlled by either additive or partially dominant gene action, depending on the B treatments. When B treatments were higher than the critical concentration of the F_1 plants, the response of F_1 was closer to that of the more sensitive parents (Paull et al., 1988a).

B tolerance in bread wheat is controlled by at least three major genes, namely, *Bo1*, *Bo2* and *Bo3* (Paull et al., 1988a). These genes were additive, non-maternal and partially dominant, based on dry matter production and tissue B concentration in F_1, F_2 and F_3 populations derived from five genotypes that differed for level of tolerance. Transgressive segregation was observed in the progeny between the moderately tolerant Halberd and tolerant G61450, suggesting that another gene, designated *Bo4*, was involved in the control of tolerance to B (Paull, 1990). Transgressive segregation was also observed for several crosses between exotic B tolerant genotypes (D. B. Moody pers comm.; Chantachume, 1995) and it is probable that there is also a *Bo5* locus.

A number of chromosomes of wheat have been reported to control tolerance to B. The Chinese Spring/Kenya Farmer substitution lines were examined and chromosome 4A was found to have a major effect in controlling the response to excess B (Paull et al. 1988a). Further information was sought by using the method of monosomic analysis (Sears, 1954) and with interspecific addition lines. Reciprocal monosomic analysis between Chinese Spring and Federation showed that chromosome 7B was the most probable location of the gene for B tolerance of Federation. Monosomic analysis between Chinese Spring and G61450 revealed that chromosome 7D was likely to be the critical chromosome conferring tolerance of G61450 (Paull, 1990). These results were further investigated by Chantachume et al. (1993) using the reciprocal backcross monosomic method (Snape and Law, 1980). The *Bo1* locus was located to

chromosome 7B of Halberd and chromosome 4A of G61450 carried *Bo4* (Chantachume et al., 1993). Paull et al. (1993) identified linkage between the *Bo4* locus of G61450 and the RFLP locus *XksuG10* located on 4AL. Reciprocal monosomic analysis of crosses between Condor and exotic tolerant genotypes, India 126, Bonza and Benventuto Inca also indicated that chromosome 4A had a significant effect in controlling B tolerance in these lines (Chantachume, 1995).

Paull et al. (1991a) tested two amphiploids produced from diploid relatives of wheat (*Agropyron elongatum* genome EE and *Agropyron junceum* genome E^bE^b) and the wheat variety Chinese Spring. These amphiploids were more tolerant to B than Chinese Spring and it was concluded that the tolerance of the related species was expressed in a wheat background. The response of the disomic addition lines of *Ag. elongatum* into Chinese Spring indicated that chromosome 7E was critical in the control of tolerance to B (Paull, 1990).

Manyowa and Miller (1991) reviewed the genetics of tolerance to high mineral concentrations in the tribe *Triticeae*. They reported potent sources of B tolerance among members of this tribe including, Imperial rye (*S. cereale*), *Ag. elongatum* and *Ae. sharonensis*. In Imperial rye and *Ae. sharonensis*, addition lines for chromosome arms 2R, 3R, 5R, 3S, 5S and 7S expressed a higher level of tolerance than Chinese Spring.

In barley, the number of genes controlling B tolerance was studied in intercrosses between Sahara 3771 (highly tolerant), CM 72 (moderately tolerant) and Stirling (intolerant) and evaluated the F_2 and F_3 progenies in solution culture containing a high level of B. Tolerance of barley to B was found to be controlled by at least three major genes in Sahara 3771 and two in CM 72 (Jenkin, 1993). In peas, two major genes controlling tolerance were identified from the evaluation of F_1, F_2 and F_3 progenies of five crosses in soil containing high concentrations of B (Bagheri, 1994). A study in a recombinant inbred population using RFLP linkage analysis revealed that one of these genes was located on chromosome group 1 (Bagheri, 1994).

2.3 Genetics of other mineral toxicities

Stress caused by an excess of other minerals, such as aluminium (Al) and manganese (Mn) in acid soils, and sodium (Na) in saline soils often reduces plants growth (Foy, 1974; Foy et al., 1988; Epstien, 1985). Examples of genetic studies of the response of plants to these nutritional imbalances are reviewed below.

2.3.1 Al toxicity

Al toxicity is the most important limitation to crop production in acid soils. Al toxicity usually inhibits root cell division and elongation, thus reducing water and nutrient uptake (Foy et al., 1967). Genetic variation for Al tolerance has been reported in many plant species (Wheeler et al., 1993) and much of the research has focused on wheat (Scott and Fisher, 1992). In a comparison between cultivars of cereals (bread wheat, barley and oats), cultivars of bread wheat and oats (*Aveana* species) with Brazilian origin were the most tolerant (Wheeler et al., 1993).

The genetic control of tolerance to Al has been reported by a number of authors with both major and minor genes described. For example, Al tolerance in wheat was found to be controlled by a major gene (Kerridge and Konstrad, 1968). However, Berzonsky (1992) suggested that several major genes and minor genes were involved in Al tolerance in some wheat crosses. Tolerance of Al in barley was found to be controlled by a single gene (Reid, 1970) or a single locus with multiple alleles (Minella and Sorrells, 1992). Rhue et al. (1978) found that Al tolerance in corn was controlled by a single locus with multiple alleles. The differences in genetic control listed above might be related to either the degree of variation between parents, or the method of genetic analysis undertaken.

A number of studies have been conducted to identify the chromosomal locations of the genes controlling tolerance to Al in wheat and numerous chromosomes have been implicated. Genes controlling Al tolerance in hexaploid wheat have been reported in the

A and D genomes (Slootmaker, 1974; Aniol and Gustafson, 1984). In a comparison between nullisomic-tetrasomic and ditelosomic lines of Chinese Spring and euploid Chinese Spring, genes for Al tolerance have been found to be located on chromosome arms 2DL, 3DL, 4DL, 4BL, 6AL, and 7AS, and chromosome 7D (Aniol and Gustafson, 1984). Analysis of addition lines of rye to a Chinese Spring wheat background indicated that there were major genes located on rye chromosomes 3R, 6RS and 4R (Aniol and Gusafson, 1984). Prestes et al. (1975) found an Al factor associated with chromosome 5D and Polle et al. (1978) located an Al tolerance gene on chromosome 4D of Chinese Spring. Takagi et al. (1983) found three major genes located on 2DL, 4DL and 5AS. In a very tolerant variety, BH1146, genes conferring tolerance were found on chromosomes 2A, 5A, 6A, 2D and 4D (Aniol, 1991). Manyowa and Miller (1991) reviewed Al stress in *Triticeae* and concluded that it was controlled by factors predominantly on chromosome 5R of *S. cereale*, $5E^bL/6E^bL$ of *Th. bessarabicum* and 5D of *T. aestivum* cv Atlas 66. In barley, Stolen and Anderson (1978) reported that an Al tolerance gene was located on chromosome 4.

2.3.2 Mn toxicity

Mn is regarded as one of the factors affecting plant growth in acid soils. Genotypic differences in Mn tolerance are related to the Mn tolerance of the shoot tissues (Marschner, 1986) and genetic variation has been reported for a number of species (Foy et al., 1988).

Much less work has been undertaken on the genetic control of tolerance to manganese toxicity in cereals, compared to Al, and the method of control is less clear. However, Foy et al. (1988) discussed a wide range of response of Mn toxicity in wheat and proposed that many genes with small effects were likely to be involved in Mn tolerance. This was supported by Moroni et al. (1991) who also claimed that the inheritance of tolerance to Mn in wheat was controlled by polygenes. Tolerance to excess Mn was reported to be controlled by factors located on chromosomes $5E^b$ and $5E^bL/6E^bL$ of *Th. bessarabicum* (Manyowa and Miller, 1991).

In alfalfa, Mn tolerance was attributed to additive gene action with little or no dominance (Dessureaux, 1959). In lettuce, from a study involving five genotypes, Mn tolerance was controlled by one to four genes (Eenink and Garrestsen, 1977). Brown and Devine (1980) found differences between reciprocal crosses of soybean lines with contrasting levels of Mn tolerance and suggested that cytoplasmic inheritance may play a role in this crop. Heenan et al. (1981) reported that tolerance in soybean cultivars was at least partially dominant. In maize, loci influencing Mn accumulation appeared to be located on chromosome 9 (Naismith et al., 1974).

2.3.3 Salinity

Stress caused by soil salinity is often found in semiarid and arid conditions, and inherently affects plants in a number of ways (Epstein, 1985). Stress may be caused by water deficit resulting from osmotic imbalance or high concentrations of toxic ions such as Na^+ and Cl^- (Marschner, 1986). Large differences in salinity or salt tolerance exist among cultivars within a crop species (Vose, 1983) and the responses, mechanisms and adaptation to salt tolerance have been reviewed by Epstien (1985), Greenway and Munns (1980) and Munns and Termaat (1986). In rice, Yeo and Flowers (1986) suggested that salt tolerance depends on a number of components and improvement could be achieved by selecting separately each component and pyramiding them through breeding.

Salt tolerance in wheat was governed by the ability to reduce Na^+ and increase K^+ accumulation in shoots (Gorham et al., 1990). Bread wheat generally shows higher salt tolerance than durum wheat (Shah et al., 1987). Comparisons between bread wheat (genome AABBDD), *Aegilops squarrosa* (the D-genome ancestor) and durum wheat (genome AABB) under salt stress conditions were reported by Shah et al. (1987) and Gorham et al. (1987). They found that bread wheat had lower Na^+ accumulation than durum wheat and concluded that the difference resulted from the difference in genomes between bread wheat and durum wheat. Gorham et al. (1987, 1990) demonstrated that the superiority was due to a gene controlling salt tolerance located on

chromosome 4D of hexaploid wheat. Dvorak et al. (1994) showed that when the *Kna1* gene, located on 4DL of bread wheat was transferred into a durum wheat background, the *Kna1* recombinant families expressed a higher level of salt tolerance than the *Kna1*- families and parental durum cultivars.

2.4 Durum wheat

2.4.1 Classification

All wheats belong to the genus *Triticum*, a member of the Gramineae family and Triticeae tribe. Most of the grasses and cereal grains within this tribe have a basic chromosome number of seven, and the lowest chromosome numbers are found in the diploids, with 14 chromosomes (seven pairs). The primary cultivated wheats, bread wheat (*Triticum aestivum* L.) and durum wheat (*Triticum turgidum* L.) are allopolyploids. Bread wheat is an allohexaploid containing three distinct genomes referred to as A, B and D (2n = 6x = 42). Durum wheat is an allotetraploid containing A and B genomes (2n = 4x = 28). The progenitors of the A and D genomes are believed to be *T. monococcum* L. and *T. tauschii* (Coss) Schmal., respectively (Chapman et al., 1976; Dvorak, 1988). The progenitor of the B genome is uncertain, but was proposed to be derived from *T. speltoides* (Tausch.) Gren. or close relatives (Kerby and Kuspira, 1987).

At the tetraploid level, two main species have been recognized, *T. timopheevi* (2n = 4x = 28, genome AAGG) and *T. turgidum* (genome AABB). *T. timopheevi* consists of two varieties, *araraticum* and *timopheevi*. Within *T. turgidum* L., six varietal groups are recognized, these including, *turgidum*, *carthlicum*, *dicoccoides*, *dicoccon*, *durum* and *polonicum*. *T. turgidum* var *durum* is regarded as the most widely cultivated tetraploid wheat (Kerby and Kuspira, 1987).

2.4.2 Association of the genetic control of some traits between durum wheat and bread wheat

Homoeoalleles

The genomes of polyploid bread wheat (A, B and D) and durum wheat (A and B) are related phylogenetically (Kerby and Kuspira, 1987; Talbert et al., 1992). Each member of one genome has a homoeologous pattern in the other genome. Homoeology among the A, B and D genomes of wheats and those of related species has long been recognized (reviewed by Gale et al., 1989). The ability of a chromosome from one genome to compensate for the loss of one from another genome was first demonstrated by Sears (1966) through the development of nullisomic-tetrasomic and other aneuploid stocks. These aneuploid lines have been used to locate genes to individual chromosomes, or chromosome arms, and more than 300 genes have been located on hexaploid wheat chromosomes, including genes for morphological and physiological traits, pest resistance, enzymes, storage proteins and DNA marker loci. Most of these occur as homoeoalleles in triplicate sets, except pest resistance loci and some mutant characters (McIntosh, 1988; Hart, 1994). It was proposed that any gene found on a wheat chromosome can be expected to be represented by homoeologous loci on homoeologous chromosomes in the two other wheat genomes and in alien genomes (Gale et al., 1989).

Differences cause by other factors

Bread wheat is significantly different from durum wheat in many phenotypic characters. The differences may be due to the addition of nuclear genes from *T. tauschii* (D genome donor), physiological changes caused by increases in ploidy level, the interaction between genes on the A, B and D genomes, or the interaction between ploidy level and the environment. Also, as bread and durum wheat have undergone separate evolutionary pathways, they have accumulated different sets of mutations.

Bread wheat has been shown to have an advantage over durum wheat in adaptability to adverse soil conditions, such as salinity or acidity. Bread wheat has a high degree of variation in tolerance to stress caused by an excess of mineral nutrients, such as Na (Shah et al., 1987) and Al (Foy and da Silva, 1991), and thus there is a greater potential to develop lines with tolerance to stress than that found in durum wheat where there was a limited range in genetic variation.

Although D-genome chromosomes contain genes conferring a number of desired agronomic characters, such as resistance to pests and pathogens (Kaloshian et al., 1991; Cox, 1994), transferring these genes from bread wheat or other alien species, into durum wheat, is not always readily achieved. Kerber (1983) found that a gene conferring resistance to *Puccinia recondita* present in *T. tauschii*, was suppressed when transferred from the D-genome to a tetraploid wheat background. Similarly, when the Al tolerant rye was crossed with CS bread wheat, the expression of Al tolerance was suppressed in the wheat background (Gustafson and Ross, 1989).

For a number of characters, phenotypic variants in one species might not necessarily be homologous to the alleles responsible for major control of similar variants in another (Moore et al., 1993). Therefore, some alleles of interest may be recognized only in specific species or varieties within species. McIntosh (1988) listed the genes controlling many characters of wheat and its relatives. Some characters were controlled by genes located on the same chromosomes in bread wheat and durum wheat and these are summarized in Table 2.2. The similarity between the species may be due to homoeoalleles or specific alleles but in many cases further confirmation is required as there have been limited experiments with durum wheat.

Table 2.2 Examples of characters controlled by the same chromosome in both bread wheat and durum wheat, summarized from McIntosh (1988).

Character/locus	Location[a]	Example of varieties or lines	
		Bread wheat	Durum wheat
Morphological			
Waxiness (*$W_1$1*)	2BS	Cornell Sel 5075	Long kernel
Purple pericarp	3A, 7B	-	-
Black glume (*Bg*)	1A	-	-
Red glume (*Rg1*)	1B	Federation 41, Red Egyptian	Ward
Hairy glume (*Hg*)	1AS	Prelude, Indian, Jones Fife	Golden Ball
Reduced height (*Rht1*)	4AS	WW15, Siete Cerros, Wren	Cocorit 71, Creso, Mida
Hybrid necrosis (*Ne1s*)	-	Gaza, Felix, Big Club	Ponca, Quanah, Iumillo
Hybrid necrosis (*Ne2*)	-	Harvest Queen	Stewart, Acme, Mindum
Lack of ligules (*lg1*)	2B	Eligulate W1342 *lg2*	Liguless durum selection
Chlorophyll-deficit mutant[b] (*cn-A1*)	7A	Chlorina-1 mutant	CDd6 mutant
Chlorophyll-deficit mutant[b] (*cn-B1*)	7B	-	CDd1, CDd2 mutant
Reaction to disease and pest			
Powdery mildew (*Pm4A*)	2AL	Steinwedel*2/Khapli	Khapli, Yuma
Yellow rust (*Yr7*)	2BL	Brock, Renard, Tommy	Iumillo *durum*
Stem rust (*Sr35*)	-	Hexaploid derivatives	Tetraploid derivatives
Hessian fly resistance (*H5*)	1A	Abe, Beru, Downy	Giorgio 331-4
Hessian fly resistance (*H6*)	5A	Adder, Benhur, Caldwell	Purdue 4385 A4-6
Hessian fly resistance (*H11*)	1AS	Kay, Lines 916, 910, 941	PI 94587
Enzymes			
Alcohol dehydrogenase (*Adh-A1a*)	-	Chinese Spring	PI 226951
Alcohol dehydrogenase (*Adh-A1b*)	-	Rageni derivatives	CI 4013

[a] Chromosomal locations on bread wheat chromosomes, [b] Klindworth et al. (1995)

2.5 RFLPs and genetic studies in wheat

2.5.1 Identification and use

Polymorphic DNA markers have become an important tool for linkage studies and mapping genes to chromosomes. Of all the types of DNA markers, restriction fragment length polymorphism (RFLP) is the most widely used (Rafalski and Tingey, 1993). The identification of RFLPs involves the extraction of DNA from the organisms under investigation, and digestion of the DNA with a restriction endonuclease to yield a mixture of restriction fragments differing in length. These fragments are separated by gel electrophoresis and transferred to a nitrocellulose filter (Southern, 1975). Individual fragments can be identified by hybridizing with an appropriately labelled DNA probe, and visualized by autoradiography. If two individuals differ in a restriction site, which produces fragments of different lengths, the bands will appeared at different locations on the autoradiograph. In this circumstance, an RFLP is demonstrated.

RFLPs are inherited in a manner similar to Mendelian genes. At a given RFLP locus, alleles are codominant and independent of the environment. Therefore, major applications of RFLPs are to map genes to chromosomes, construct linkage maps and to tag genes for selection during the breeding process. The characterization of a large number of RFLPs has led to the construction of RFLP linkage maps in tomatoes (Bernatzky and Tanksley, 1986), maize (Helentjaris et al., 1986), barley (Heun et al., 1991; Klienhofs et al., 1993; Graner et al., 1994), rice (McCouch et al., 1988) and wheat (see Section 2.5.2). Practical applications of RFLPs for marker-based mapping of both qualitative and quantitative loci in plant breeding have been reviewed by many authors (see Section 2.5.3).

2.5.2 RFLP based linkage maps in wheat

Compared to diploid species such as tomato, maize, rice and barley, genetic maps of hexaploid wheat are relatively incomplete. However, wheat has a major advantage over many diploid species because the availability of aneuploid stocks allows the DNA probes to be assigned to individual chromosomes or chromosome arms before the linkage analysis is carried out (Sharp et al., 1989). Most DNA clones used to detect wheat RFLP loci have been obtained from *T. aestivum*, *T. tauschii*, barley, oats and rye (Hart, 1994).

RFLPs have been assigned to wheat chromosome arms (Sharp et al., 1989; Anderson et al., 1992) and RFLP-based maps exist for hexaploid wheat (Chao et al., 1989; Liu and Tsunewaki, 1991; Devos et al., 1992; 1993) and its D-genome progenitor, *T tauschii* (Gill et al., 1991b) (for examples, see Table 2.3). Results from cytogenetic (Sears, 1956; Dvorak, 1988), isozyme (Hart, 1987) and RFLP (Devos et al., 1992; 1993; Van Deynze et al., 1995) analyses indicated substantial conservation of genes among homoeologous chromosomes of Triticeae. Thus, it was concluded that the same set of probes may be used for RFLP genetic mapping in all species within Triticeae (Kam-Morgan et al., 1989).

2.5.3 Examples of application to plant breeding

In theory, it has been proposed that applications of molecular markers in plant breeding include marker assisted breeding, accelerated backcrossing, pyramiding genes, analysis and selection of quantitative traits, identification of hybrids, selection for resistance to pests and diseases and analysis of alien chromosome segments (Beckman and Soller, 1983; 1986; Bartel et al., 1989; Tanksley et al., 1989). Some of these applications have been achieved in wheat, such as the combination of RFLPs and cytogenetic manipulation of the wheat genome for introgression of alien genes and tagging of disease resistance genes (Langridge, 1994). The use of RFLPs to analyse quantitative trait loci (QTL) has been reported in many diploid crops.

Table 2.3 Examples of RFLP maps of bread wheat and its relatives.

Homoeologous group	Crop	No. of loci	Reference
1 to 7	Bread wheat	204	Liu and Tsunewaki (1991)
1 to 7	Barley	155, 587	Heun et al. (1991), Langridge et al. (1995)
1 to 7	*T. tauschii*	152, 58	Gill et al. (1991a), Lagudah et al. (1991)
1	Bread wheat and its relatives	98	Van Deynze et al. (1995)
1	Barley, rye	17	Wang et al. (1992)
2	Bread wheat, barley, rye	59	Devos et al. (1993)
3	Bread wheat, barley, rye	25, 16	Devos et al. (1992), Devos and Gale (1993)
4	Bread wheat	40	Mickelson-Young et al. (1995)
5	Bread wheat	38	Xie et al. (1993)
7	Bread wheat	29	Chao et al. (1989)
7	Rye	22	Rognli et al. (1992)

Cytogenetic manipulation

In bread wheat, the availability of aneuploid lines and the establishment of the genetic maps have greatly facilitated manipulation of chromosomes and genes (Law et al., 1987). Gale et al. (1989) concluded that RFLPs can be used for the identification of aneuploid genotypes, to increase the precision in intervarietal chromosome manipulation and to assist in the identification of alien chromosomes and of introgressed segments of the wild species. For example, Paull et al. (1994) identified the chromosome segment containing the stem rust resistance gene, *Sr22*, introgressed from *T. boeoticum* to chromosome 7A of bread wheat variety Schomburgk, by the use of RFLPs and near isogenic lines. This investigation demonstrated a far larger segment of *T. boeoticum* had been transferred to bread wheat than previously thought and that there had been almost no recombination of this segment with the 7A of bread wheat despite an intensive crossing program.

Identification of qualitative and quantitative trait gene(s)

Examples of tagging disease resistance genes have been reported by many researchers. In rice, RFLP markers for blast resistance genes have been demonstrated on chromosomes 6 and 12 (Yu et al., 1991). Heun (1992) used the QTL mapping method to identify loci associated with quantitative resistance to powdery mildew in barley. In wheat, genes conferring resistance to powdery mildew have been mapped onto several chromosomes arms such as, *Pm1* on 7AL, *Pm2* on 2DL, *Pm3b* on 1AS and *Pm4a* on 2AL, by aneuploid analysis (summarized by McIntosh, 1988) and confirmed by RFLP analysis (Ma et al., 1994).

There are several approaches to the use of RFLP markers to detect quantitative trait loci (QTL). In combination with the extensive RFLP-based maps that are available, phenotypic means for the progeny in each of the marker classes are compared. An estimation of the effect of each allele on the phenotypic variation of the character under investigation can be made by t-test or analysis of variance (Stuber, 1992; Zhikang et al., 1995). Significant differences between phenotypic means of the alternative RFLPs suggest that a QTL may be linked to the markers (Lander and Bostein, 1989).

QTL analysis using LOD scores have been developed by Lander and Bostein (1989). LOD is defined as the ratio of maximum likelihood estimates of the probable location of QTLs relative to the markers. Paterson et al. (1988) demonstrated the use of complete RFLP linkage maps and LOD scores to map QTLs associated with fruit mass, fruit pH and soluble solid content in an interspecific cross of tomatoes. Heun (1992) used both LOD values and comparisons of means of the subpopulations, to identify a QTL for powdery mildew resistance of barley on chromosomes 1S and 7 in an F_1-derived doubled haploid population. Zhikang et al. (1995) identified QTLs responsible for transgressive segregation for heading date and plant height in a cross between two semidwarf rice varieties by the use of LOD scores and analysis of variance of 2418 F_4 lines and 113 RFLP markers.

2.6 Conclusions and objectives

B toxicity in rainfed crops grown in South Australia, Western Australia and Victoria, has been identified for many species such as barley, bread wheat, durum wheat and grain legumes. An extensive breeding program in bread wheat has focused on producing tolerant varieties, with the release of BT-Schomburgk, Barunga and Frame.

Durum wheat was introduced into South Australian agriculture in 1991. Little information was known of the adaptation of durum to high concentrations of B in soil in these areas. Furthermore, there was no information of the genetic control of B tolerance in this species.

With the importance of cereal production to South Australian agriculture, this project has three basic objectives. The first was to identify the degree of genetic variation for response to B toxicity in durum wheat and determine whether it is large enough to introduce into an otherwise adapted variety. The second objective was to identify the mode of inheritance and number of genes controlling B tolerance, and thus determine appropriate strategies for breeding and selecting for B tolerance. The third objective was to identify the chromosomal locations of genes conferring B tolerance in durum wheat. Identifying chromosomes controlling B tolerance would assist in establishing linkage maps thereby allowing the identification of a closely linked marker which could be used for selection.

Chapter 3

Genetic variation for tolerance to high concentrations of boron in durum wheat

3.1 Introduction

A large range of genetic variation for response to high concentrations of B has been reported in bread wheat, barley and peas and improvements in tolerance can be made through selection and breeding. In these species, genetic variation for B tolerance was found within the commercial varieties in South Australia (Paull et al., 1992a; Jenkin, 1993, Bagheri et al., 1992).

In contrast to other crops, most durum varieties were reported to have a limited range of B tolerance, and were identified as sensitive to very sensitive (Yau et al., 1995). Brooks (1991) examined the adaptation of durum wheat to South Australia and found that durum can outyield bread wheat in some, generally higher yielding, areas suggesting that durum wheat has the potential to be cultivated in these conditions. An industry has been rapidly developed in these areas since his report was published. Alternatively, when durum was grown at lower yield sites, such as a high B site, Two Wells, all durums showed severe symptoms of B toxicity and it was concluded that the effect of B toxicity was one of major problems for adaptation of durum to South Australia. Small but significant variation for tissue concentrations of B was found among the commercial and landrace durum genotypes (Brooks, 1991). Therefore, there is the potential to study the genetic variation for B tolerance in durum wheat.

This chapter describes a series of experiments which aims to identify the extent of genetic variation between durum genotypes to provide the possibility of improvement of B tolerance through breeding. The objectives of this chapter were to:-

(1) assess the variation in B tolerance among a selection of adapted durum wheat varieties and exotic germplasm originating from Asia and North Africa;

(2) compare the level of tolerance in durum with bread wheat and

(3) examine the response of durum to increasing levels of B concentrations both in soils and solution.

3.2 Preliminary study of the response of durum wheat to high levels of B

3.2.1 Introduction

In bread wheat, a large range of variation in response to high concentrations of B was found within a collection of Australian and overseas varieties (Moody et al., 1988; Paull, 1990). Varieties were classified into five categories including; tolerant: G61450, moderately tolerant: Halberd, moderately sensitive: Schomburgk, sensitive: (W1xMMC) and very sensitive: Kenya Farmer, when grown in high B soils (Paull, 1990). Chantachume et al. (1995) developed a filter paper method to measure the response of seedling root growth under high concentrations of B and found the same ranking for these varieties.

In durum, little information on the variability of response to B was available. Yau et al. (1995) screened nineteen advanced lines of durum originating from West Asia and North Africa and reported a narrow range of variation for tolerance to B compared to bread wheat. All durums tested displayed the same, or a lower, level of tolerance than Schomburgk, a moderately B sensitive bread wheat.

A preliminary experiment was undertaken to investigate the response of durum to high concentrations of B, to determine the degree of genetic variation within the current adapted durum varieties and advanced lines and to identify a method of screening for B tolerance for a large number of genotypes.

3.2.2 Materials and methods

Genotypes

Twelve durum and three bread wheat genotypes were tested in soil and filter paper experiments. Seeds of durum wheat were obtained from Dr R. Hare, NSW Agriculture, Tamworth and from Australian Winter Cereal Collection, Tamworth, NSW. Seeds of the three bread wheat varieties were obtained from Dr J. G. Paull,

University of Adelaide. The name, origin and pedigree of each of the genotypes is shown in Table 3.2.1. Three replicates of fifty seeds of each genotype were weighed, imbibed on moist filter paper in petri dishes, stored at 2-4° C for 48 h and then placed at 15-18° C for 24 h, to ensure uniform germination, prior to sowing.

Filter paper experiment

A filter paper bioassay described by Chantachume et al. (1995) was used to study root and shoot growth in response to B. Solutions containing 500 μM $Ca(NO_3).4H_2O$ and 2.5 μM $ZnSO_4.7H_2O$ and boric acid at the rates of 0 and 100 mgB L^{-1} (designated as B0 and B100, respectively) were prepared. Sheets of filter paper (Ekwip® grade R6 size 36x42 cm) were soaked in these solutions for 2 min and allowed to drain for 2 min. Fifteen germinated seeds were placed in a single row along the centre of each paper with a spacing of 2 cm between each seed. The paper was then rolled up, covered with aluminium foil and stored vertically at 18° C.

The genotypes and B treatments were arranged as a split plot with three replications. After 12 days, root and shoot growth were assessed by measuring both length and fresh weight. Root length was measured as the longest seminal root and shoot length as the coleoptile length.

Glasshouse experiment

The screening was conducted in boxes (2m x 1m x 0.25m) as used by Moody et al. (1988) for screening bread wheat. The soil used was a bulk sample of silty clay loam texture from the surface (0-10 cm) of a red-brown earth (Typic Haploxeralf) obtained from the CSIRO Glenthorne Research Farm, O'Halloran Hill, South Australia (Paull et al., 1988b). Boron was applied to the soil at the concentration of 100 mg kg^{-1} soil. The concentration of extractable B in hot $CaCl_2$ in this soil was 58 mg kg^{-1} (Spouncer et al., 1992).

Table 3.2.1 Pedigree, country of origin and description of durum and bread wheat genotypes used in this experiment.

Genotypes	Pedigree	Country	Description
Durum wheat			
Souri Ac 60	Landrace	Tunisia	Sodicity tolerant[a]
Wollaroi	TAM1B-17/Kamilaroi sib//Rokel Sel./ Kamilaroi sib	Australia	Semi-dwarf variety
Minieh 72	Landrace	Egypt	Mn efficient[a]
TD 9456	Landrace	Syria	Sodicity tolerant[a]
Rokel'S'	Mexicali'S'/Flamingo'S'	Mexico	Semi-dwarf variety
Seville	Landrace	Algeria	Sodicity tolerant[a]
Gasvi	Landrace	Egypt	Sodicity tolerant[a]
RH 880009	Yallaroi//Tam 1B-17/Kamilaroi sib	Australia	NSW advanced line
Capeiti	Eiti/Cappelli	Italy	-
Yallaroi	Guillemot seln. No. 3/Kamilaroi sib	Australia	Semi-dwarf variety
RH 880008	Yallaroi//Tam 1B-17/Kamilaroi sib	Australia	NSW advanced line
Yavaros'S'	Jori'S' C69/Anhinga'S'//Flamingo'S'	Mexico	Semi-dwarf variety
Ruff'S'	Jori'S'/3/LD357E/Tehvacan60//Guillemot'S'	Mexico	Semi-dwarf variety
Bread wheat			
G61450	Mentana/Kenya//Quaderna	Greece	Tolerant to B[b]
Halberd	Scimitar/Kenya C46042//Bobin/3/Insignia49	Australia	Moderately tolerant to B[b]
Schomburgk	W3589/Oxley/2/2*Warigal/3/2*Aroona	Australia	Moderately sensitive to B[b]
Kenya Farmer	Gaza/2*Bobin//Button/Kenya 73D2I1C	Kenya	Very sensitive to B[b]

[a, b] Reference from Brooks (1991) and Paull (1990), respectively.

Seven seeds of each genotype were planted in rows, spaced 6 cm between rows and 4 cm between plants. The experiment was arranged in a randomized complete block design with three replications. Plant height was measured at six weeks after sowing. Expression of leaf symptoms was rated as the length of necrotic symptoms relative to total leaf length for the youngest (YEB), second youngest (YEB+1) and third youngest (YEB+2) leaves at seven weeks after sowing. Data were analysed statistically by analysis of variance and parameters were calculated for correlation coefficients.

3.2.3 Results

Seed size

Genotypes used in this study differed in seed size (Table 3.2.2). Wollaroi, Rokel'S', Souri Ac 60 and Gasvi durum and Kenya Farmer bread wheat were among the largest seed group (230-255 mg/50 seeds). RH 880008, Minieh 72 and Yavaros'S' had the smallest seed (164-168 mg/50 seeds).

Filter paper experiment

Root growth

Root growth of durum and bread wheat decreased significantly in the presence of B in filter paper (Table 3.2.2). There was significant variation in root length at B0, but the relative difference between genotypes was much less than at B100. At B100, G61450 had the longest roots (7.1 cm and 68% relative to B0) and Kenya Farmer had the shortest roots, (1.7 cm and a relative root length of 10%). Although there was a statistically significant interaction between genotypes and B treatments, all of the durums studied had root lengths at the high B treatment within the range from G61450 to Kenya Farmer and most were similar to Schomburgk. Root lengths and relative root lengths of the durums ranged between 2.7-5.1 cm and 17-35%, respectively. The results for root weight were similar to root length.

Table 3.2.2 Response of 12 durum and three bread wheat genotypes to high concentrations of B in the filter paper and soil experiments.

Genotypes	seed weight (mg/50sd)	Filter paper experiment												Glasshouse experiment			
		Root length (cm)			Root weight (mg plant^{-1})			Shoot length (cm)			Shoot weight (mg plant^{-1})			Height	Leaf symptom (%)		
		B0	B100	RRL	B0	B100	RRW	B0	B100	RSL	B0	B100	RSW	(cm)	YEB+2	YEB+1	YEB
Durum wheat																	
Souri Ac 60	230	13.9	4.8	35	53	21	40	12.3	10.0	81	129	133	103	31.2	36	29	19
Wollaroi	255	15.1	5.1	34	45	12	27	7.9	6.9	88	69	67	97	30.6	32	25	21
Minieh 72	166	15.5	4.1	31	46	10	22	8.5	8.1	95	137	87	63	32.1	34	26	21
TD 9456	206	13.1	4.6	30	44	12	27	10.8	8.3	76	111	82	74	23.4	39	27	22
Rokel'S'	231	15.6	4.5	29	49	11	22	8.6	6.9	80	73	64	88	26.6	32	27	26
Seville	177	13.6	3.9	29	37	11	30	7.6	6.6	86	99	74	74	34.4	28	18	17
Gasvi	231	17.5	4.3	25	51	15	29	8.0	7.7	97	93	98	105	35.7	27	15	13
RH 880009	172	15.1	3.3	22	43	9	21	8.1	6.3	78	76	63	83	30.6	21	27	19
Capeiti	209	12.8	2.8	22	33	9	27	10.9	5.6	51	107	51	48	31.1	30	24	19
Yallaroi	225	15.3	3.1	20	42	11	26	6.3	7.3	117	67	58	87	29.6	27	20	22
RH 880008	164	15.8	2.8	18	44	8	18	7.8	5.8	73	77	57	74	27.3	28	22	18
Yavaros'S'	168	16.2	2.7	17	43	6	14	7.3	4.9	68	82	52	63	26.9	27	26	20
Bread wheat																	
G61450	195	10.5	7.1	68	23	17	74	8.2	6.4	78	76	63	83	34.5	15	7	5
Schomburgk	176	13.2	3.4	26	31	9	29	6.6	5.2	79	66	49	74	30.1	25	18	15
Kenya Farmer	250	17.8	1.7	10	43	6	14	10.7	2.4	22	158	31	20	23.9	90	71	39
LSD (P<0.05)	12	2.8		7	7		12	2.1		32	16		23	6.3	8	14	8

RRL = relative root length [(root length at B100/root length at B0) x 100], RRW = relative root weight, RSL = relative shoot length, RSW = relative shoot weight

Shoot growth

Compared to root growth, B had a small effect on shoot growth of durum wheat (Table 3.2.2). Genotypes displayed significant variation in shoot length and weight when grown in both B0 and B100. Therefore, the effect of B on shoot growth of each genotype was determined on the basis of relative shoot growth. In bread wheat, the tolerant G61450 and moderately sensitive Schomburgk showed approximately 70-80% of shoot length and shoot weight at B100 relative to the control (B0), whereas at B100 Kenya Farmer produced only 20% of the control. In durum wheat, most of genotypes showed about 70-110% of shoot growth at B100 compared to control, with the exception of Capeiti, Yavaros'S' and Minieh 72, where relative shoot growth ranged between 48 to 63%

Glasshouse experiment

Plant height

Plant height of all genotypes measured at six weeks after sowing, ranged between 23 and 36 cm and no variation was found between the tolerant (G61450) and moderately sensitive (Schomburgk) checks (Table 3.2.2).

Symptoms of B toxicity

Genotypes were measured for severity of symptoms of B toxicity at seven weeks after sowing (Table 3.2.2 and Plate 3.1). The appearance of leaf symptoms in durum was similar to bread wheat. The symptoms consisted of chlorotic and necrotic lesions developing from the tips, along the margins towards the base of the leaves. In general, symptoms were most severe on the older leaves. The ranking of genotypes was consistent for YEB, YEB+1 and YEB+2. Kenya Farmer showed the most severe symptoms (90% of total first leaf length, YEB+2) and G61450 expressed the least severe symptoms (15%). All durum genotypes were ranked between Schomburgk and Kenya Farmer (20-40%).

Plate 3.1 Comparison of the response of bread wheat (central row) and durum wheat grown in soil with 100 mgB kg^{-1}.

(a) durum wheat rated as moderately sensitive v bread wheat (G61450, in the central row) rated as tolerant

(b) durum wheat rated as moderately sensitive v bread wheat (Kenya Farmer, in the central row) rated as very sensitive

(a)

(b)

Correlation between parameters

The correlations between parameters measured from both filter paper and glasshouse experiments were calculated (Table 3.2.3). All correlation coefficients between seed weight and other characters were not statistically significant. The response of plants to high concentrations of B was therefore independent of seed size. Both root and shoot growth at B100 showed positive significant correlations with relative growth as is to be expected when correlating a ratio with one of the variables included in the ratio. Significant negative correlations resulted between symptom expression and root parameters. Correlations between symptoms and shoot parameters were also negative, but not statistically significant.

Table 3.2.3 Correlation coefficients between characters including root growth, shoot growth and leaf symptom expression for bread and durum wheat grown in high concentrations of B in filter paper and soil.

Characters	Seed wt.	RRL	RRW	RSL	RSW	Symptom[a]
Root growth at B100						
Root length	0.13	0.95**	0.83**	0.43	0.63**	-0.56*
Root weight	0.33	0.70**	0.73**	0.41	0.70**	-0.49*
Shoot growth at B100						
Shoot length	0.06	0.40	0.31	0.71**	0.76**	-0.31
Shoot weight	0.12	0.32	0.28	0.48*	0.67**	-0.25

*, ** Significant at P<0.05 and 0.01, respectively. [a] Data were average from three leaves.

RRL = relative root length, RRW = relative root weight

RSL = relative shoot length, RSW = relative shoot weight

3.2.4 Discussion

A significant reduction in seedling root and shoot growth of bread and durum wheats occurred when seedlings were grown in filter paper treated with a high concentration of B. Significant variation occurred in root and shoot growth among bread wheat genotypes in the B100 treatment in filter paper. The ranking of these three bread wheat checks was consistent with previous observations in high B soils (Paull et al., 1992a), solution culture (Nable, 1988) and by the filter paper method (Chantachume et al., 1995). Significant variation in root and shoot growth in response to B was observed among the durum genotypes, although the range of variation was much less than that for the bread wheats.

The growth of the first seminal root and of shoot (coleoptile) of durum and bread wheats grown at high B in filter paper were negatively correlated with the expression of symptoms of B toxicity when the same varieties were grown in high B soil in a glasshouse. In this experiment, the correlation coefficient between root length and symptom expression was higher than that between shoot length and symptoms, suggesting that root length is a better parameter than shoot length for discrimination between tolerant and sensitive genotypes. A highly significant correlation occurred between root length at B100 and relative root length ($r = 0.95^{**}$) indicating that B100 can be used as a single treatment to screen for B tolerant genotypes, without a control treatment. However, a relatively low correlation coefficient between root length and symptom expression was found in this study (0.56^{**}). This is in contrast to that reported in bread wheat ($r = 0.78^{**}$) (Chantachume et al., 1995) and may be due to the low variation in response of the durum genotypes used in this study. The high correlation reported by Chantachume et al. (1995) was consistent with those of bread wheat used in this study. For example, root lengths of G61450 and Kenya Farmer at B100 were 7.1 and 1.7 cm, and symptom expression of the first leaf of these genotypes was 15 and 90%, respectively.

The experiment examining genetic variation of durum in response to high concentrations of B contrasted with the results reported for wheat, barley, medics and peas (Nable, 1988; Paull et al., 1988b; Bagheri et al., 1992). Only a small range in response to B occurred within Australian and CIMMYT varieties and adapted lines. All of the durums were similar to Schomburgk and rated as sensitive to moderately sensitive. The lack of variation may arise from the fact that these durum genotypes were developed in areas where B toxicity has not been reported. For example, the current Australian durum varieties were bred at Tamworth, NSW, and evaluated in surrounding regions where no problems with B have been recognized (Hare, 1994b). Therefore, there is an urgent need to improve the B tolerance in durum for growing in areas where high B is a major problem. The next step in the identification of tolerant genotypes is to examine accessions from overseas germplasm, especially landrace cultivars originating from areas where high tolerance to B have been found in other crops, such as countries of Asia and Asia Minor (Moody et al., 1988, Paull et al., 1992a). The level of tolerance sought should be the same as or higher than that of the bread wheat Halberd, which was rated as a moderately tolerant and successfully used as a donor parent for improving the B tolerance of Schomburgk (Moody et al., 1993).

In conclusion, a low level of genetic variation for B tolerance was found in adapted durum genotypes originating from Australia, Italy, Mexico and some landrace cultivars collected from Algeria, Egypt, Tunisia and Syria. Nevertheless, this experiment confirmed the efficiency of the filter paper method. This technique is non-destructive, time and cost efficient and can screen a large number of genotypes (Chantachume et al., 1995). The B100 treatment was appropriate for discriminating between tolerant and sensitive genotypes and can be adopted for evaluating further the response of exotic germplasm of durum to high concentrations of B. Once the tolerant genotypes have been identified, seedlings can be transferred to normal soils for crossing or seed production.

3.3 Identifying sources of B tolerance in exotic germplasm

3.3.1 Introduction

The results of the previous section demonstrated that no useful genetic variation for B tolerance was available in Australian and adapted introduced durum varieties. Therefore, there was a need to identify sources of B tolerance in overseas germplasm. The major aim for this experiment was to identify sources of B tolerance from germplasm collected from areas where B tolerance predominated in hexaploid wheat, barley and peas (Moody et al., 1988; Bagheri et al., 1994). Screening a large number of accessions should enable the identification of the geographical origin of tolerant accessions and allow a comparison of the range of variation within durum and bread wheats.

3.3.2 Materials and methods

Genotypes

A total of 249 accessions collected from North Africa and Asia, and 51 varieties or advanced lines from Australia, Italy and CIMMYT were tested for response to B. Seeds of all accessions were obtained from the Australian Winter Cereals Collection, Tamworth, New South Wales. Seeds of varieties and advanced lines were obtained from a collection maintained at the Waite Institute. Three bread wheat genotypes with known levels of response to B (G61450, Halberd and Schomburgk) were included as checks. The response to B and pedigree of G61450, Halberd and Schomburgk are shown in Table 3.2.1. All seed was pre-germinated before sowing to ensure uniform germination.

Screening procedure

Initial screening

Response to B was assessed by the filter paper method, described in Section 3.2.2. Filter papers were soaked with boric acid solution at the concentration of 100 mgB L^{-1}. Seven to ten germinated seeds of each genotype were sown on each paper. The screening consisted of two replicates. Three bread wheat checks, G61450, Halberd and Schomburgk, were included every fifty entries. After 12 days, the length of the longest root of each seedling was measured and the average seedling root length of each genotype was calculated and compared with the checks.

Second screening

Six durum accessions identified as tolerant, moderately tolerant, moderately sensitive and sensitive were selected (Table 3.3.3) and the seedlings were transplanted to normal soil for seed multiplication. Residual seed harvested from the transplanted plants was retained and multiplied further to form the seed stock for genetic studies described in later chapters.

To obtain confirmation of response and compare the range of response to B to Yallaroi and the bread wheat genotypes, namely, G61450 (T), Halberd (MT), Schomburgk (MS) and Kenya Farmer (S), seeds harvested from each genotype were tested for response to B in filter paper at B0 and B100 treatments. Fifteen seeds were grown in each B treatment and genotype combination. The experiment was arranged as a split plot design with three replications. Root length was measured at 12 days after sowing, and the mean seedling root length of each accession was calculated.

3.3.3 Results

When screened at the B100 filter paper treatment, the root lengths of Schomburgk, Halberd and G61450 ranged between 3.2-4.6, 5.8-7.2 and 8.1-8.8 cm, respectively (data not shown). According to the response of bread wheat checks, durum accessions were classified into four categories, namely, sensitive, moderately sensitive, moderately tolerant and tolerant (Table 3.3.1).

Table 3.3.1 Scheme of classification of 300 durum genotypes, relative to bread wheat checks, for response to B, on the basis of root length when grown in filter papers containing 100 mgB L^{-1}.

Root length (cm)	Category	Bread wheat
≤ 3	sensitive (S)	-
> 3 - 5	moderately sensitive (MS)	Schomburgk
> 5 - 8	moderately tolerant (MT)	Halberd
> 8	tolerant (T)	G61450

A narrow range in response to B in terms of root growth in high B concentration was measured among the adapted genotypes and most of the accessions of durum. All varieties/lines and accessions originating from Australia, Italy, CIMMYT and African countries were rated as sensitive to moderately sensitive to B (Table 3.3.2). Only seven lines, or 2% of the total population, originally collected from China (2), India (3) and Iraq (2), were classified as moderately tolerant or tolerant. Two tolerant, two moderately tolerant, one moderately sensitive and one sensitive accessions were selected for further study. The accession numbers, names, countries of origin and root lengths at B100 in the first screening are shown in Table 3.3.3.

Table 3.3.2 Distribution of response to B in durum genotypes, according to countries of origin, based on root lengths in filter papers containing 100 mgB L^{-1}.

Country	Response to B				No. of lines	%MT-T
	S	MS	MT	T		
Africa						
Algeria	12	17	0	0	29	0
Ethiopia	10	13	0	0	23	0
Morocco	8	10	0	0	18	0
Tunisia	6	7	0	0	13	0
Total	*36*	*47*	*0*	*0*	*73*	*0*
Asia						
Afghanistan	6	8	0	0	14	0
China	2	8	2	0	12	17
India	5	5	1	2	13	23
Iran	3	10	0	0	13	0
Iraq	6	6	2	0	14	14
Syria	24	30	0	0	54	0
Turkey	15	31	0	0	46	0
Total	*61*	*98*	*5*	*2*	*166*	*4.2*
Others						
Australia	1	23	0	0	24	0
Italy	3	4	0	0	7	0
Mexico	12	8	0	0	20	0
Total	*16*	*35*	*0*	*0*	*51*	*0*
Total	**113**	**180**	**5**	**2**	**300**	***2.3***
Frequency (%)	***37.7***	***60.0***	***1.6***	***0.7***	***100***	***2.3***

When selected accessions were progeny tested and compared with the bread wheat checks, significant differences in root length and a significant B treatment x genotype interaction were found in both durum and bread wheats (Figure 3.1). At B0, the root length of durum and bread wheat ranged between 11 and 14 cm. At B100, genotypes were classified into four distinct classes. G61450, AUS 10110 and AUS 10105, the group with the longest roots, ranged between 6-7 cm. Halberd, AUS 10344 and AUS 14010 produced root lengths between 5-6 cm, Schomburgk, AUS 10348 and Yallaroi had root lengths between 3-4 cm and Kenya Farmer and AUS 13244 had the shortest roots (1-2 cm). An example of a comparison between root length of bread wheat and durum wheat is shown in Plate 3.2.

Table 3.3.3 Australian Wheat Collection accession number, name, country of origin and mean root length in B100 of the selected durum accessions.

Accession No.	Name	Country of origin	Root length (cm)[a]
AUS 10110	AUS 10110	India	8.7
AUS 10105	AUS 10105	India	8.2
AUS 10344	Niloticum	Iraq	5.6
AUS 14010	Lingzhi Baimong Baidamai	China	5.3
AUS 10348	Erythrospermum	Iraq	3.7
AUS 13244	Gandum	Afghanistan	1.5

[a] root length grown at B100 treatment at initial screening (Table 3.3.2)

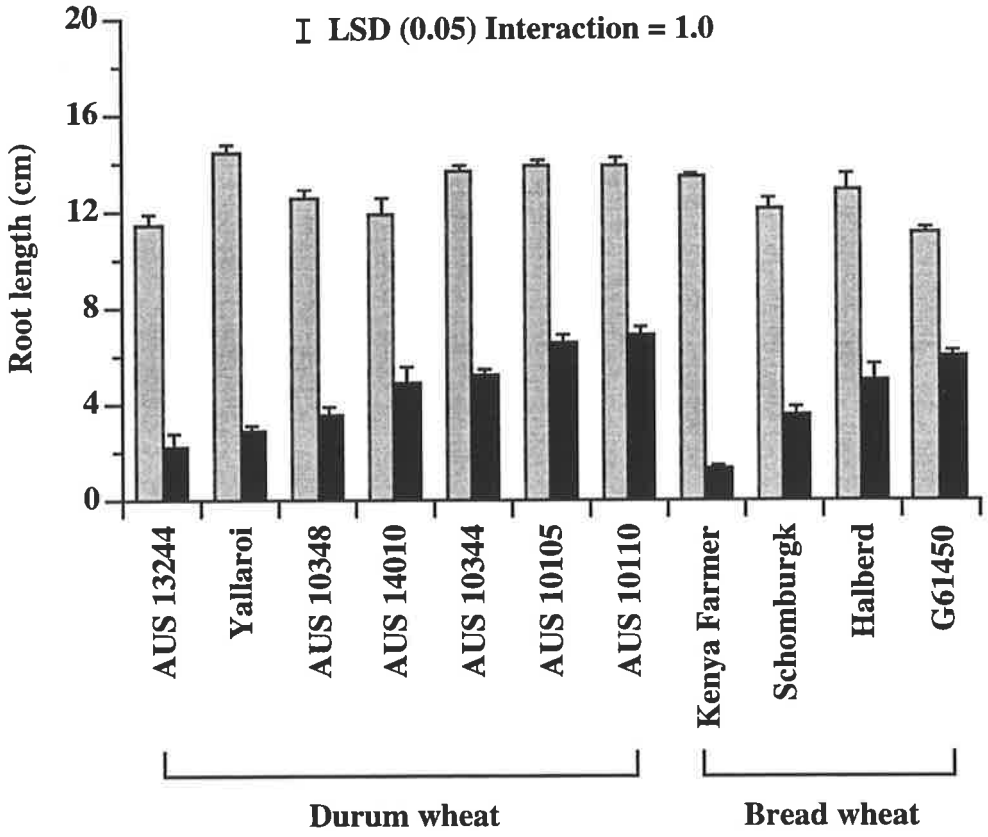


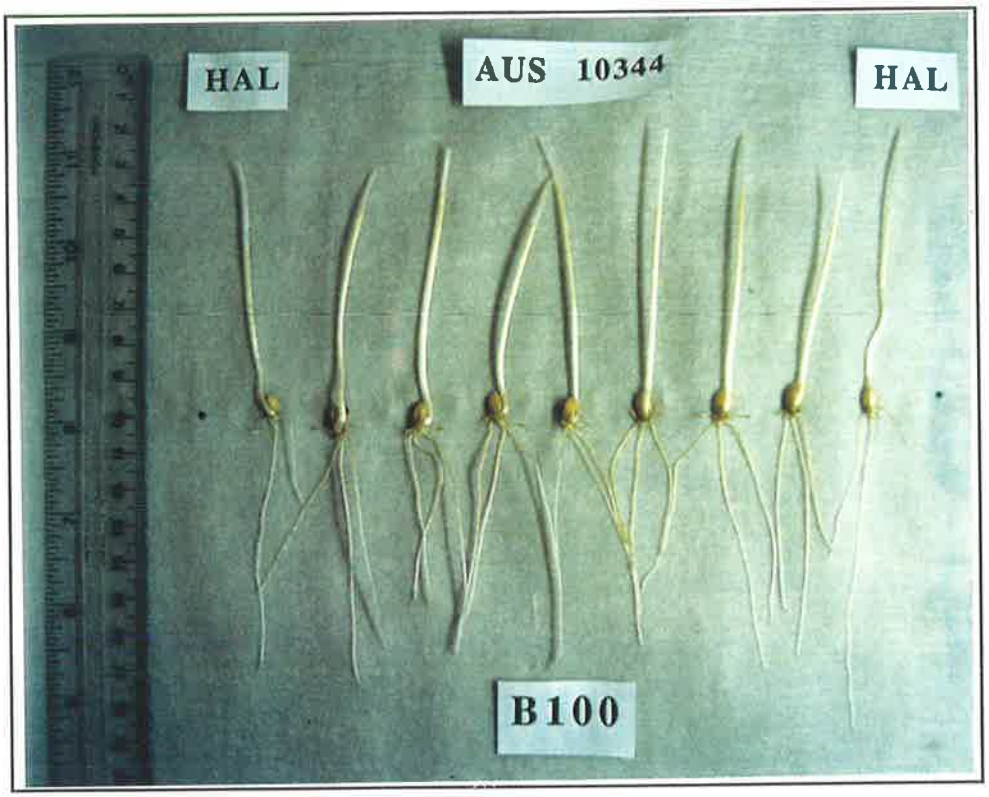
Figure 3.1 Root length of durum and bread wheat genotypes tested in filter papers at B0 (□) and B100 (■) treatments in the second screening. Vertical bars represent the standard error of three replicates.

Plate 3.2 Comparison of root length between bread wheat and durum wheat. From left to right: Seedling no. 1 and 9 = Halberd (moderately tolerant bread wheat), no. 2-8 = durum wheat, grown in filter paper containing 100 mgB L^{-1}.

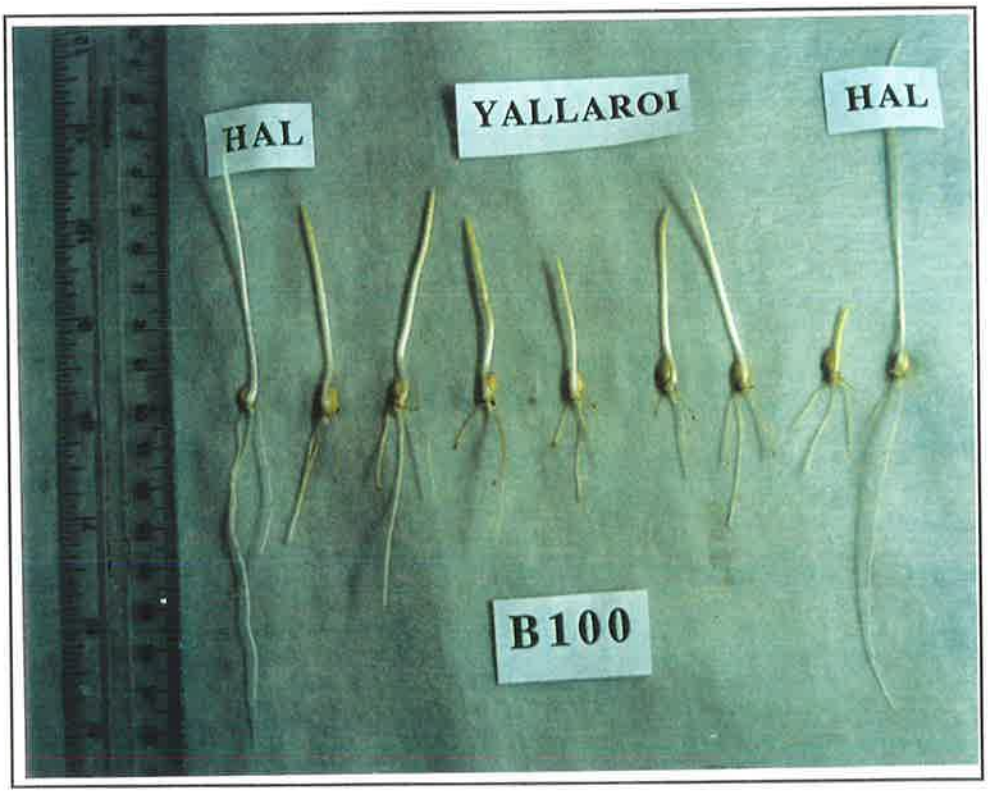
(a) Halberd v AUS 10344

(b) Halberd v Yallaroi

(a)



(b)



3.3.3 Discussion

A large range in root lengths of seedlings grown in a high concentration of B occurred among the durum accessions originating from diverse geographical locations. The B treatment used in this screening was able to discriminate between tolerant and sensitive genotypes. For example, the root length of accessions classified as tolerant (AUS 10110) and sensitive (AUS 13244) at B100 were 7.0 and 2.0 cm, respectively, and a similar level of discrimination was achieved for the bread wheat checks (Figure 3.1).

Genetic variation in response to B was demonstrated among the exotic durum germplasm with seven accessions (2% of the population), originating from India, Iraq and China, being classified as moderately tolerant and tolerant. B tolerant material from other crops has also been identified from these geographical regions. Moody et al. (1988) screened 1579 accessions of bread wheat and classified a high proportion of lines (37% from Iraq and 58% from India) as moderately tolerant or tolerant. Paull et al. (1992b) screened overseas pea accessions and identified only two lines more tolerant to B than the moderately tolerant Australian varieties. These two lines also originated from India. A number of reports in the literature have described areas of India having B toxicity problems (e.g. Chauhan and Asthana, 1979; Chauhan and Powar, 1978). The results of the durum screening were consistent with those found in bread wheat, barley, medics and peas with a majority of B tolerant accessions originating in Asian countries, suggesting that high concentrations of B may be present in soil and have a major influence upon the adaptation of plants in that region. It can be proposed that the germplasm of other crops originating from these regions is likely to provide a high level of variation for this character.

Compared to bread wheat, durum wheat had smaller range in variation for response to B. The most tolerant durums were rated the same as G61450. Moody et al. (1988) identified some overseas genotypes as more tolerant than G61450. Furthermore, the most sensitive durum, AUS 13244, had significantly shorter roots than Schomburgk,

but slightly longer than Kenya Farmer (Figure 3.1). Paull et al. (1992a) concluded that the variation in response to B found in bread wheat was controlled by at least four genes. The number of genes controlling B tolerance in durum is investigated further in Chapter 4. The smaller range of response to B in durum wheats may be due to response being controlled by fewer genes than in bread wheat.

This experiment showed that genetic variation for response to high concentrations of B does exist in durum wheat. The solution for overcoming B toxicity would be breeding and selection of B tolerant varieties. However, more information is needed on the mechanism of tolerance in this species and how it can be incorporated into intolerant but adapted varieties to facilitate the breeding process.

3.4 Variability of tolerance to high concentrations of B in durum; a comparison of soil and filter paper screening

3.4.1 Introduction

Preliminary investigations identified a number of genotypes as moderately tolerant and tolerant to B. However, the results were based on single or two levels of B in filter paper. To investigate in more detail the response to B of durum wheat, a range of B concentrations in filter paper were used to confirm that the genotype x B interaction within durum is a result of variation in tolerance. The genotypes were also grown in soil at different levels of applied B. The response at the whole plant level was measured and compared with the response of root growth in filter paper.

The objective of this study was to investigate the extent of variation among durum genotypes at the whole-plant and root growth level, in response to a range of B concentrations.

3.4.2 Materials and methods

Genotypes

Seven genotypes were grown in a glasshouse experiment and nine genotypes in a filter paper experiment (Table 3.4.1). To ensure uniform germination, all seeds were pregerminated before sowing. Three extra genotypes, namely, Abyssinian 29, Wollaroi and Capeiti, were included because they showed good agronomic characters when grown in the field (Brooks, 1991).

Glasshouse experiment

The soil used was a bulk sample of silty clay loam texture from the surface (0-10 cm) of a red-brown earth (Typic Haploxeralf) obtained from the CSIRO Glenthorne Research Farm, O'Halloran Hill, South Australia (Paull et al., 1988b). Boron was applied to the soil at concentrations of 40, 60 and 80 mg kg^{-1} (designated B40, B60

Table 3.4.1. Genotypes tested for response to B, together with their country of origin and responses to high concentrations of B based on preliminary investigations (Section 3.3).

Genotype	Name and pedigree	Origin	B response[1]
AUS 10110	Table 3.3.3	India	T
AUS 10105	Table 3.3.3	India	T
AUS 10344	Table 3.3.3	Iraq	MT
AUS 14010	Table 3.3.3	China	MT
Abyssinan 29	landrace	Ethiopia	-
Wollaroi	Table 3.2.1	Australia	MS
AUS 10348	Table 3.3.3	Iraq	MS
Capeiti	Table 3.2.1	Italy	MS
Langdon (LDN)	Mindum/Carleton,Ld194/2/Khapli/3/Ld308,Heiti/Stewart/2/Mindum/Carleton/4/Stewart/5/Carleton	USA	-
Yallaroi	Table 3.2.1	Australia	MS

[1] Response of genotypes in 100 mgB L^{-1} in filter paper from Section 3.3 T = tolerant, MT = moderately tolerant, MS = moderately sensitive.

and B80, respectively). Untreated soil was used as a control (designated B0). The B0, B40, B60 and B80 treatments were grown in trays (30 x 40 x 12 cm) containing 13 kg of soil. The trays were maintained at 70% of field capacity by watering to weight with de-ionised water.

In each tray, four seedlings from each genotype were planted in rows, spaced 6 cm between rows and 6 cm between plants. The experiment was arranged in a split plot design with three replications. Plants were grown for seven weeks. The above ground portion was harvested at ground level, oven dried at 80° C for 48 h, weighed, digested in nitric acid and analysed for B by inductively coupled plasma spectrometry (Zarcinas et al., 1987). During plant growth, height was measured at six weeks after sowing. Expression of leaf symptoms was rated as the length of necrotic symptoms relative to total leaf length for the youngest expanded blade (YEB), second youngest (YEB+1) and third youngest (YEB+2) leaves at six weeks after sowing.

Prior to statistical analysis, data for dry matter yield and shoot B concentrations were subjected to logarithmic (log_{10}) and square root transformations, respectively.

Filter paper experiment

A filter paper experiment, as described in Section 3.2, was used. B treatments were applied as boric acid into the solution at the rates of 0, 25, 50, 75, 100 and 150 $mgBL^{-1}$ (designated as B0, B25, B50, B75, B100 and B150, respectively). Fifteen seeds of each genotype were grown with one genotype per filter paper. The genotypes and B treatments were arranged as a split plot design with three replications. Root growth at 12 days after sowing was assessed by measuring the longest root of each seedling.

3.4.3 Results

Glasshouse experiment

Plant height

Plant height of the seven durum genotypes, at six weeks after sowing, was not affected by B treatments (Table 3.4.2). Differences between the heights of the genotypes were significant at $P<0.05$, whereas the interaction with B treatment was not significant. Plant height of most genotypes was between 34 to 40 cm with the exception of Yallaroi, which was shorter than the other genotypes because of its semi-dwarf habit.

Symptom of B toxicity

Plants were measured for severity of symptoms of B toxicity at six weeks after sowing. In general, the severity of symptoms increased with the level of applied B and symptoms were most severe on the oldest leaf, YEB+2 (Figure 3.2). The ranking of genotypes was consistent for all leaves measured. Symptoms were similar for AUS 14010, AUS 10344, AUS 10105 and AUS 10110, being less severe than for the other three genotypes. Langdon generally developed the most severe symptoms while Yallaroi and AUS 10348 were either in an intermediate group or similar to Langdon, depending upon the leaf and the treatment.

Dry matter yield and tissue B concentrations

Dry matter yield decreased significantly with an increase in the concentration of B applied to the soil and significant differences ($P<0.01$) occurred among the genotypes while the treatment x genotype interaction was also significant (Table 3.4.3). There was little difference between genotypes at B0 and B40. At B60 and B80, dry matter yields of tolerant genotypes (AUS 10110, AUS 10105, AUS 10344 and AUS 14010) were significantly higher than those for AUS 10348, Yallaroi and Langdon.

Table 3.4.2. Plant height at six weeks after sowing (cm) of seven genotypes grown at four levels of applied B.

Genotype	Plant height (cm)				
	B0	B40	B60	B80	mean
AUS 10110	38	40	42	39	*40*
AUS 10105	39	38	40	41	*39*
AUS 10344	35	34	40	38	*37*
AUS 14010	40	38	42	40	*40*
AUS 10348	37	36	35	37	*36*
Langdon	32	33	37	34	*34*
Yallaroi	28	26	26	23	*26*
Mean	*36*	*35*	*37*	*36*	*36*

LSD ($P < 0.05$) Genotype 3.0.

B treatment and the B treatment x Genotypes interaction were not significant.

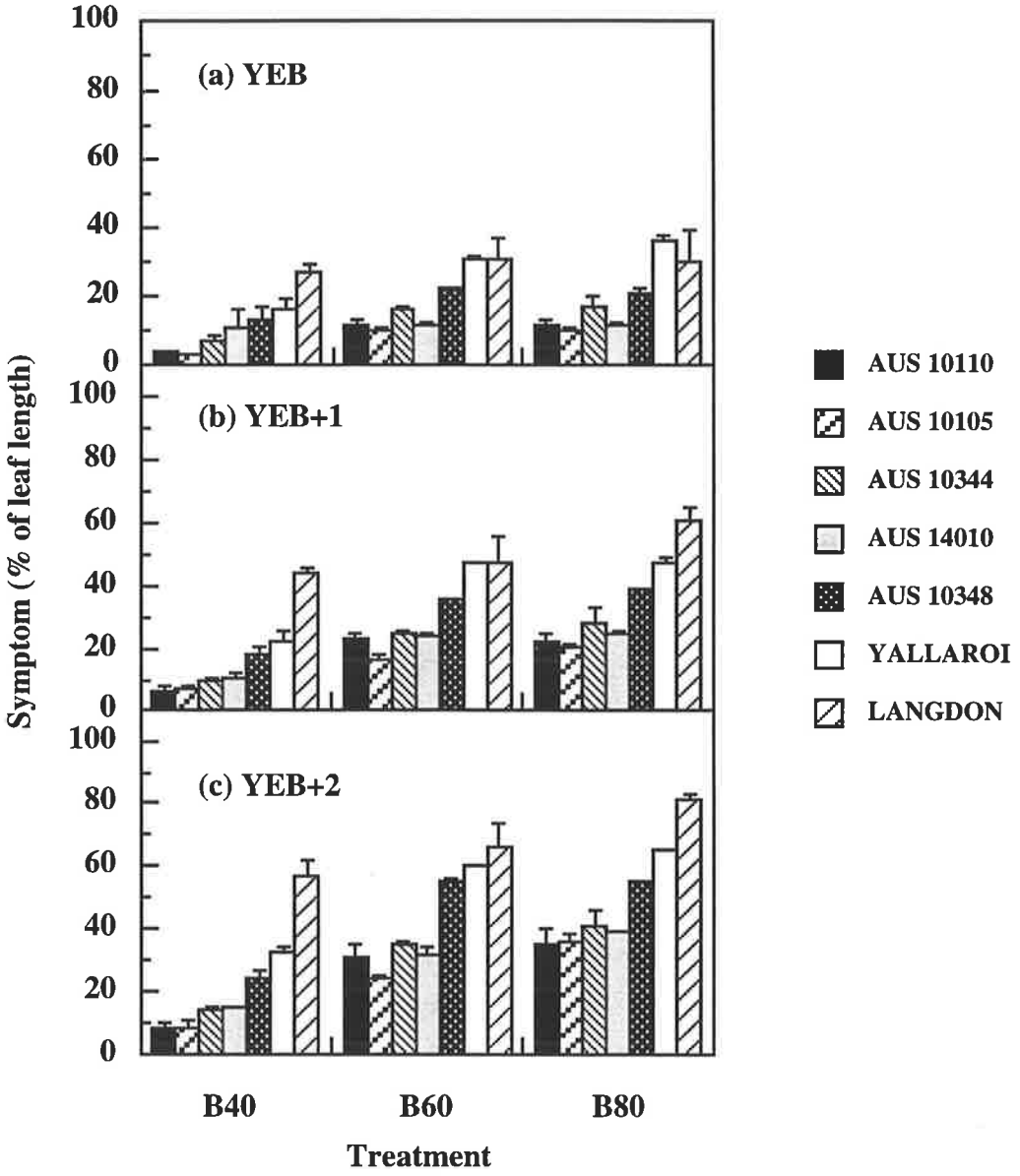


Figure 3.2. Severity of symptoms of B toxicity expressed as a percentage of total leaf length for the first three leaves: (a) YEB, (b) YEB+1 and (c) YEB+2 of seven genotypes grown at 40, 60 and 80 mg kg^{-1} applied boron. Vertical bars represent standard errors of the means of the three replications.

Table 3.4.3. Dry matter yield (mg row^{-1}) of seven genotypes grown at four levels of applied B. The significance values refer to the logarithmically transformed data, presented in brackets.

Genotype	yield (mg row^{-1})				
	B0	B40	B60	B80	mean
AUS 10110	2238 (3.35)	2097 (3.32)	1794 (3.25)	1428 (3.15)	*1889 (3.27)*
AUS 10105	2126 (3.33)	2042 (3.31)	1905 (3.28)	1641 (3.22)	*1726 (3.28)*
AUS 10344	1542 (3.19)	1248 (3.10)	1144 (3.06)	1299 (3.11)	*1308 (3.11)*
AUS 14010	1996 (3.30)	1737 (3.24)	1817 (3.26)	1294 (3.11)	*1711 (3.23)*
AUS 10348	1988 (3.30)	1500 (3.18)	902 (2.96)	869 (2.94)	*1314 (3.09)*
Langdon	1291 (3.11)	1004 (3.00)	740 (2.87)	516 (2.71)	*887 (2.92)*
Yallaroi	1454 (3.16)	1211 (3.08)	804 (2.91)	624 (2.80)	*1023 (2.99)*
Mean	*1805 (3.25)*	*1548 (3.18)*	*1180 (3.08)*	*1096 (3.01)*	*1409 (3.13)*

LSD ($P < 0.05$) B treatment 0.05 Genotype 0.08 Interaction 0.16

LSD ($P < 0.01$) B treatment 0.08 Genotype 0.11 Interaction 0.21

When the data were converted to relative yields, that is yield at B treatment/control, to take account of any variation in inherent vigour of genotypes, the same four genotypes were identified as tolerant. For example, the relative yields of AUS 10110, AUS 10105, AUS 10344 and AUS 14010 at B80 compared to B0 were between 65-80% while those of AUS 10348, Yallaroi and Langdon were between 40-45% (data not shown).

Shoot B concentrations increased significantly with increased B treatments (Table 3.4.4). At B0, there was no significant variation in B concentrations among genotypes and values ranged from 10 mg kg^{-1} in AUS 14010 to 22 mg kg^{-1} in Langdon. At higher B treatments, the B concentrations of tolerant genotypes (AUS 10110, AUS 10105, AUS 10344 and AUS 14010) were always lower than the sensitives genotypes (AUS 10348, Yallaroi and Langdon).

Filter paper experiment

Root length in filter paper

At 12 days after sowing, root length was markedly decreased by increasing B concentrations in filter papers (Table 3.4.5). Root growth of sensitive genotypes (eg. AUS 10348, Yallaroi and Langdon) was most affected by B supply, whereas AUS 10110, AUS 10344 and AUS 14010 showed less reduction. Although there was significant variation at B25, the differences in root length between tolerant and sensitive genotypes expressed as a percentage of the control were most obvious at higher levels of B. For example, AUS 10110 showed 64% and 42% of maximum root length (B0) at B100 and B150 respectively, whereas Yallaroi was 15% and 11% at these levels (Table 3.4.5 and Plate 3.3).

Root length of three other genotypes, namely, Abyssinian 29, Wollaroi and Capeiti were similar to AUS 10348 and Yallaroi and together these comprised the sensitive group. Although no glasshouse experimental data were available for the first three

Table 3.4.4 Concentrations of boron in shoots (mg kg^{-1}) of seven genotypes grown at four levels of applied B. The significance values refer to square root transformed data, presented in brackets.

Genotype	Boron concentration (mg kg^{-1})				
	B0	B40	B60	B80	mean
AUS 10110	18 (4.24)	222 (14.90)	823 (28.69)	995 (31.54)	*514 (19.84)*
AUS 10105	11 (3.32)	125 (11.18)	549 (23.43)	750 (27.39)	*359 (16.33)*
AUS 10344	11 (3.32)	131 (11.45)	486 (22.05)	685 (26.17)	*328 (15.75)*
AUS 14010	10 (3.16)	160 (12.65)	544 (23.32)	879 (29.65)	*398 (17.20)*
AUS 10348	14 (3.74)	288 (16.97)	1533 (39.15)	1743 (41.75)	*894 (25.40)*
Langdon	22 (4.69)	417 (20.42)	1389 (37.27)	1995 (44.67)	*956 (26.76)*
Yallaroi	17 (4.12)	320 (17.89)	1387 (37.24)	2094 (45.76)	*954 (26.25)*
Mean	*15 (3.80)*	*238 (15.07)*	*959 (30.16)*	*1036 (35.28)*	*629 (21.08)*

LSD ($P < 0.05$) B treatment 2.06 Genotype 1.25 Interaction 2.80

LSD ($P < 0.01$) B treatment 3.12 Genotype 1.66 Interaction 3.93

Table 3.4.5. Root length (cm) for nine genotypes grown in solution culture in filter papers at six levels of applied B. Relative root lengths expressed as percentage of B0 are presented in brackets.

Genotype	Root length (cm)/ relative root length (% of B0)					
	B0	B25	B50	B75	B100	B150
AUS 10110	12.9	13.1 (102)	12.3 (95)	9.9 (77)	8.3 (64)	5.4 (42)
AUS 10344	13.2	12.5 (95)	10.7 (81)	8.0 (61)	5.3 (40)	4.0 (30)
AUS 14010	13.0	11.8 (91)	9.4 (72)	7.3 (56)	5.3 (41)	3.2 (25)
Abyssinian 29	18.1	12.6 (70)	8.9 (49)	7.0 (39)	4.0 (22)	2.8 (15)
Wollaroi	16.3	13.8 (85)	9.4 (58)	8.1 (50)	4.5 (27)	2.6 (16)
AUS 10348	13.7	9.8 (72)	8.2 (60)	6.5 (47)	3.5 (26)	2.0 (15)
Capeiti	13.0	10.3 (79)	7.5 (58)	5.8 (45)	3.0 (23)	1.8 (14)
Langdon	15.3	10.8 (71)	7.4 (48)	5.7 (37)	3.3 (22)	2.2 (14)
Yallaroi	14.4	9.5 (66)	6.6 (46)	4.8 (33)	2.1 (15)	1.6 (11)

LSD ($P < 0.05$) B treatment 0.7 Genotype 0.8 Interaction 1.2

LSD ($P < 0.01$) B treatment 1.2 Genotype 1.2 Interaction 2.3

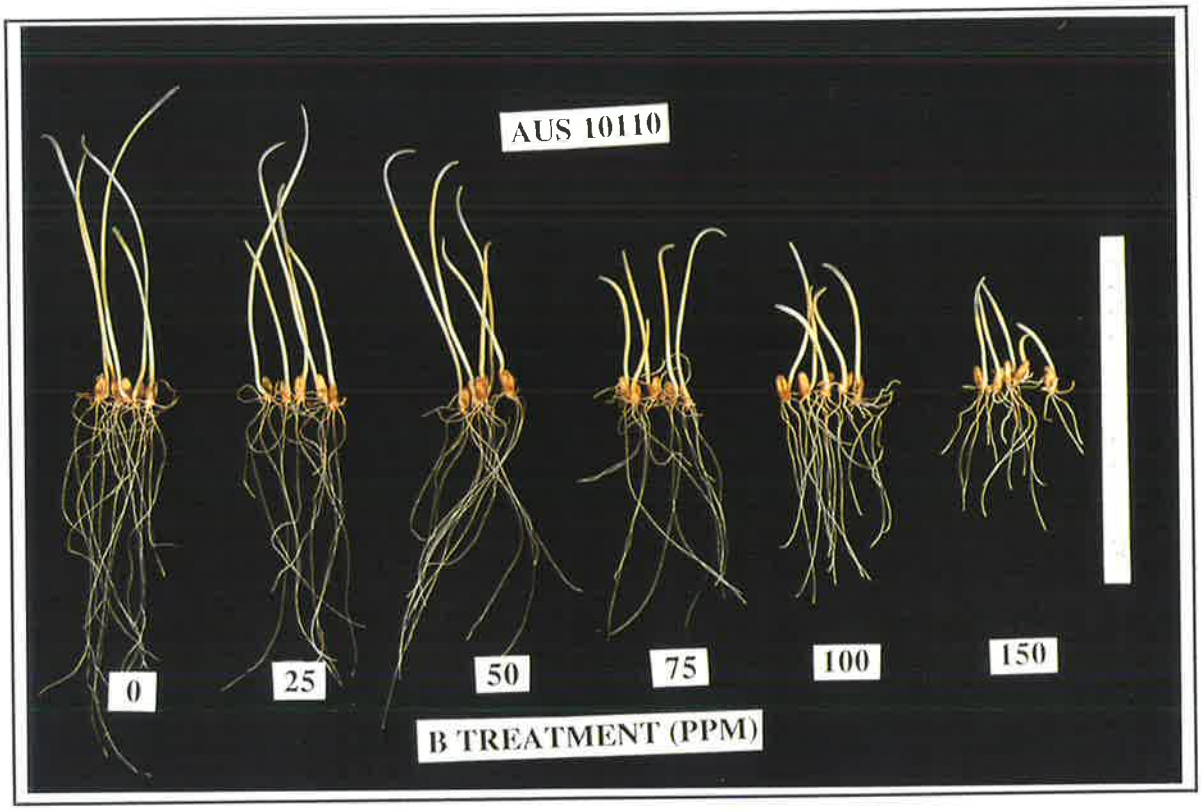
Plate 3.3 Comparison of the response of two genotypes grown at six B treatments.

(a) AUS 10110

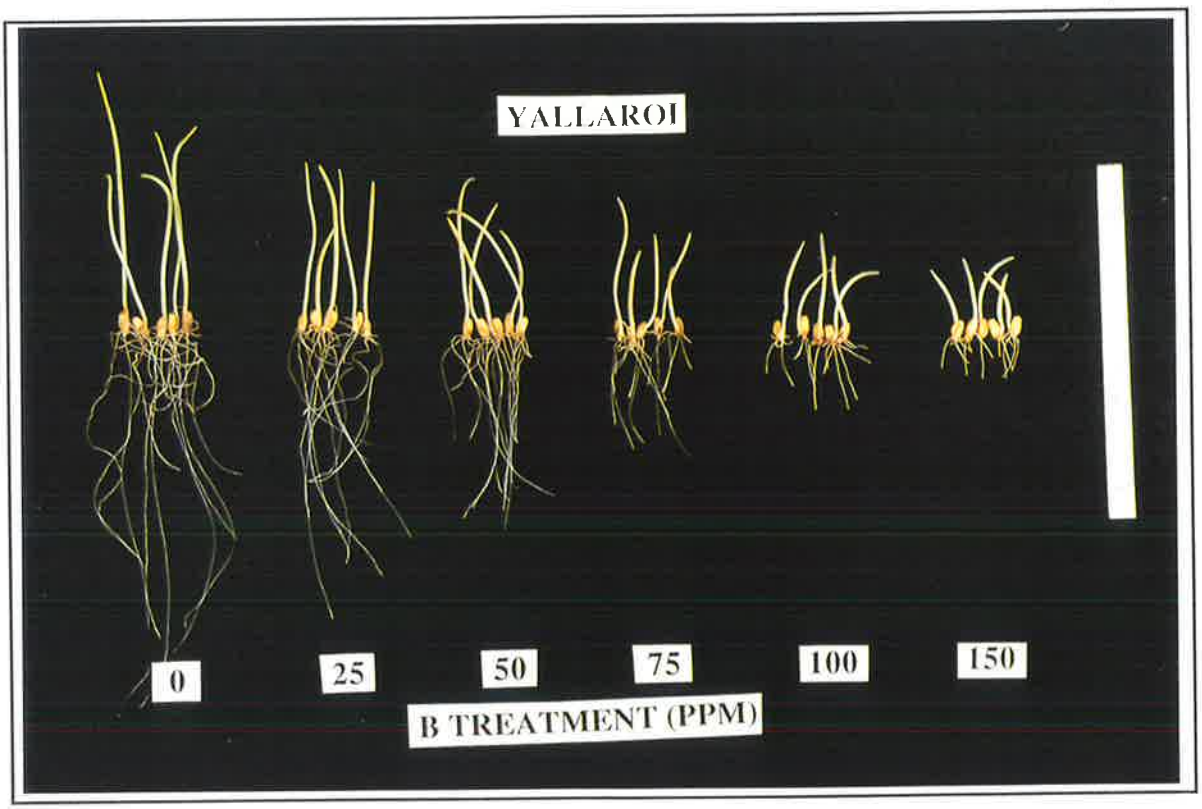
(b) Yallaroi

Treatments from left to right: B0, B25, B50, B75, B100 and B150.

(a)


AUS 10110
0
25
50
75
100
150
B TREATMENT (PPM)

(b)


YALLAROI
0
25
50
75
100
150
B TREATMENT (PPM)

genotypes, these results were consistent with preliminary field and glasshouse screening (A.J. Rathjen, unpublished; Brooks, 1991).

Correlations between parameters

There were highly significant correlations among the parameters in the glasshouse experiment in B60 and B80, namely, symptom expression, shoot B concentrations and dry matter yield (Table 3.4.6 and Figure 3.3). These parameters were also significantly correlated with root length at the B50, 75, 100 and B150 treatments in filter paper (Table 3.4.6 and Figure 3.4). The correlations between root length at B100 and shoot B concentrations at B60 and B80 were also high but not significant (-0.67 and -0.78, respectively, Table 3.4.6), probably because of the small number of genotypes tested.

Table 3.4.6 Correlation coefficients between root length of seedlings grown in filter paper and symptom expression, dry matter yield and shoot B concentration of plants grown in a glasshouse experiment for six durum genotypes (AUS 10110, AUS 10344, AUS 14010, AUS 10348, Langdon and Yallaroi).

Characters	Symptom[a]		B concentration		Dry matter yield	
	B60	B80	B60	B80	B60	B80
Glasshouse experiment						
Symptom (B60)			0.86*	0.96**	-0.89*	-0.98**
Symptom (B80)			0.79	0.92**	-0.85*	-0.95**
B concentration (B60)					-0.75	-0.78
B concentration (B80)					-0.85*	-0.95**
Filter paper experiment						
Root length (B50)	-0.90*	-0.91*	-0.73	-0.86*	0.77	0.92**
Root length (B75)	-0.85*	-0.89*	-0.66	-0.81*	0.79	0.90*
Root length (B100)	-0.87*	-0.88*	-0.67	-0.78	0.84*	0.88*
Root length (B150)	-0.85*	-0.84*	-0.72	-0.81*	0.77	0.87*

*, ** significant at P<0.05 and 0.01, respectively

[a] Data were the average of three leaves.

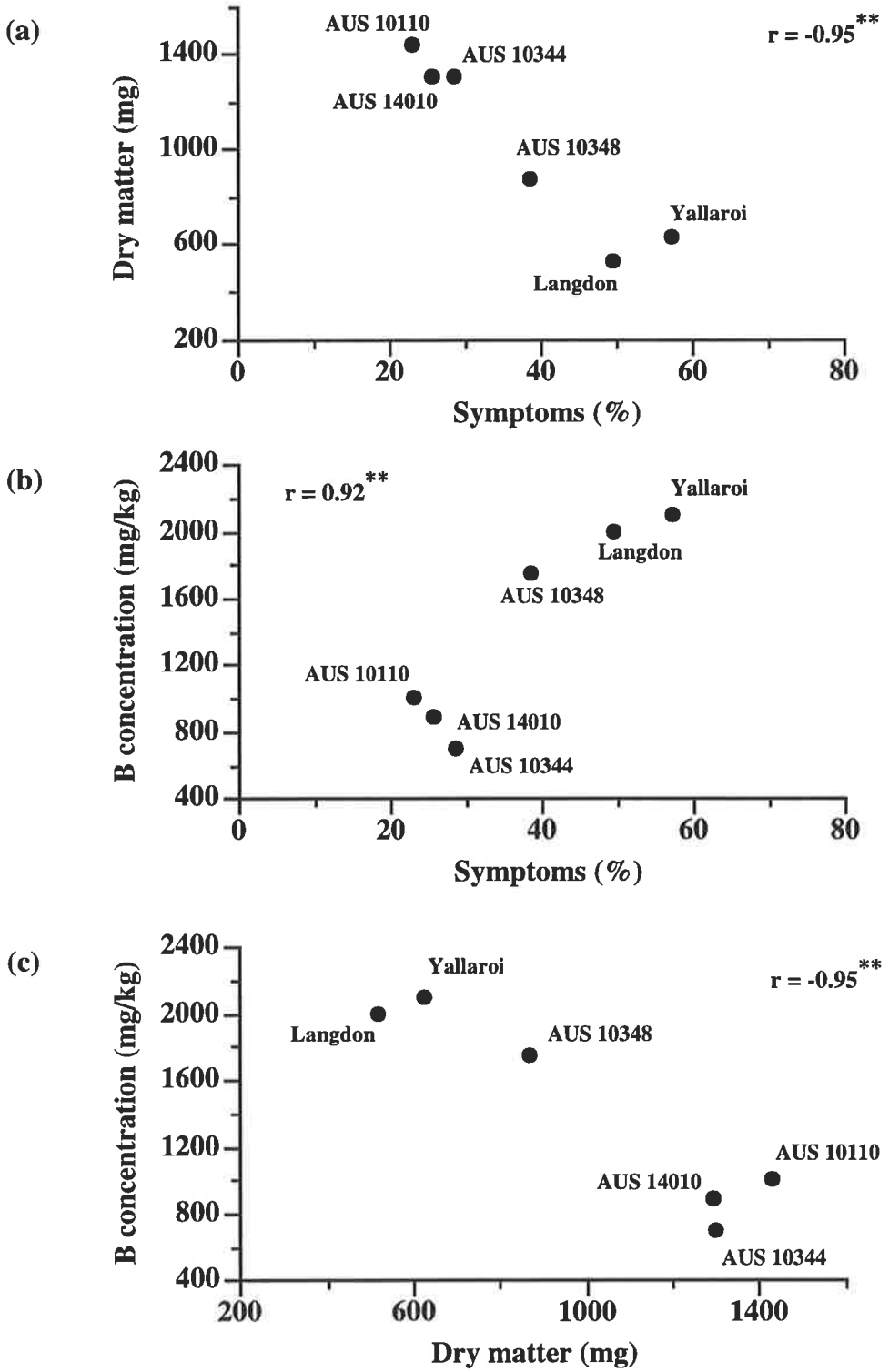


Figure 3.3 Relationships between symptoms of B toxicity, dry matter yield and shoot B concentrations for six durum genotypes grown in the B80 treatment of a glasshouse experiment.

(a) symptoms v dry matter, **(b)** symptoms v B concentration **(c)** dry matter v B concentration

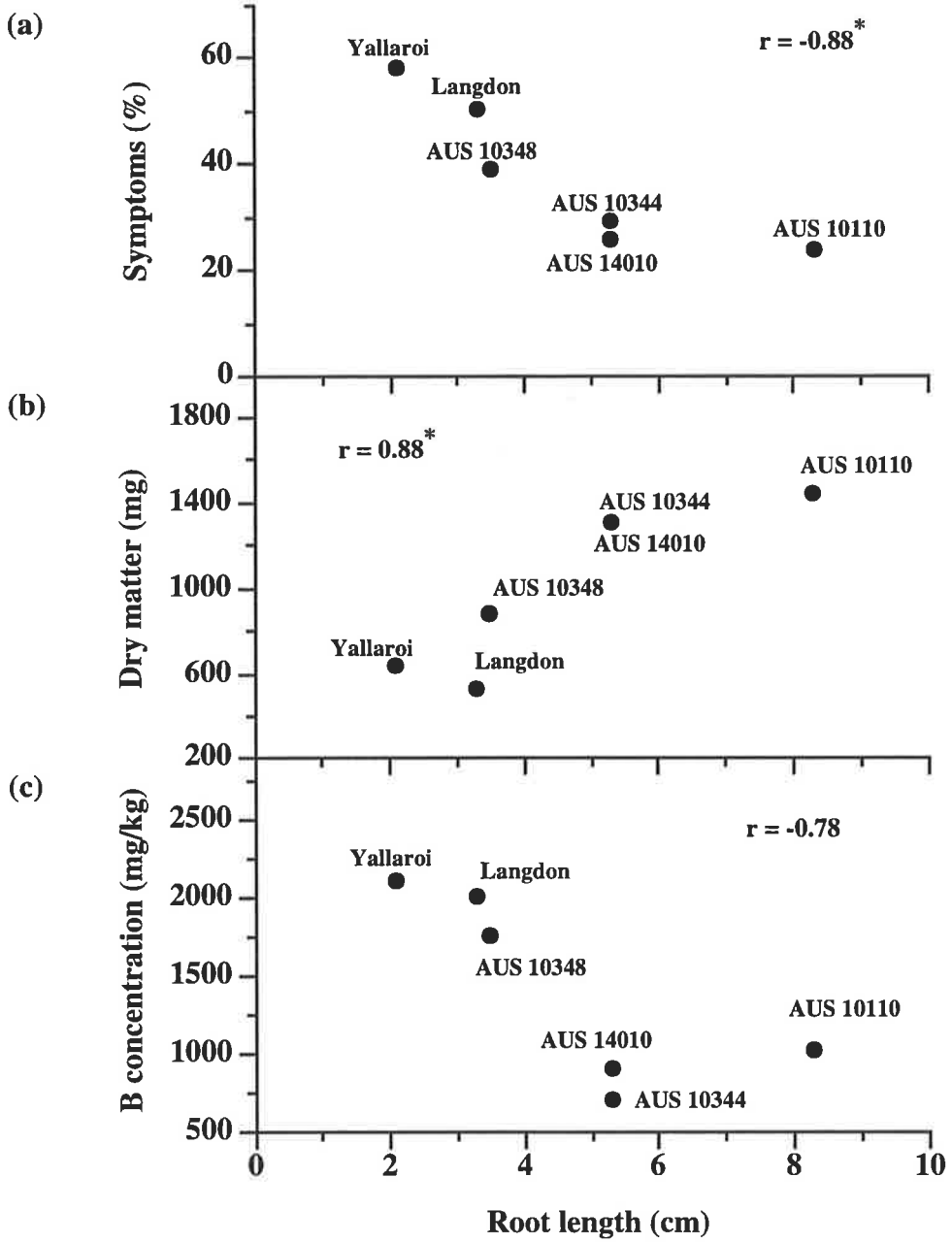


Figure 3.4 Relationships between root lengths for six durum genotypes when grown at B100 in filter paper and symptoms of B toxicity, dry matter yield and shoot B concentrations grown at B80 treatment in a glasshouse experiment.

(a) root length at B100 v symptoms at B80

(b) root length at B100 v dry matter at B80

(c) root length at B100 v shoot B concentration at B80

3.4.4 Discussion

In this study, durum wheat genotypes displayed variation in response to high concentrations of B, both in glasshouse and filter paper experiments. In the glasshouse experiment, tolerant genotypes displayed less severe symptoms of B toxicity, lower B concentrations in shoots and higher dry matter yields than the sensitive genotypes. Significant dry matter yield reductions associated with high tissue B concentrations have been reported for many crops, for example, bread wheat, barley and peas (Paull et al., 1988b, Bagheri et al., 1992). The expression of B tolerance of wheat, barley, peas and medics was reported to be controlled by exclusion of B from the plants (Nable et al., 1990a; Paull et al., 1992b). Since the results obtained with durum are consistent with those of other crops, a similar mechanism is likely to control B tolerance in durum. Based on symptom expression, shoot dry weight and concentration of B in shoots, the genotypes can be classified into two major groups: Tolerant: AUS 10110, AUS 10105, AUS 10344 and AUS 14010 and Sensitive: AUS 10348, Yallaroi and Langdon.

In the filter paper experiment, root growth also varied among genotypes, with tolerant genotypes developing longer roots than sensitive genotypes at high B treatments, although there was little difference between the genotypes in the control treatment. This result confirmed that root growth at high concentrations of B plays an important role in expression of B tolerance in durum wheat as found in bread wheat and barley (Nable, 1988; Nable et al., 1990a). The differences between genotypes were not attributed to internal seed B content (Nable and Paull, 1990) or endogeneous auxin in seed, but were proposed to be due to differences in permeability of root cell membranes (Huang and Graham, 1990).

The ranking of genotypes based on root growth was consistent with the results from the glasshouse experiment, with the exception of AUS 10110. For example, the correlation coefficients for root length at B75 and tissue B concentration at B80 when

AUS 10110 was included and excluded were -0.81 and -0.95, respectively (data not shown). This genotype had the longest roots at all levels of applied B in filter paper but had slightly higher shoot B concentrations compared to AUS 10344 and AUS 14010, when grown in the high B soils. Possible explanations could be as follows. Firstly, AUS 10110 has very large leaves compared to the other genotypes. Since this line has superior root growth at high levels of B it is likely to have greater transpiration and therefore to absorb and translocate more B from the soil, compared to other tolerant genotypes. Secondly, the filter paper method was a short term screening and the measurement only accounted for the effects of B on root elongation and cell division, while an experiment of longer duration in the glasshouse allowed plants to express differences in tolerance to B on shoot growth and nutrient uptake as well as root growth.

The reduced dry matter yield observed for plants grown in high B soils is likely to be the result of reduction in both root and shoot growth (Paull, 1985; Holloway and Alston, 1992). The results from this experiment also confirmed that genotypes that had greater root growth at high B in filter paper also produce higher dry matter yields in soil. Based on filter paper screening, genotypes were classified as; tolerant: AUS 10105 and AUS 10110, moderately tolerant: AUS 10344 and AUS 14010, moderately sensitive: Abyssinian 29, Wollaroi, AUS 10348, Capeiti, Langdon and Yallaroi. The classification of genotypes as tolerant, moderately tolerant, moderately sensitive was based on the result of Section 3.3, in which AUS 13244 was identified as sensitive and Yallaroi as moderately sensitive (Figure 3.1). Therefore, genotypes with a lower level of tolerance than AUS 10344 and AUS 14010 but similar to Yallaroi were classified as moderately sensitive.

3.5 Discussion

Solution techniques have been used widely in screening for tolerance to mineral toxicities and to elucidate the mechanisms and genetics of tolerance to stresses. Root growth measurement has been used successfully for screening for Al tolerance in bread wheat (e.g. Rengel and Jurkic, 1992). Konzak et al. (1976) used a filter paper solution method, and were able to classified wheat cultivars into four distinct levels of Al tolerance by root measurement and visual estimates of root growth.

Two methods, namely soil and filter paper screening, have been used for screening bread wheat for response to B. For screening with very sensitive genotypes such as Kenya Farmer, soil screening is advocated (Paull et al., 1990). The presence of the gene responsible for sensitivity to B in Kenya Farmer can be detected easily visually since this genotype produce a 'mid-leaf necrosis' even under low B treatments. Paull et al. (1993) located the gene controlling mid-leaf necrosis on the long arm of chromosome 4A and this trait can be used as a genetic marker for linkage analysis with other characters in bread wheat. For screening of lines ranked tolerant or very tolerant, the filter paper method was found to be more appropriate (Chantachume, 1995). To discriminate between tolerant and very tolerant genotypes, very high concentrations of soil B are required. In these conditions, some plants failed to establish within the rating time (5 to 7 weeks after planting), or showed no variation for symptom expression due to an interaction of B and other environmental factors. In durum wheat, filter paper screening is being proposed to be more appropriate than soil screening for genetic study of B tolerance for the following reasons:-

(1) none of genotypes screened in soils developed mid-leaf necrosis,

(2) there was a large degree of variation of plant type, development and maturity. For example, all tolerant genotypes identified were landraces and showed far greater variation in leaf size, plant height and maturity, compared with that found among the adapted varieties, and

(3) a very clear distinction between the genotypes classified of tolerant and sensitive was shown in root lengths. This ranking of genotypes was consistent to that found in soil.

A limited level of variation for B tolerance was found within commercial varieties or lines agronomically adapted to South Australian conditions such as those bred in New South Wales and CIMMYT varieties. Genotypes originating from the Mediterranean basin, Europe and Africa were also classified as sensitive to moderately sensitive to B, indicating that durums from these areas were probably grown in non-toxic soils or that the parental material had limited genetic variability. Sources of variation to B originated predominately from Asia, but only seven landraces were more tolerant than current Australian varieties. Similar findings for durum have also been observed for other characters such as micronutrient efficiency and disease resistance. For example in South Australia, where toxicity to B and sodium (Na), along with deficiencies of manganese (Mn), zinc (Zn) and copper (Cu) are problems for crop growth (Reuter et al., 1988), commercial durum varieties have been found to be sensitive to Mn deficiency and none of them showed a high level of efficiency as that found in bread wheat and rye varieties (Graham, 1984, 1988). Kaur et al. (1988) screened more than 1200 wheat genotypes and reported that only two durum lines, originating from India, were Mn efficient. Another example of the contrasting variation between bread and durum wheats in response to disease is provided by susceptibility to crown rot (*Fusarium graminearum* Group 1). Commercial durums were susceptible to crown rot while resistance was found in bread wheat varieties such as Gala (Dodman and Wildermuth, 1985) and Molen (Van Wyk et al., 1988). At present, no useful genetic variation for resistance to this disease has been identified in durum but an attempt to transfer resistance genes from a Chinese bread wheat to durum is being made in the CIMMYT breeding program (Rajaram et al., 1993). The results from the study reported here suggest that substantial genetic variability for B tolerance is available in landrace genotypes from Central and Eastern Asia and therefore sources of variability for other characters might also exist in the landrace populations from these areas.

Very significant differences between the tolerant and sensitive durum genotypes were shown by a number of characters such as shoot B concentration, dry matter production and severity of symptom expression. All of these characters showed a high correlation to root growth when grown under high B conditions. The significant difference in root growth suggested that, when grown in B-toxic soils, tolerant genotypes might develop deeper root systems than sensitive genotypes. This may be of considerable agronomic importance for arid and semi-arid conditions, where water stress often occurs during the early and late stages of crop growth. The restriction of root growth by high concentrations of B in soil (Holloway and Alston, 1992) means that plants will be prone to premature wilting due to water stress, followed by restricted nutrient uptake and susceptibility to diseases and pests. Root growth at an early stage of development also has major implications for persistence and growth of plants under mediterranean-type environments (Tuner and Begg, 1981). Therefore, genetic variation in root growth in response to B should be exploited to produce durums better adapted to B toxic soils and less susceptible to moisture stress.

The results from this study indicated that considerable genetic variation exists in exotic germplasm of durum and, based on the experience with bread wheat (Paull et al., 1991b; Moody et al., 1993), it should be possible to transfer tolerance to sensitive varieties. Nevertheless, these tolerant genotypes were not locally adapted and thus have poor grain yield potentials. The genetic control of the tolerance in relation to the Australian variety, Yallaroi, is investigated in the next chapter to devise efficient breeding and selection strategies.

Chapter 4

Genetic control of B tolerance in durum wheat

4.1 Introduction

A number of studies in wheat and barley showed that tolerance to high concentrations of B was controlled by several major genes, acting additively (Paull et al., 1991a; Chantachume, 1995; Jenkin, 1993). Since the results from screening durum (Chapter 3) demonstrated a comparable range of genetic variation to that of bread wheat, it was proposed that durum is likely to have the same genetic system for B tolerance as that found in bread wheat. In this chapter, a set of durum genotypes chosen on the basis of response to B were investigated for their number of genes controlling their response to high concentrations of B.

In the previous chapter, it was shown that B tolerance is expressed and can be measured by root length of seedlings grown in filter paper. The tested genotypes were generally ranked in the same order as found in the B enriched soil (Section 3.4). Thus, the filter paper method can be used to study large numbers of plants such as in segregating populations.

The response of parents with a range of tolerance and F_1, F_2 and F_3 generations were examined in a filter paper containing B solutions. The objectives of this study were to identify the mode of inheritance and number of genes involved in determining B tolerance in durum wheat. An understanding of the genetic control of durum wheat to high concentrations of B would facilitate breeding tolerant varieties.

4.2 General materials and methods

Genotypes and seed production

Genotypes were selected based on their root length when grown in filter paper (Section 3.3). They were classified as tolerant: T (AUS 10110 and AUS 10105), moderately tolerant: MT (AUS 10344 and AUS 14010), moderately sensitive: MS (AUS 10348 and Yallaroi) and sensitive: S (AUS 13244). A single seedling from each of these genotypes was transplanted into a potting mix and the seeds harvested from each plant were progeny tested (Figure 3.1). Seeds from this source were then used for crossing and were multiplied for use in further experiments. Name, country of origin, pedigree and response to B of each line is presented in Table 3.2.1 and 3.3.3 (Chapter 3).

The seven genotypes were crossed in all combinations including reciprocals. F_1 plants were grown in a potting mix to produce the F_2 generation. F_2 populations from all the combinations were tested for their response to B. F_3 populations were obtained from selected F_2 plants which had been tested for response to B and transplanted to normal potting mix. F_3 seed from crosses involving AUS 10105 (T) and AUS 13244 (S) were obtained only from unselected F_2 plants grown in the potting mix.

Screening Procedure

The response to B of parents, F_1, F_2 and F_3 populations was examined by the use of the filter paper method, as described in Chapter 3. The B concentrations were varied with generations and cross combinations, and are described in the materials and methods of individual sections in this chapter.

Genetic analysis

The response of F_1 hybrids was measured and compared to the parents by analysis of variance. As there was a limited number of seeds being tested in the F_1 generation, genetic analyses were performed in the F_2 and F_3 generations. Seedling root lengths of

the F_2 plants and F_2 derived F_3 families were measured and the distribution of the response was calculated. As the response to B was measured as a quantitative character, the number of genes controlling the response to B was estimated on the basis of the variance of the segregating populations compared to the expected variance of the one and two gene models (Chantachume, 1995). The expected variance was calculated from the variance components of the parents and the F_1 hybrids. The variance components of the segregating generations were partitioned in terms of an additive-dominance model (Mather and Jinks, 1977), described as follow;

$$V_{F2} = \frac{1}{2}D + \frac{1}{4}H + E \qquad \text{(Mather and Jinks (1977), page 45)}$$

$$V_{F3} = \frac{3}{4}D + \frac{3}{16}H + E \qquad \text{(Mather and Jinks (1977), page 58)}$$

where - V_{F2} and V_{F3} are the variances of F_2 and F_2 derived F_3 populations, respectively.

- D is the additive component of the variance, defined as d^2 for one gene segregation and $(d_a^2+d_b^2)$ for two gene segregation.

- H is the dominance component of the variance, defined as h^2 for one gene segregation and $(h_a^2+h_b^2)$ for two gene segregation.

- d is the departure from the mid-point (m) of the means of each homozygous genotype (AA and aa) (See Figure 4.1a).

- d_a is the departure of AA from the mid-point of AA and aa, and d_b is the departure of BB from the mid-point of BB and bb.

- h is the departure from the mid-point of the heterozygous genotypes (Aa) (See Figure 4.1a).

- h_a and h_b are the departures from the mid-point of the heterozygous genotypes AaBb, AaBB, AABb and aaBb and the homozygous intermediate genotypes aaBB and AAbb.

- E is the environmental variance.

The environmental variance was calculated as

$$E = \frac{1}{4}V_{P1} + \frac{1}{4}V_{P2} + \frac{1}{2}V_{F1}$$

where - V_{P1} and V_{P2} are the variances of the parents

- V_{F1} is the variance of the F_1 hybrid.

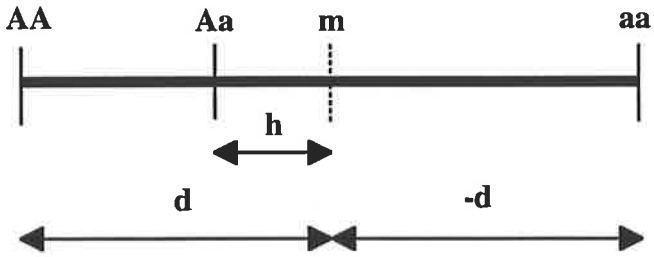


Figure 4.1a The d and h metrics of the allelic difference A-a. Deviations are measured from the mid-parent, m, midway between the two homozygous AA and aa. Aa may lie on either side of m and the sign of h will vary accordingly (Mather and Jinks, 1977).

One gene segregation

$$V_{F2} = \frac{1}{2}d^2 + \frac{1}{4}h^2 + E$$
$$V_{F3} = \frac{3}{4}d^2 + \frac{3}{16}h^2 + E$$

Two genes segregation

With the assumptions of no linkage and no epistasis, the estimations of the expected variances were

$$V_{F2} = \frac{1}{2}(d_a^2 + d_b^2) + \frac{1}{4}(h_a^2 + h_b^2) + E$$
$$V_{F3} = \frac{3}{4}(d_a^2 + d_b^2) + \frac{3}{16}(h_a^2 + h_b^2) + E$$

The confidence intervals of the observed variances for F_2 or F_3 populations were calculated as suggested by Dr D. G. Pederson (pers comm.), described as

$$(V_o \times df)/\chi^2_a \leq \text{Confidence interval} \leq (V_o \times df)/\chi^2_b$$

where - V_o is the observed variance of F_2 or F_3 populations

- df is the degrees of freedom of n-1
- n is the number of plants of an F_2 population or number of F_2 derived F_3 families
- χ^2_a and χ^2_b are the lower and upper level chi-square values at P = 0.95, df = n-1.

For both the F_2 and F_3 generations, populations were identified as deviating significantly from the expected variances for single or two genes models when the expected variance was outside the range of the confidence interval of the observed variance (D.G. Pederson, pers comm.).

F_2 derived F_3 populations

The results for the screening of F_2 derived F_3 families were interpreted in two steps.

- First, a comparison between the observed variance and the expected variance for one and two gene model was made.
- Second, families were classified into three categories (homozygous tolerant, segregating and homozygous sensitive) for the crosses that did not deviate from the single gene model, and four categories (homozygous tolerant, homozygous intermediate, segregating and homozygous sensitive) for the crosses that did not deviate from the two genes model. The observed frequency of each category was compared to the expected frequency by chi-square analysis.

For the classification of families, the mean and variance of individual families were compared to the confidence interval of the parental mean and the LSD of the parental variance, which were calculated as suggested by Dr D. G. Pederson (pers comm.). The confidence interval of each parental mean was calculated as

$$\text{confidence interval} = m \pm t\alpha_1 \times \sqrt{(V_P \times (\frac{1}{n_1} + \frac{1}{n_2}))}$$

where - m is the mean of the parent.

- n_1 is the number of plants within the family.

- n_2 is the number of plants of the parent which were tested.

- $t\alpha_1$ is the t-value at the probability of $0.05/n_2$ and degrees of freedom of $(n_1-1) + (n_2-1)$.

- V_P is the variance of the parent.

The LSD of the parental variances was calculated as

$$\text{LSD of the parental variance} = V_P \times F\alpha_1$$

where V_P is the variance of a parent.

$F\alpha_1$ is the F-value at the probability of $0.05/n_3$ and degrees of freedom of (n_1-1), $((n_2-1) + (n_3-1))$, where

n_1 is the number of plants within a family,

n_2 and n_3 are the number of plants within each of the two parents.

When the mean of a family was within the range of the confidence interval of either of the parents and the variance of the family was less than the LSD of the parental variance, the family was assigned as either homozygous sensitive or homozygous tolerant. When the mean of a family was higher than the sensitive but lower than the tolerant parent and had a variance less than LSD of the parental variance, the family was classified as homozygous intermediate. Families which had variance greater than those of the parents were classified as segregating (D.G. Pederson, pers comm.).

Following the classification of individual families, chi-square analysis was used for testing the goodness of fit of the observed segregation ratio to values expected for several models. F_2 derived F_3 populations were tested for the monogenic segregation ratio of 1 homozygous tolerant : 2 segregating : 1 homozygous sensitive and for the digenic segregation ratio of 1 homozygous tolerant : 2 homozygous intermediate : 12 segregating : 1 homozygous sensitive.

Chi-square analysis was not performed at the F_2 generation because response to B was expressed as a quantitative trait and there was no clear cut point for distinguishing between alternative categories.

4.3 Response of F_1 hybrids to high concentrations of B

4.3.1 Introduction

In Chapter 3, significant variation for the response to B between genotypes was found for several characters, namely, severity of leaf symptoms, dry matter production and tissue B concentrations when grown in soils containing high B. These characters showed a high correlation with root growth in filter paper containing high concentrations of B.

This experiment was conducted using a range of genotypes, including tolerant, moderately tolerant, moderately sensitive and sensitive genotypes and the F_1 hybrids derived from these genotypes. The objectives were to examine the response of F_1 hybrids derived from parents possessing different levels of B tolerance, and to identify the optimum level of B concentration for screening parents and their F_1 hybrids by using the filter paper screening method. The results were to be applied to the screening of segregating populations such as F_2 and F_3 generations. The response of the F_1 hybrids, relative to the parents, also provided information on the degree of dominance of the genes conferring tolerance to B.

4.3.2 Materials and methods

Genotypes

AUS 10105 (T), AUS 10110 (T), AUS 10344 (MT), AUS 14010 (MT), AUS 10348 (MS), Yallaroi (MS), AUS 13244 (S) and the F_1 hybrids from all combinations of these genotypes, including reciprocals, were tested. All parental genotypes, except AUS 13244, had been tested in filter paper and glasshouse experiments reported in Chapter 3. AUS 13244 was tested in a filter paper experiment and its response in high concentrations of B in soils was confirmed (data not shown).

Response of F_1 hybrids from T x MS and MT x MS to different concentrations of B

This experiment aimed at investigating the mode of gene action of B tolerance when tested at a number of concentrations of B. Parents and F_1 hybrids from the crosses AUS 10110 (T) x Yallaroi (MS) and AUS 10344 (MT) x Yallaroi (MS) were grown in filter paper soaked with four levels of applied B. The B treatments were 0, 50, 100 and 150 mgB L^{-1} designated as B0, B50, B100 and B150, respectively. B treatments and genotypes were arranged as a split plot design with three replications. Four seeds of each parental genotype (P_1 and P_2), F_1 hybrid (P_1xP_2) and reciprocal (P_2xP_1) were sown in each paper. The experiment was conducted for 12 days and the root length of each seedling was measured.

Response of F_1 hybrids derived from seven parents to high concentrations of B

Parental lines including AUS 10105 (T), AUS 10110 (T), AUS 10344 (MT), AUS 14010 (MT), AUS 10348 (MS), Yallaroi (MS) and AUS 13244 (S) were crossed in all combinations including reciprocals. Parents and F_1 hybrids were tested for their response to B by using the filter paper method.

Parents and F_1 hybrids were tested at B100. Crosses involving more tolerant combinations (AUS 10105, AUS 10110, AUS 10344, AUS 14010) were also tested at B150 and the more sensitive combinations (AUS 10348, Yallaroi and AUS 13244) in a B50 treatment. Four seeds of each parent, F_1 and reciprocal F_1 were sown on each paper. For each treatment, genotypes were arranged as a randomized completed block with two replications.

F_1 seeds of the crosses AUS 10105 x AUS 13244 and AUS 10110 x Yallaroi were not available. Only one replicate of the crosses AUS 10344 x AUS 14010, AUS 10110 x AUS 14010, AUS 10105 x AUS 14010 and AUS 10105 x AUS 10110 was tested in this experiment due to restricted seed supply.

At 12 days after sowing, the root lengths of F_1 hybrids were measured and compared to

parental lines, for each combination.

4.3.3 Results

Response of F_1 hybrids between T x MS and MT x MS to different concentrations of B

Parents and F_1 hybrids exhibited maximum root length at B0 and the differences between genotypes were not significant. Root lengths of both parents and F_1 hybrids were reduced with increasing concentrations of B and significant treatment x genotype interactions were observed ($P<0.01$) for both crosses (Table 4.3.1). The difference between F_1 hybrids and their parents at high concentrations of B was therefore not due to differences in inherent vigour of seedlings. At higher B treatments, the root lengths of the F_1 hybrids were intermediate to their parents and no significant differences between the reciprocal pairs were observed.

The response of F_1 hybrids varied according to the B treatment and parental combination. When expressed relative to the control, the F_1 hybrids trended from a tolerant to a sensitive response as the concentration of B increased (Figure 4.2).

At all levels of applied B, F_1 hybrids from the cross involving the more tolerant parent AUS 10110 (T), had longer roots than F_1 hybrids from AUS 10344 (MT) x Yallaroi (MS) (Figure 4.2). For example, the root lengths of the F_1 hybrids of AUS 10110 (T) x Yallaroi (MS) and AUS 101344 (MT) x Yallaroi (MS) at B100 were 50 and 37% of the B0 treatment, respectively.

Response of F_1 hybrids derived from seven parents to high concentrations of B

The range of tolerance to high concentrations of B was demonstrated for seven parents at B100 and B150, but no significant difference between the moderately sensitive and sensitive parents was found in the B50 treatment (Table 4.3.2). At B100, AUS 10105 (T) and AUS 10110 (T) were the most tolerant, and AUS 10344 (MT) and AUS 14010 (MT) were intermediate and more tolerant than AUS 10348 (MS) and Yallaroi (MS). AUS 13244 (S) had the shortest roots but was not significantly different from Yallaroi

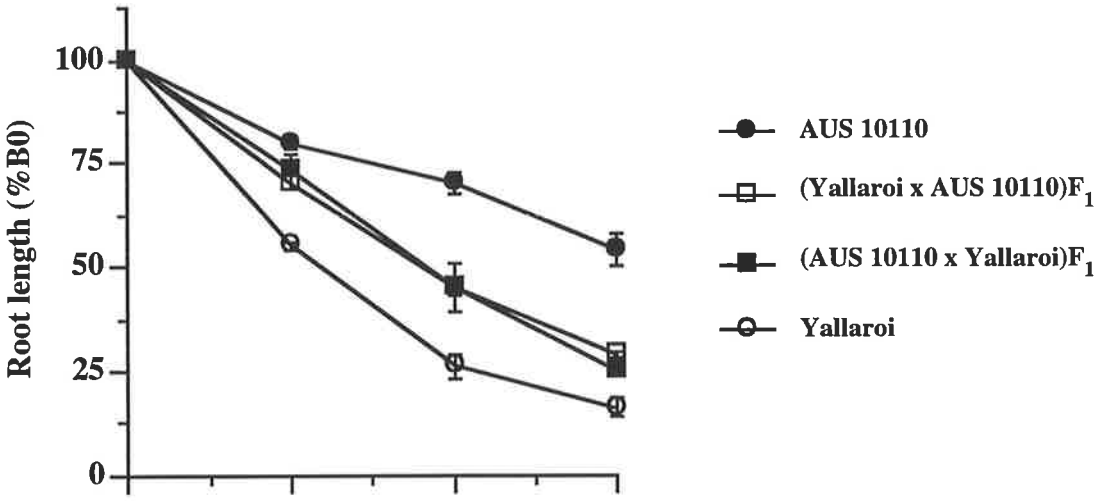
at either the B50 or B100 treatments.

Root lengths of F_1 hybrids for the crosses among the more sensitive parents (AUS 10348 (MS), Yallaroi (MS) and AUS 13244 (S)) tested at B50 and the more tolerant parents (AUS 10105 (T), AUS 10110 (T), AUS 10344 (MT) and AUS 14010 (MT)) tested at B100 were not significantly different from the two parents. At B100 and B150 most of the other F_1 hybrids were intermediate to the parents. No difference between reciprocal crosses was established.

Table 4.3.1 Mean root length (cm) for three genotypes and their F_1 hybrids, including reciprocals, grown in filter papers at four of B concentrations.

Parent/cross	Root length (cm)			
	B0	B50	B100	B150
AUS 10110 (T)	12.9	10.3	9.0	6.9
AUS 10110 x Yallaroi	13.0	9.1	5.8	3.7
Yallaroi x AUS 10110	12.1	8.9	5.4	3.0
Yallaroi (MS)	14.1	7.8	3.7	2.2
LSD interaction (P<0.05)			1.2	
(P<0.01)			1.5	
AUS 10344 (MT)	14.1	9.7	6.8	4.5
AUS 10344 x Yallaroi	14.0	7.8	5.0	2.8
Yallaroi x AUS 10344	13.8	7.9	4.0	2.2
Yallaroi (MS)	15.2	7.8	2.9	1.8
LSD interaction (P<0.05)			1.3	
(P<0.01)			1.9	

(a) AUS 10110 x Yallaroi



(b) AUS 10344 x Yallaroi

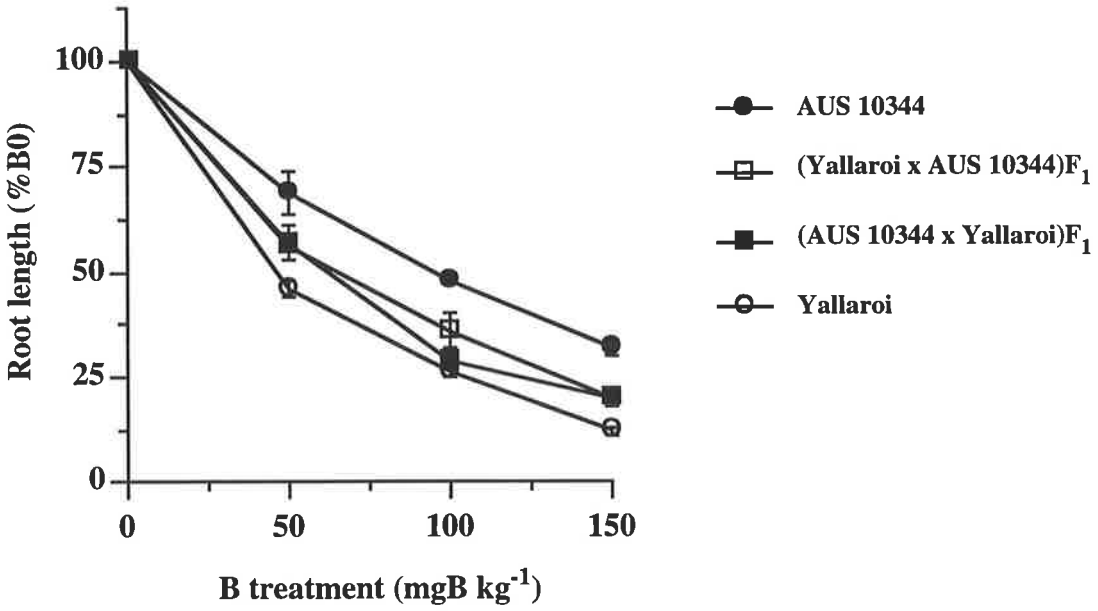


Figure 4.2 Mean root length (% of control) of parents and F_1 hybrids grown at four B concentrations. Vertical bars represent the standard error of the mean of three replicates.

(a) AUS 10110 (T) x Yallaroi (MS)

(b) AUS 10344 (MT) x Yallaroi (MS)

Table 4.3.2 Mean root length (cm) of parents (P_1 and P_2), F_1 hybrids (P_1xP_2) and reciprocal crosses (P_2xP_1) tested at B50, B100 and B150 treatments.

B	Cross		Mean root length (cm)				LSD
	P_1	P_2	P_1	P_2	P_1xP_2	P_2xP_1	(P<0.05)
150	AUS 10105 (T)	AUS 10110 (T)	7.6	7.2	7.8	7.7	ns[a]
		AUS 10344 (MT)	7.4	4.6	5.9	5.8	0.6
		AUS 14010 (MT)	7.5	4.2	5.3	5.8	-[b]
	AUS 10110 (T)	AUS 10344 (MT))	7.3	3.6	4.2	4.9	1.2
		AUS 14010 (MT)	7.5	3.5	5.9	5.2	2.0
100	AUS 10105 (T)	AUS 10110 (T)	9.0	10.2	8.9	9.8	-[b]
		AUS 10344 (MT)	7.8	5.8	6.7	6.8	ns
		AUS 14010 (MT)	9.7	6.6	8.5	8.6	-[b]
		AUS 10348 (MS)	8.4	4.8	6.9	7.6	2.7
		Yallaroi (MS)	8.5	3.3	7.4	6.9	1.2
	AUS 10110 (T)	AUS 10344 (MT)	8.5	6.8	7.2	6.5	ns
		AUS 14010 (MT)	8.4	6.5	7.4	7.9	-[b]
		AUS 10348 (MS)	9.2	5.1	7.9	7.1	2.6
		AUS 13244 (S)	8.4	3.3	6.0	5.4	1.9
	AUS 10344(MT)	AUS 14010 (MT)	6.6	6.6	6.8	6.6	-[b]
		AUS 10348 (MS)	7.9	4.6	5.1	5.7	2.2
		Yallaroi (MS)	7.3	3.7	5.6	6.0	2.2
		AUS 13244 (S)	7.1	2.6	5.6	5.9	1.5
	AUS 14010 (MT)	AUS 10348 (MS)	7.3	4.8	6.9	6.6	2.3
		Yallaroi (MS)	7.0	3.3	6.9	6.4	2.2
		AUS 13244 (S)	6.4	2.8	6.0	5.4	2.2
	AUS 10348 (MS)	Yallaroi (MS)	5.0	3.8	4.8	4.8	0.4
		AUS 13244 (S)	5.2	2.7	3.7	4.2	2.0
	Yallaroi (MS)	AUS 13244 (S)	3.7	3.2	3.8	3.8	ns
50	AUS 10348 (MS)	Yallaroi (MS)	9.4	7.8	9.5	8.2	ns
		AUS 13244 (S)	8.6	7.0	6.3	7.6	ns
	Yallaroi (MS)	AUS 13244 (S)	8.1	7.0	7.2	7.8	ns

[a] not significant at P<0.05, [b] data were obtained from one replication.

4.3.4 Discussion

The response of F_1 hybrids of durum wheat compared to their parental lines indicated that B tolerance measured by root length when grown in high concentrations of B is a heritable trait. Significant differences between F_1 and parental lines were shown for most of the crosses used in this study. At B0, no variation of root length was detected between F_1 hybrids and parents, hence the variation in root growth at high concentrations of B could not be attributed to the difference between seedling vigour of F_1 hybrids and parents.

There was no significant difference between any of reciprocal crosses at all levels of applied B, demonstrating that the inheritance of B tolerance in durum wheat is not cytoplasmically inherited.

The expression of tolerance to B was largely controlled by additive effects. The results from both experiments showed that at B100, most F_1 hybrids from the crosses derived from contrasting levels of tolerance, such as tolerant or moderately tolerant x moderately sensitive or sensitive, were intermediate to the two parents. This finding was also reported in bread wheat (Paull et al., 1988a) and peas (Bagheri, 1994). The concentrations of B in the shoots of F_1 hybrids derived from five bread wheat varieties, differing in their sensitivity to high concentrations of B in soils, were consistently intermediate to the parents (Paull et al., 1988a). However, the nature of the expression was dependent on the external concentration of B. As found in AUS 10110 (T) x Yallaroi (MS) (Figure 4.2), root lengths of F_1 hybrids were similar to the more tolerant parent (AUS 10110) at B50, intermediate to the two parents at B100 and similar to the sensitive parent (Yallaroi) at B150. This indicated that incomplete-dominant gene action was involved and the degree of dominance depended on external B concentrations. Such a response is consistent with the hypothesis of Knight (1973) that for a quantitative trait the response of an F_1 hybrid relative to its parents, will vary according to the environment conditions.

The existence of several levels of tolerance among the parental lines and the variation between F_1 hybrids from different cross combinations suggested that more than one gene was likely to be involved. The segregation of F_2 and F_3 progenies is examined in the next sections, to determine in more detail the genotypes of the parents. If these lines possess the same genes for tolerance, no segregation will be observed. On the other hand, if genotypes contain different genes for tolerance, segregation or transgressive segregation will be observed.

The results suggested that B100 was appropriate to screen progeny derived from tolerant or moderately tolerant x moderately sensitive or sensitive parents and B150 was appropriate to screen progeny from tolerant x moderately tolerant parents as these treatments provided the maximum discrimination between the parents. The filter paper screening could not distinguish the response of F_1 hybrids and parents from the moderately sensitive x sensitive cross Yallaroi (MS) x AUS 13244 (S) because of the similarity of the responses of Yallaroi (MS) and AUS 13244 (S), but significant variation between AUS 10348 (MS) and AUS 13244 (S) was found at the B100 treatment.

4.4 Response of F_2 and F_2 derived F_3 families derived from seven durum genotypes to high concentrations of B

4.4.1 Introduction

B tolerance of bread wheat was determined to be under simple genetic control (Paull et al., 1991a). A large range of response to B exists within Australian commercial cultivars of this species, thus enabling the rapid breeding of alternative tolerant varieties. For example, the development of a B tolerant variety, BT-Schomburgk, was achieved by backcrossing with Schomburgk, a moderately sensitive variety as the recurrent parent and Halberd, a moderately tolerant variety as the donor parent. A significant yield advantage of BT-Schomburgk over Schomburgk was evident when both were grown in B toxic soils (Moody et al., 1993).

In contrast to bread wheat, very little genetic variation in response to B occurred within commercial durum varieties (Chapter 3). The lines which have a potential to increase the level of B tolerance of Australian durum varieties were landraces which possess many undesirable characters and have a low yield potential. Therefore, transferring B tolerance from these lines into adapted genotypes would be require an extensive backcrossing program.

This section describes a study of the genetic control of B tolerance in durum wheat. The nature of inheritance was investigated and the genetic relationships between the exotic B tolerant lines and the Australian variety, Yallaroi, were also examined. An understanding of the genetic system in this species would increase the efficiency of breeding and selection for B tolerance.

The objectives of the experiments were to identify genetic relationships between genotypes with different levels of response to B and to determine the number of genes controlling B tolerance. Based on the results found in bread wheat, the genetic relationships among the genotypes used in this study were expected to be:-

(a) a difference of a single gene for the crosses between tolerant (T) x moderately tolerant (MT), moderately tolerant (MT) x moderately sensitive (MS) and moderately sensitive (MS) x sensitive (S) genotypes,

(b) a difference of two genes for the crosses between tolerant (T) x moderately sensitive (MS) and moderately tolerant (MT) x sensitive (S) genotypes.

4.4.2 Materials and methods

Genotypes

Seven parental lines (AUS 10110 (T), AUS 10105 (T), AUS 10344 (MT), AUS 14010 (MT), AUS 10348 (MS), Yallaroi (MS) and AUS 13244 (S)), the F_2 generation from all combinations and the F_3 generation from selected combinations were tested for their response to B. Since the results from F_1 hybrids showed no difference between reciprocals, F_2 seeds for each cross were obtained from the bulked seeds of the reciprocals. The F_3 derived lines were obtained from individual F_2 plants which had been tested at high B and transplanted into normal potting mix. F_3 derived lines from crosses involving AUS 10105 (T) and AUS 13244 (S) were obtained from individual unselected F_2 plants grown in a potting mix.

Screening procedure

Filter paper screening was used to study the response of the F_2 and F_3 generations to high concentrations of B. The B treatment was B150 for crosses among tolerant genotypes (AUS 10105 (T), AUS 10110 (T), AUS 10344 (MT) and AUS 14010 (MT)) and B100 for the other crosses.

The F_2 seeds were sown at a spacing of 2 cm between seeds and 11 F_2 seeds and two seeds of each parent per paper. A total of 12 papers, or 132 F_2 seeds plus 24 seeds of each of the parental lines, were sown for each combination. For the F_3 generation, the F_3 seeds and parental genotypes were sown with 13 seeds of each family or parental line per paper. About 60-100 F_3 families and 20-26 seeds of each parental genotype

were tested for each cross.

Identifying the number of genes controlling B tolerance

Root lengths of seedlings of the F_2 plants and F_3 families were measured and the observed variances were compared with the variances expected for one and two gene models in both generations as described in Section 4.2 and used by Chantachume (1995). Previous investigations (Chantachume, 1995) and the F_1 analysis (Section 4.3) indicated that B tolerance was controlled by additive effects and low levels of dominance effects were observed at B100 for most crosses or at B150 for tolerant x moderately tolerant combinations. Therefore, the expected variances were calculated from the following equations (described in Section 4.2) on the assumptions of no epistasis, no linkage and no dominance (h, h_a and $h_b = 0$, then the component H = 0).

One gene segregation

$$V_{F2} = \frac{1}{2}d^2 + E$$
$$V_{F3} = \frac{3}{4}d^2 + E$$

where - V_{F2} and V_{F3} are variances of F_2 and F_2 derived F_3 populations, respectively
- d is the departure from the mid-point (m) of the mean of each homozygous genotype (AA and aa).
- E is the environmental variance.

Since the results from Section 4.3 indicated that the F_1 hybrid was intermediate to the parents and F_1 hybrids were not tested in this screening, the variance of the F_1 was estimated from the average variance of the two parents ($V_{F1} = \frac{1}{2}(V_{P1} + V_{P2})$).
Therefore $E = \frac{1}{4}V_{P1} + \frac{1}{4}V_{P2} + \frac{1}{2}(\frac{1}{2}(V_{P1} + V_{P2}))$ or $= \frac{1}{2}V_{P1} + \frac{1}{2}V_{P2}$

Two genes segregation

$$V_{F2} = \frac{1}{2}(d_a^2 + d_b^2) + E$$
$$V_{F3} = \frac{3}{4}(d_a^2 + d_b^2) + E$$

If d is the departure from mid-point (m) of the homozygous genotypes AABB and aabb (Figure 4.1b), and $d_a = d_b = d/2$ then the variances of the F_2 and F_3 generations can be estimated as,

$$V_{F2} = \frac{1}{4}d^2 + E$$

$$V_{F3} = \frac{3}{8}d^2 + E$$

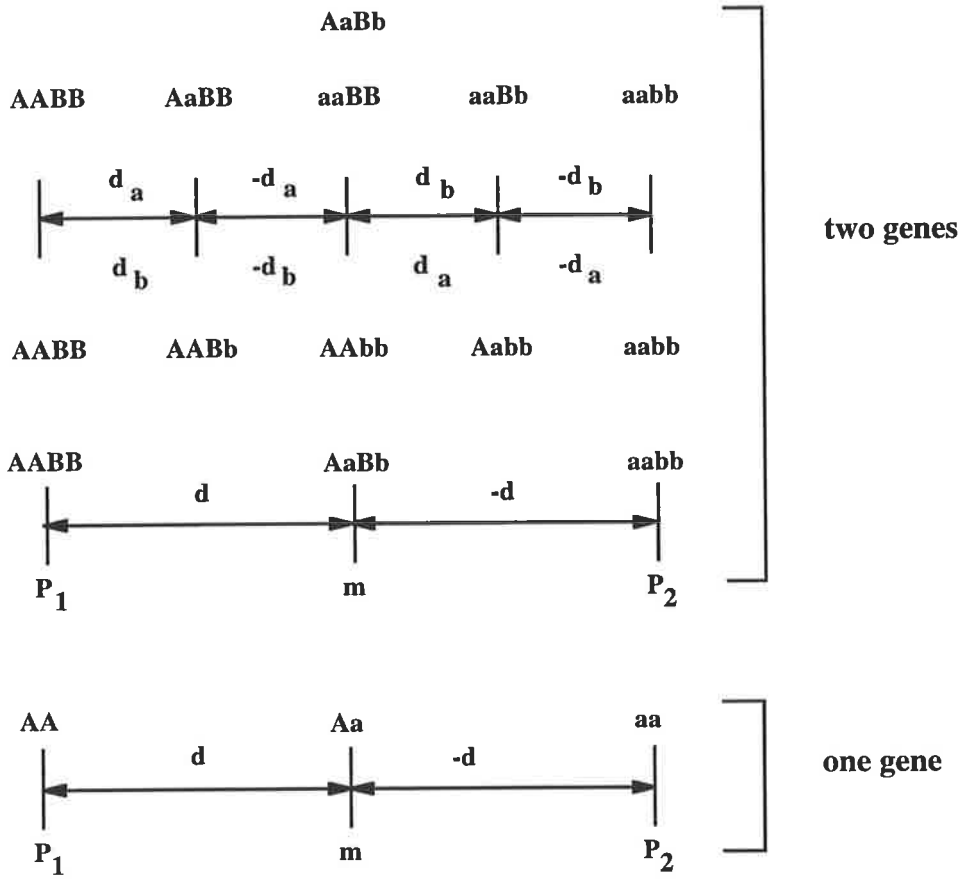


Figure 4.1b Diagram showing the definitions of genetic parameters used in this study. The parameter m is the mid-point (m) between parents (P_1 and P_2). Based on the assumption of no dominance, d is defined as either the departure of AA from the mid-point of AA and aa for a one gene model, or the departure of AABB from the mid-point between AABB and aabb for a two genes model. For the two genes model, d is also defined as (d_a+d_b) and if $d_a = d_b$ then d_a and $d_b = d/2$.

Relationship between F_2 and F_3

The relationship between the F_2 parents and F_2 derived F_3 progeny was analysed by

regression analysis using StatView® program version 2.1 for Macintosh computers.

4.4.3 Results

Response of F_2 populations to high concentrations of B

Parental lines and the F_2 of the 21 populations tested in this study showed a large range of response (Table 4.4.1). Parents were classified into four groups, tolerant: T (AUS 10105 and AUS 10110), moderately tolerant: MT (AUS 10344 and AUS 14010), moderately sensitive: MS (AUS 10348 and Yallaroi) and sensitive: S (AUS 13244). It was not possible to classify the F_2 plants into discrete categories for two reasons. First, continuous segregation was observed from crosses involving parents having contrasting levels of tolerance, such as tolerant (T) x moderately sensitive (MS) or moderately tolerant (MT) x sensitive (S) (Figure 4.3 and 4.4). Second, an overlap in response of the parental lines was found in the crosses involving lines with similar levels of response (Table 4.4.1, Figure 4.5c, 4.6a-c). Therefore, the estimation of the number of genes control B tolerance was assessed in the F_2 generation by the comparison of observed and expected variances and not by chi-square analysis.

Tolerant (T) x Moderately tolerant (MT)

The observed variance of the F_2 was in agreement with the variance expected for segregation at a single gene for the crosses, AUS 10105 (T) x AUS 10344 (MT), AUS 10105 (T) x AUS 14010 (MT) and AUS 10110 (T) x AUS 10344 (MT). The observed variance of AUS 10110 (T) x AUS 14010 (MT) was higher than the range expected for both the one and two gene models (Table 4.4.2).

Tolerant (T) x Moderately sensitive (MS)

The observed variances of AUS 10110 (T) x AUS 10348 (MS) and AUS 10110 (T) x Yallaroi (MS) did not deviate significantly from the two genes model, but the variance of AUS 10105 (T) x AUS 10348 (MS) fitted the single gene model. The observed variance of AUS 10105 (T) x Yallaroi (MS) was higher than the expected variances for

segregation at both one and two genes (Table 4.4.2).

Tolerant (T) x Sensitive (S)

The crosses between T x S lines (AUS 10110 (T) x AUS 13244 (S) and AUS 10105 (T) x AUS 13244 (S)) segregated with a larger range than that found in T x MS crosses (Figure 4.3a and 4.4a). This suggested that it is likely that these parents differed for more than two genes. The comparison of observed and expected variances was inconclusive due to the limited population size tested; a larger population would be required to compare observed and expected variances for segregation at two and three genes.

Moderately tolerant (MT) x Moderately sensitive (MS)

The observed variances from AUS 14010 (MT) x AUS 10348 (MS) and AUS 14010 (MT) x Yallaroi (MS) were not significantly different from the expected variance for a single gene model. The observed variance of AUS 10344 (MT) x AUS 10348 (MS) was significantly higher than the expected variances for both one and two gene models and this was likely to be the result of transgressive segregation (Figure 4.5c). The F_2 variance of AUS 10344 (MT) x Yallaroi (MS) fitted a two gene model (Table 4.4.2), but there is reason to believe that this observation was influenced by the inconsistent behavior of AUS 10344 (MT). In this cross, AUS 10344 (MT) expressed a mean root length of 8.6 cm compared with a range of 5-7 cm when tested with other crosses under the same condition. This unusually high value for the root length resulted in a high value for d in this cross and hence influenced the expected variances for the one and two gene models.

Moderately tolerant (MT) x Sensitive (S)

The observed variance of AUS 10344 (MT) x AUS 13244 (S) was consistent with a two genes model but the variance of AUS 14010 (MT) x AUS 13244 (S) was consistent with a one gene model (Table 4.4.2).

Moderately sensitive (MS) x Sensitive (S)

It was not possible to classify the segregants from the crosses between moderately sensitive and sensitive parents, due to overlap of the parental lines. The expected variances for both one and two gene models were within the range of the confidence interval of observed F_2 variances for AUS 10348 (MS) x AUS 13244 (S) and Yallaroi (MS) x AUS 13244 (S) (Table 4.4.2).

Crosses between lines of the same level of tolerance to B

The segregation patterns (Figure 4.6) and comparison of variances for the cross AUS 10105 (T) x AUS 10110 (T) (Table 4.4.2) was consistent with the expectation of no segregation. Transgressive segregation was observed in the crosses AUS 10348 (MS) x Yallaroi (MS) and AUS 10344 (MT) x AUS 14010 (MT) (Figure 4.6a and 4.6b, respectively).

Table 4.4.1 Mean root length (cm), standard deviation and number of plants of parents and the F_2 generation grown in filter paper containing 100 or 150 mgB L^{-1}.

Cross		B	P_1			P_2			F_2		
P_1	P_2		mean	SD[a]	no[b]	mean	SD	no	mean	SD	no
AUS 10105 (T)	AUS 10110 (T)	100	7.5	1.1	17	7.9	0.8	22	7.9	1.1	119
	AUS 10344 (MT)	150	6.6	1.1	22	3.5	0.7	20	4.8	1.4	132
	AUS 14010 (MT)	150	6.3	1.0	20	3.2	0.8	23	5.1	1.4	128
	AUS 10348 (MS)	100	9.7	1.5	20	3.6	0.8	22	7.2	2.5	132
	Yallaroi (MS)	100	8.0	1.0	20	2.7	0.8	23	5.4	2.6	127
	AUS 13244 (S)	100	8.0	1.0	22	1.8	0.6	19	6.2	2.2	131
AUS 10110 (T)	AUS 10344 (MT)	150	6.1	1.2	22	3.8	0.5	24	5.5	1.5	131
	AUS 14010 (MT)	150	6.3	0.8	19	3.3	0.4	19	5.3	1.9	131
	AUS 10348 (MS)	100	7.9	0.9	24	3.3	0.9	23	6.1	1.6	132
	Yallaroi (MS)	100	7.3	0.6	22	2.7	0.6	23	4.1	1.5	127
	AUS 13244 (S)	100	7.8	1.3	22	2.0	0.5	19	5.9	2.0	131
AUS 10344 (MT)	AUS 14010 (MT)	100	5.1	0.5	19	5.0	0.7	20	4.5	1.3	128
	AUS 10348 (MS)	100	6.1	0.9	24	3.0	0.8	22	5.7	1.8	132
	Yallaroi (MS)	100	8.6	1.1	24	2.9	0.9	24	6.2	1.7	131
	AUS 13244 (S)	100	6.7	0.9	24	1.5	0.5	20	4.7	1.6	131
AUS 14010 (MT)	AUS 10348 (MS)	100	7.9	1.1	24	3.8	0.8	19	5.3	2.0	115
	Yallaroi (MS)	100	4.5	0.8	23	2.6	0.9	23	3.5	1.2	117
	AUS 13244 (S)	100	7.4	1.0	23	1.8	0.6	15	4.7	1.9	127
AUS 10348 (MS)	Yallaroi (MS)	100	3.4	0.7	24	2.7	0.8	21	4.0	2.1	130
	AUS 13244 (S)	100	3.9	0.9	19	2.4	0.6	23	4.0	0.8	119
Yallaroi (MS)	AUS 13244 (S)	100	3.4	0.9	24	2.2	0.6	21	3.5	0.9	130

[a] SD = Standard deviation of mean, [b] no = number of plants tested

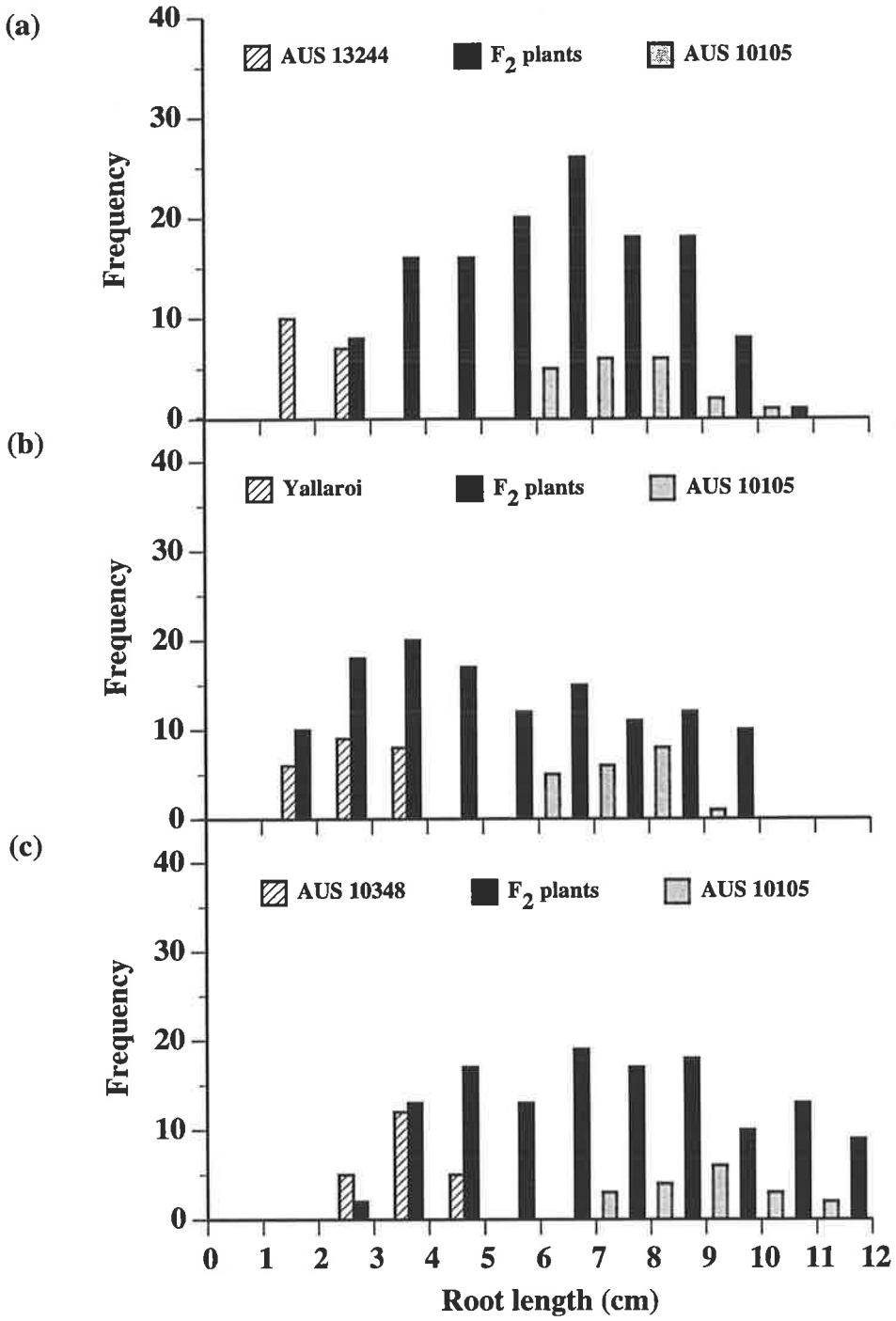


Figure 4.3 Response of parents and F_2 plants grown at the B100 treatment for the crosses involving the tolerant line AUS 10105 (T) with sensitive (S) or moderately sensitive (MS) lines;

(a) AUS 10105 (T) x AUS 13244 (S); mean (SD in parentheses) for AUS 10105 and AUS 13244 were 8.0 (1.0) and 1.8 (0.5), respectively.

(b) AUS 10105 (T) x Yallaroi (MS); mean (SD in parentheses) for AUS 10105 and Yallaroi were 8.0 (1.0) and 2.7 (0.8), respectively.

(c) AUS 10105 (T) x AUS 10348 (MS); mean (SD in parentheses) for AUS 10105 and AUS 10348 were 9.7 (1.5) and 3.6 (0.8), respectively.

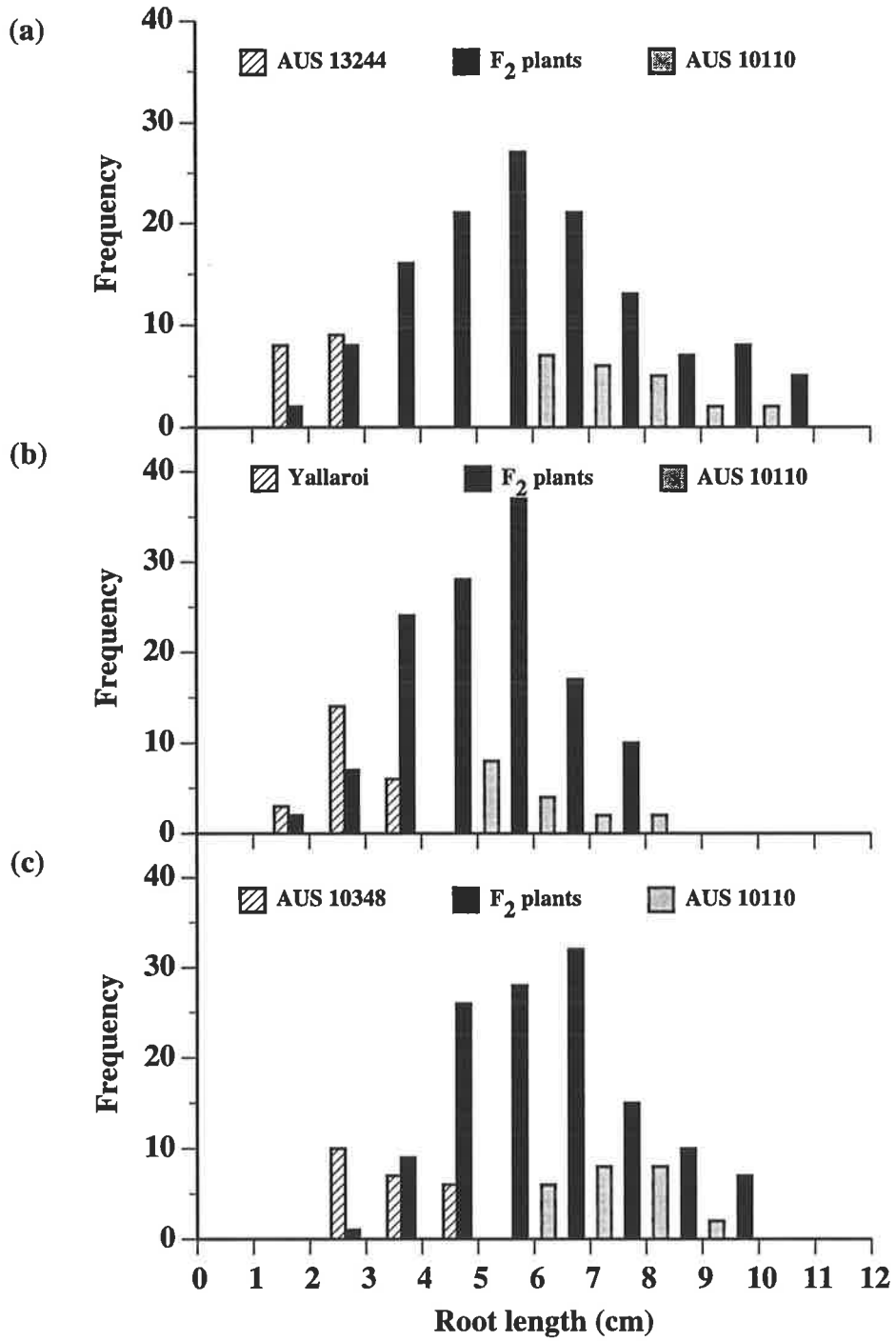


Figure 4.4 Response of parents and F_2 plants grown at the B100 treatment for the crosses involving the tolerant line AUS 10110 (T) with sensitive (S) or moderately sensitive (MS) lines;

(a) AUS 10110 (T) x AUS 13244 (S); mean (SD in parentheses) for AUS 10110 and AUS 13244 were 7.8 (1.3) and 2.0 (0.5), respectively.

(b) AUS 10110 (T) x Yallaroi (MS); mean (SD in parentheses) for AUS 10110 and Yallaroi were 7.3 (0.6) and 2.7 (0.6), respectively.

(c) AUS 10110 (T) x AUS 10348 (MS); mean (SD in parentheses) for AUS 10110 and AUS 10348 were 7.9 (0.9) and 3.3 (0.9), respectively.

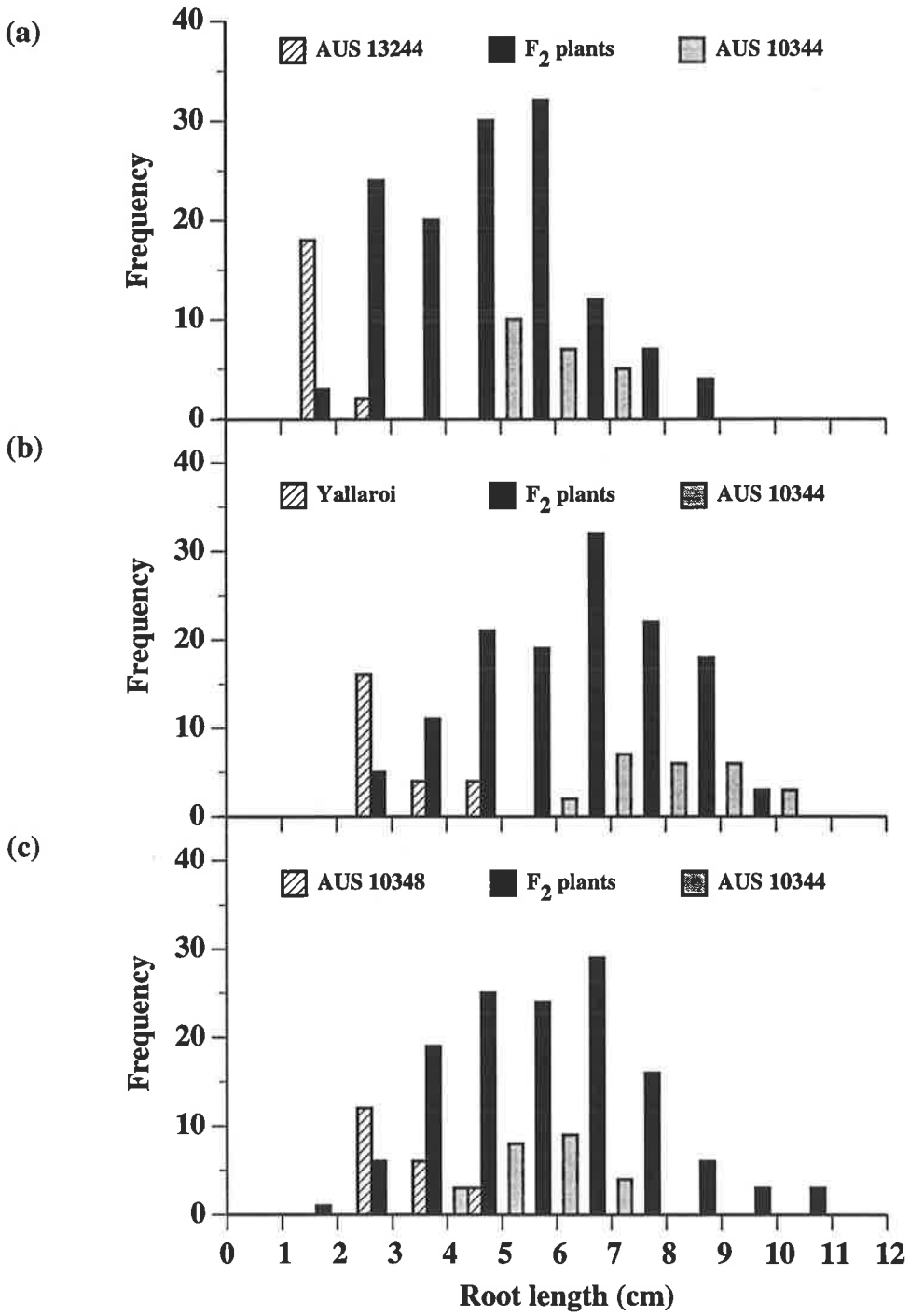


Figure 4.5 Response of parents and F_2 plants grown at the B100 treatment for the crosses involving the moderately tolerant line AUS 10344 (MT) with sensitive (S) or moderately sensitive (MS) lines;

(a) AUS 10344 (MT)x AUS 13244 (S); mean (SD in parentheses) for AUS 10344 and AUS 13244 were 6.2 (1.0) and 1.5 (0.5), respectively.

(b) AUS 10344 (MT) x Yallaroi (MS); mean (SD in parentheses) for AUS 10344 and Yallaroi were 8.6 (1.1) and 2.9 (0.9), respectively.

(c) AUS 10344 (MT) x AUS 10348 (MS); mean (SD in parentheses) for AUS 10344 and AUS 10348 were 6.1 (0.9) and 3.1 (0.8), respectively.

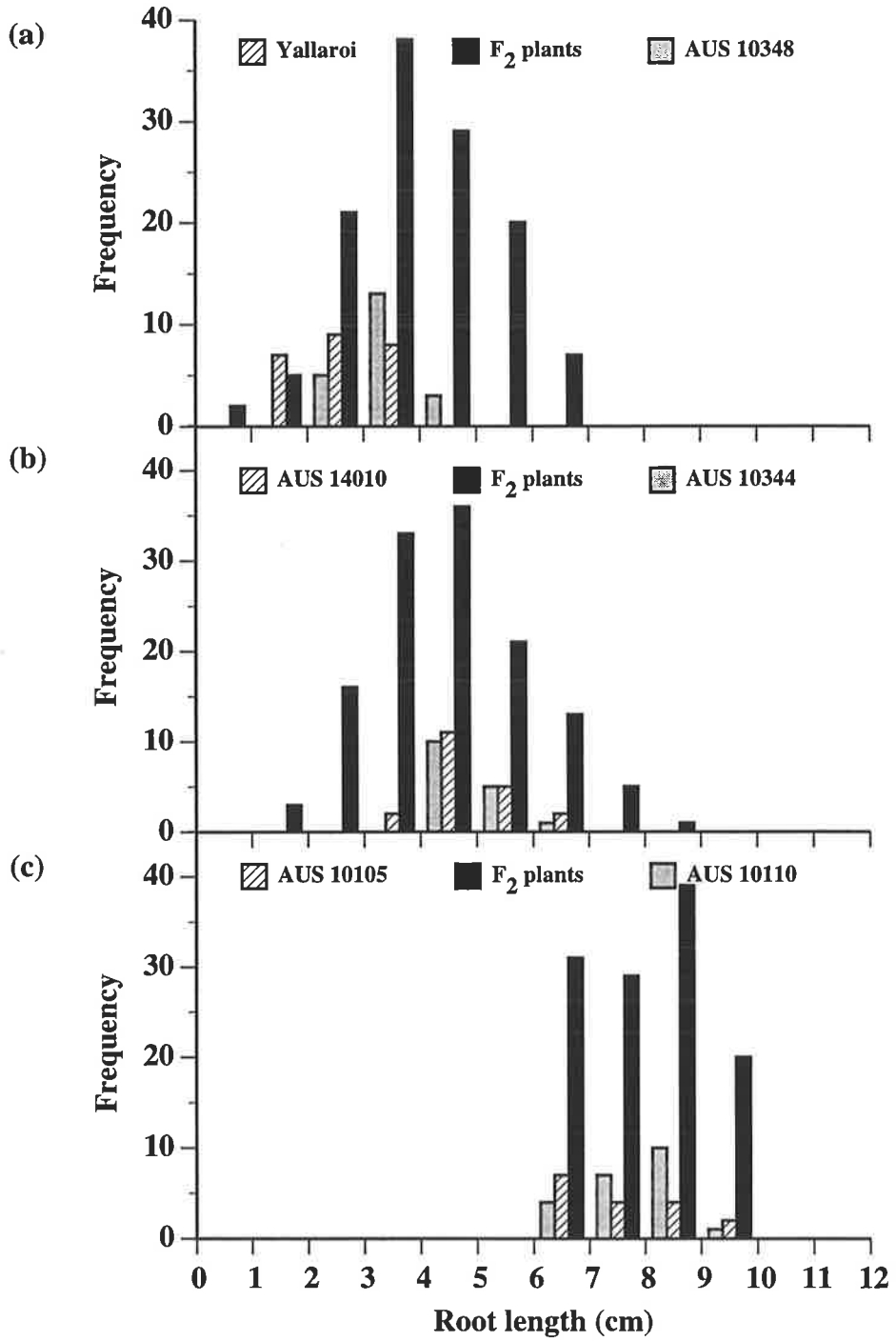


Figure 4.6 Response of parents and F_2 plants grown at B100 treatment for the crosses between lines of the same level of tolerance to B;

(a) AUS 10348 (MS) x Yallaroi (MS); mean (SD in parentheses) for AUS 10348 and Yallaroi were 3.4 (0.7) and 2.7 (0.8), respectively.

(b) AUS 10344 (MT) x AUS 14010 (MT); mean (SD in parentheses) for AUS 10344 and AUS 14010 were 5.1 (0.5) and 5.0 (0.7), respectively.

(c) AUS 10110 (T) x AUS 10105 (T); mean (SD in parentheses) for AUS 10110 and AUS 10105 were 7.9 (0.8) and 7.5 (1.1), respectively.

Table 4.4.2 Observed variances of parents and F_2 populations and estimated parameters and variances expected of the F_2 generation for segregation at one or two genes when grown in filter paper containing high concentrations of B. CI is the confidence interval of the observed variance of the F_2 populations.

Cross		Observed variance				Estimated parameters[a]			Expected variance	
P_1	P_2	V_{P1}	V_{P2}	V_{F2}	CI	E	m	d	1 gene	2 genes
AUS 10105 (T)	AUS 10110 (T)	0.7	1.2	1.2	1.5-0.9	0.9	7.7	0.2	0.9	0.9
	AUS 10344 (MT)	1.1	0.5	1.9	2.5-1.5	0.8	5.0	1.5	1.9	1.4
	AUS 14010 (MT)	1.0	0.6	2.0	2.7-1.6	0.8	4.8	1.5	2.0	1.4
	AUS 10348 (MS)	2.3	0.6	6.0	7.7-4.8	1.5	6.6	3.1	6.2	3.8
	Yallaroi (MS)	1.0	0.6	6.7	8.9-5.4	0.8	5.3	2.6	4.3	2.5
	AUS 13244 (S)	1.0	0.3	4.0	5.1-3.2	0.6	4.9	3.1	5.4	3.0
AUS 10110 (T)	AUS 10344 (MT)	1.5	0.2	2.1	2.8-1.7	0.9	5.2	1.4	1.9	1.4
	AUS 14010 (MT)	0.6	0.2	3.6	4.8-2.9	0.4	4.8	1.5	1.5	0.9
	AUS 10348 (MS)	0.9	0.8	2.5	3.3-2.0	0.8	5.6	2.3	3.4	2.1
	Yallaroi (MS)	0.4	0.3	1.7	2.3-1.4	0.4	5.0	2.3	3.0	1.7
	AUS 13244 (S)	1.6	0.2	4.2	5.6-3.4	0.9	4.9	2.9	5.3	3.1
AUS 10344 (MT)	AUS 14010 (MT)	0.3	0.5	1.8	2.3-1.5	0.4	5.0	0.1	0.6	0.4
	AUS 10348 (MS)	0.9	0.6	3.3	4.4-2.6	0.7	4.6	1.5	1.9	1.3
	Yallaroi (MS)	1.2	0.8	2.8	3.8-2.3	1.0	5.8	2.8	4.9	3.0
	AUS 13244 (S)	0.8	0.2	2.6	3.4-2.1	0.5	4.1	2.6	3.9	2.2
AUS 14010 (MT)	AUS 10348 (MS)	1.2	0.5	3.8	4.2-2.6	0.8	5.8	2.0	2.9	1.9
	Yallaroi (MS)	0.7	0.8	1.4	1.8-1.1	0.7	3.6	0.9	1.2	0.9
	AUS 13244 (S)	0.9	0.4	3.8	5.0-3.0	0.6	4.6	2.8	4.5	2.6
AUS 10348 (MS)	Yallaroi (MS)	0.4	0.7	1.7	2.2-1.3	0.6	3.1	0.4	0.7	0.6
	AUS 13244 (S)	0.8	0.3	0.7	0.9-0.5	0.5	3.1	0.7	0.8	0.7
Yallaroi (MS)	AUS 13244 (S)	0.9	0.3	0.9	1.2-0.7	0.6	2.8	0.6	0.8	0.7

[a] E = environmental variance, m = mid-point between parents,
d = departure of parents from the mid-point

Response of the F_3 generation to high concentrations of B

Single gene segregation

The comparison of observed and expected variances of F_3 populations were consistent with segregation at a single gene for the crosses between T x MT (AUS 10105 (T) x AUS 10344 (MT), AUS 10105 (T) x AUS 14010 (MT), AUS 10110 (T) x AUS 10344 (MT) and AUS 10110 (T) x AUS 14010 (MT)), MT x MS (AUS 10344 (MT) x Yallaroi (MS), AUS 14010 (MT) x AUS 10348 (MS) and AUS 14010 (MT) x Yallaroi (MS)) and MS x S (AUS 10348 (MS) x AUS 13244 (S)) lines (Table 4.4.3). Chi-square analysis based on the classification of F_2 derived families according to the response of individual plants within the families (Table 4.4.4, Figure 4.7 and Plate 4.1) was also consistent with these results (Table 4.4.5).

Two genes segregation

The observed variances of the F_3 generation were not significantly different from the expected variances for a two genes model for the crosses between T x MS (AUS 10105 (T) x AUS 10348 (MS), AUS 10105 (T) x Yallaroi (MS) and AUS 10110 (T) x Yallaroi (MS)) and MT x S (AUS 10344 (MT) x AUS 13244 (S)) lines. The confidence intervals of the variances of the F_3 for the crosses AUS 10110 (T) x AUS 10348 (MS) and AUS 14010 (MT) x AUS 13244 (S) were in between the variances expected for one gene and two genes models but were closer to the two than the one gene model (Table 4.4.3). Chi-square analyses also support two genes segregation in these crosses (Table 4.4.6, Figure 4.8 and Plate 4.2).

Transgressive segregations

The response of segregation in the F_3 generation confirmed transgressive segregation observed in the F_2 for the MT x MT (AUS 10344 x AUS 14010), MT x MS (AUS 10344 (MT) x AUS 10348 (MS)) and MS x MS (AUS 10348 x Yallaroi) crosses (Table 4.4.3 and 4.4.4). This suggested a different complement of genes controlling

response to B between the parental lines involved in these crosses. Further study was undertaken for the first two crosses. The families with the longest and the shortest roots were selected to develop test cross populations. The development of these populations and their results are described in Section 4.5.

Response of F_2 derived F_3 families v F_2 parent

Mean root lengths for each F_2 derived F_3 family were plotted against the root length of the F_2 parents (Table 4.4.7 and Figure 4.9). The slopes of the fitted lines ranged between 0.30 to 0.76 and r^2 ranged from 0.10 to 0.52. The relationship of parent and offspring was lowest for the cross AUS 10110 (T) x AUS 10344 (MT) and highest for the cross AUS 10110 (T) x AUS 10348 (MS). In general, the regression coefficient was between 0.30 to 0.49 for the crosses proposed to segregate at a single gene (except for AUS 10110 x AUS 10344), and 0.61 to 0.76 for the crosses proposed to segregate at two genes.

Table 4.4.3 Observed variances of parents and F_2 derived F_3 populations and estimated parameters and expected variances of the F_3 when grown in filter paper containing high concentrations of B. CI is the confidence interval of the observed variance of the F_3.

Cross		Observed variance				Estimated parameters[a]			Expected variance	
P_1	P_2	V_{P1}	V_{P2}	V_{F3}	CI	E	m	d	1 gene	2 genes
AUS 10105 (T)	AUS 10344 (MT)	0.5	0.5	1.7	2.2-1.3	0.5	5.0	1.2	1.7	1.1
	AUS 14010 (MT)	0.4	0.7	1.3	1.7-1.0	0.5	4.3	1.2	1.7	1.1
	AUS 10348 (MS)	0.3	0.3	3.6	4.8-2.9	0.3	6.5	2.7	6.0	3.1
	Yallaroi (MS)	0.4	0.5	3.5	4.3-2.7	0.4	6.6	3.1	7.6	4.0
AUS 10110 (T)	AUS 10344 (MT)	0.8	0.7	1.9	2.4-1.5	0.8	4.5	1.4	2.1	1.4
	AUS 14010 (MT)	0.8	0.9	2.0	2.6-1.6	0.9	4.5	1.3	2.2	1.5
	AUS 10348 (MS)	0.9	0.4	3.1	4.5-2.8	0.6	5.4	2.3	4.8	2.7
	Yallaroi (MS)	2.3	1.0	5.0	6.5-3.8	1.6	6.5	3.3	9.9	5.8
AUS 10344 (MT)	AUS 14010 (MT)	0.5	0.7	2.2	2.9-1.7	0.6	7.9	0.5	0.8	0.7
	AUS 10348 (MS)	0.5	0.4	3.8	5.0-3.1	0.5	4.6	0.9	1.0	0.8
	Yallaroi (MS)	0.7	0.7	2.3	3.1-1.9	0.7	4.9	1.7	2.9	1.8
	AUS 13244 (S)	0.5	0.3	2.4	3.0-1.6	0.4	6.0	2.2	4.1	2.2
AUS 14010 (MT)	AUS 10348 (MS)	0.6	0.3	2.8	3.7-2.2	0.5	6.4	1.6	2.5	1.5
	Yallaroi (MS)	0.4	0.4	2.5	3.3-2.0	0.4	4.1	1.7	2.6	1.5
	AUS 13244 (S)	0.7	0.3	2.6	3.4-2.0	0.5	5.2	2.0	3.7	2.1
AUS 10348 (MS)	Yallaroi (MS)	0.4	0.2	1.2	1.6-0.9	0.3	4.0	0.3	0.4	0.3
	AUS 13244 (S)	0.8	0.4	0.8	1.2-0.7	0.6	4.2	0.7	1.0	0.8

[a] E = environmental variance, m = mid-point between parents, d = departure of parents from the mid-point

Table 4.4.4 Mean root length (cm) of parents and F_2 derived F_3 populations grown in filter papers containing high concentrations of B.

Cross		B	P_1		P_2		F_3	LSD[b]
P_1	P_2		mean	CI[a]	mean	CI	mean	
AUS 10105 (T)	AUS 10344 (MT)	150	6.3	5.3-7.3	3.8	2.8-4.9	5.0	1.4
	AUS 14010 (MT)	150	5.5	4.6-6.5	3.1	2.0-4.2	4.2	0.7
	AUS 10348 (MS)	100	9.2	8.3-10.1	3.7	2.8-4.6	6.0	0.8
	Yallaroi (MS)	100	9.7	8.7-10.7	3.5	2.5-4.5	4.9	1.2
AUS 10110 (T)	AUS 10344 (MT)	150	5.9	4.7-7.1	3.2	2.0-4.3	3.9	1.9
	AUS 14010 (MT)	150	5.8	4.6-6.9	3.2	1.9-4.4	4.5	2.3
	AUS 10348 (MS)	100	7.7	6.5-8.9	3.1	2.1-4.0	4.5	0.9
	Yallaroi (MS)	100	9.8	8.1-11.6	3.2	1.9-4.5	6.4	2.5
AUS 10344 (MT)	AUS 14010 (MT)	100	8.4	7.2-9.4	7.5	6.3-8.6	7.1	1.8
	AUS 10348 (MS)	100	5.5	4.4-6.5	3.7	2.7-4.7	5.1	1.1
	Yallaroi (MS)	100	6.7	5.5-7.8	3.2	2.1-4.3	4.6	1.8
	AUS 13244 (S)	100	8.2	7.2-9.3	3.2	2.3-4.1	5.3	0.7
AUS 14010 (MT)	AUS 10348 (MS)	100	8.1	7.0-9.2	4.8	3.9-5.7	6.8	1.5
	Yallaroi (MS)	100	5.8	4.9-6.8	2.4	1.4-3.4	4.2	1.1
	AUS 13244 (S)	100	7.2	6.2-8.3	3.1	2.2-4.0	2.6	0.7
AUS 10348 (MS)	Yallaroi (MS)	100	4.3	3.3-5.3	3.7	2.7-4.6	4.5	0.6
	AUS 13244 (S)	100	5.0	3.8-6.2	3.5	2.5-4.5	3.9	1.1

[a] CI = confidence interval of the parental mean

[b] LSD of the parental variance

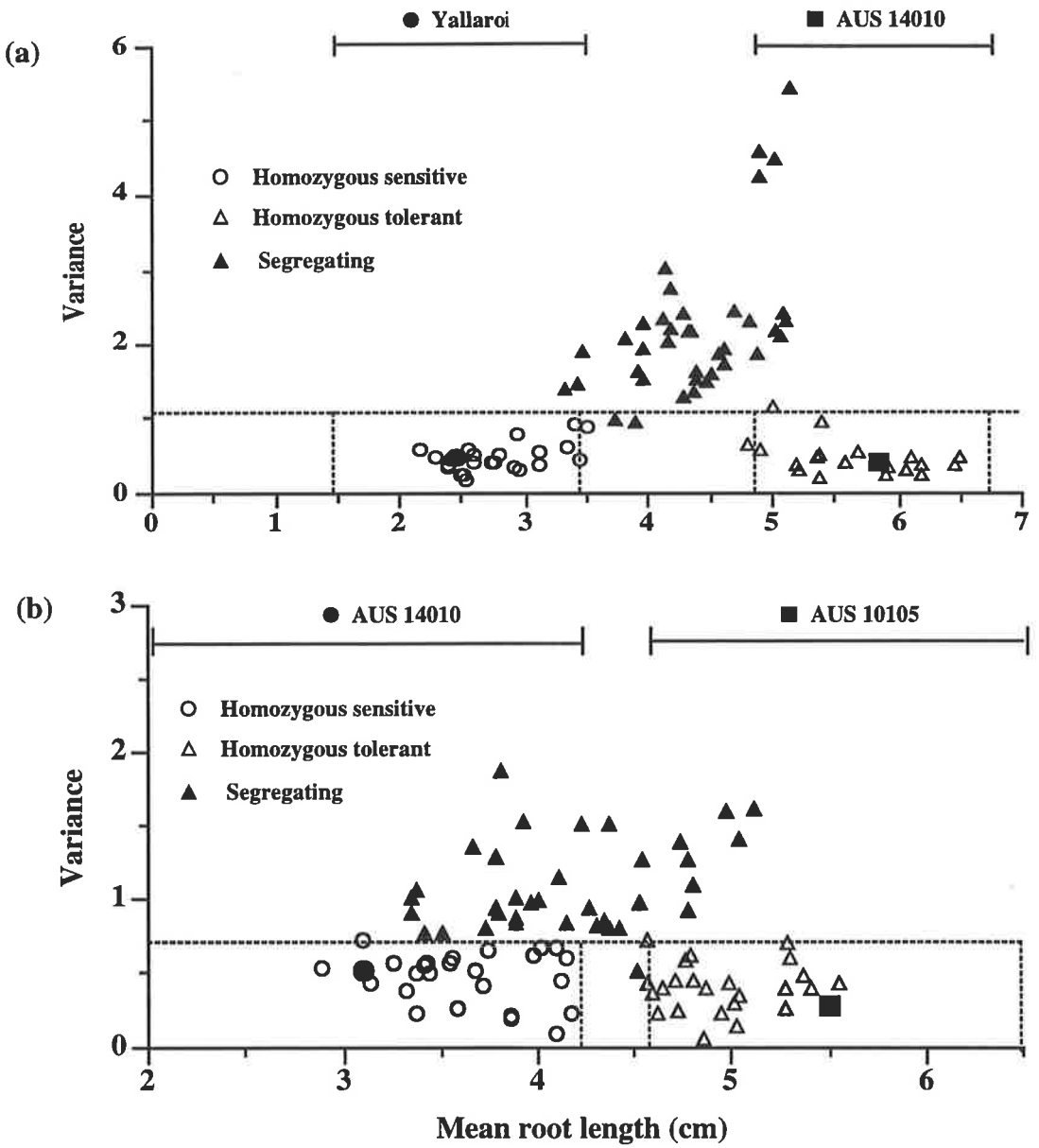


Figure 4.7 Examples of the mean root length and variances of parents and F_3 families of the crosses expected to segregate at a single gene,

(a) AUS 14010 (MT) x Yallaroi (MS); tested at B100. The confidence intervals of the means of AUS 14010 and Yallaroi were 4.9-6.8 and 1.4-3.4 cm, respectively. LSD of the parental variance was 1.1, indicated by the horizontal dashed line.

(b) AUS 10105 (T) x AUS 14010 (MT); tested at B150. The confidence intervals of the means of AUS 10105 and AUS 14010 were 4.6-6.5 and 2.0-4.2 cm, respectively. LSD of the parental variance was 0.7, indicated by the horizontal dashed line.

Table 4.4.5 Chi-square analysis for response to B of F_2 derived F_3 families from the crosses between tolerant x moderately tolerant (T x MT), moderately tolerant x moderately sensitive (MT x MS) and moderately sensitive x sensitive (MS x S) lines, expected to segregate at a single gene.

Cross	B	Model[a]		Number of F_3 families			$\chi 2$	P(df=2)
				Tolerant	Segregating	Sensitive		
T x MT								
AUS 10105 x AUS 10344	150	1:2:1	Exp.	22.75	45.5	22.75		
			Obs.	24	47	20	0.45	0.80
AUS 10105 x AUS 14010	150	1:2:1	Exp.	22	44	22		
			Obs.	24	39	25	1.15	0.56
AUS 10110 x AUS 10344	150	1:2:1	Exp.	27.25	54.5	27.25		
			Obs.	23	55	31	1.18	0.55
AUS 10110 x AUS 14010	150	1:2:1	Exp.	22.5	45	22.5		
			Obs.	19	46	25	0.84	0.66
MT x MS								
AUS 10344 x AUS 10348	100	1:2:1	Exp.	25.75	51.5	25.75		
			Obs.	Transgressive segregation observed			-	-
AUS 10344 x Yallaroi	100	1:2:1	Exp.	23	46	23		
			Obs.	23	47	22	0.06	0.97
AUS 14010 x AUS 10348	100	1:2:1	Exp.	27.25	54.5	27.25		
			Obs.	30	53	26	0.38	0.83
AUS 14010 x Yallaroi	100	1:2:1	Exp.	20	40	20		
			Obs.	18	40	22	0.40	0.82
MS x S								
AUS 10348 x AUS 13244	100	1:2:1	Exp.	18.75	37.5	18.75		
			Obs.	18	33	24	2.04	0.36

[a] expected ratio was homozygous tolerant : segregating : homozygous sensitive = 1:2:1.

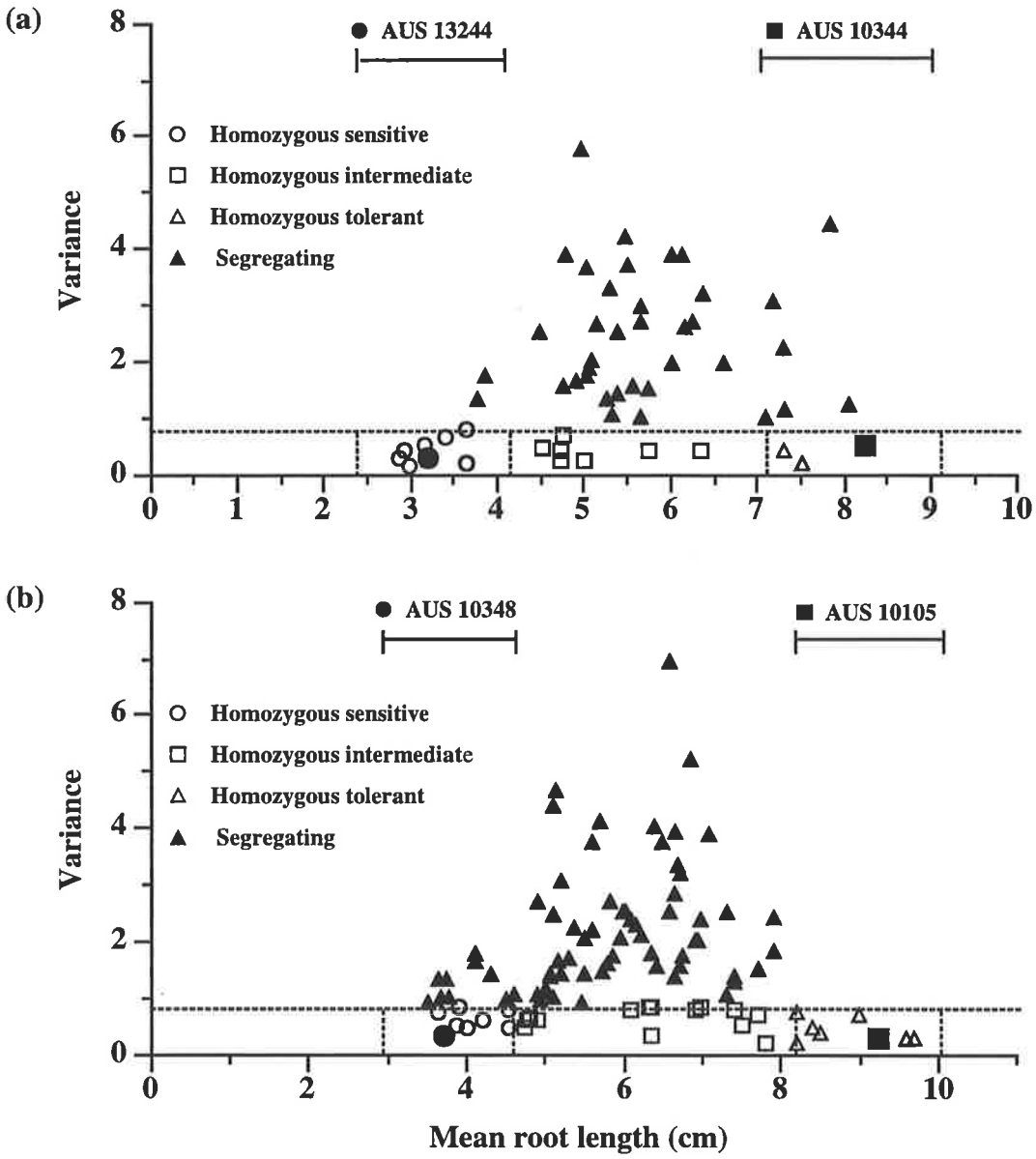


Figure 4.8 Examples of the mean root length and variances of parents and F_3 families of crosses expected to segregate at two genes,

(a) AUS 10344 (MT) x AUS 13244 (S); tested at B100. The confidence intervals of the means of AUS 10344 and AUS 13244 were 7.2-9.3 and 2.3-4.1 cm, respectively. LSD of the parental variance was 0.7, indicated by the horizontal dashed line.

(b) AUS 10105 (T) x AUS 10348 (MS); tested at B100. The confidence intervals of the means of AUS 10105 and AUS 10348 were 8.3-10.1 and 2.8-4.6 cm, respectively. LSD of the parental variance was 0.8, indicated by the horizontal dashed line.

Table 4.4.6 Chi-square analysis for response to B of F_2 derived F_3 families from the crosses between tolerant x moderately sensitive (T x MS) and moderately tolerant x sensitive (MT x S) lines, expected to segregate at two genes.

Cross	Model[a]		Number of F_3 families[b]				χ^2	P
			Tol	Int	Seg	Sens		(df=3)
T x MS								
AUS 10105 x AUS 10348	1:2:12:1	Exp.	5.6	11.3	67.5	5.6		
		Obs.	7	14	62	7	1.79	0.62
AUS 10105 x Yallaroi	1:2:12:1	Exp.	6.2	12.4	74.2	6.2		
		Obs.	8	10	72	9	2.31	0.51
AUS 10110 x AUS 10348	1:2:12:1	Exp.	7.1	14.2	85.6	7.1		
		Obs.	12	20	76	6	6.88	0.07
AUS 10110 x Yallaroi	1:2:12:1	Exp.	4.1	8.1	48.7	4.1		
		Obs.	3	14	42	6	6.39	0.09
MT x S								
AUS 10344 x AUS 13244	1:2:12:1	Exp.	3.2	6.4	38.2	3.2		
		Obs.	2	7	35	7	5.29	0.15
AUS 14010 x AUS 13244	1:2:12:1	Exp.	5.1	10.1	60.7	5.1		
		Obs.	6	4	64	7	4.73	0.19

[a] expected ratio was homozygous tolerant : homozygous intermediate : segregating: homozygous sensitive = 1:2:12:1.

[b] Tol = homozygous tolerant, Int = homozygous intermediate, Seg = segregating, Sens = homozygous sensitive

Plate 4.1 Examples of response of F_2 and F_2 derived F_3 families of AUS 14010 x Yallaroi, tested at B100.

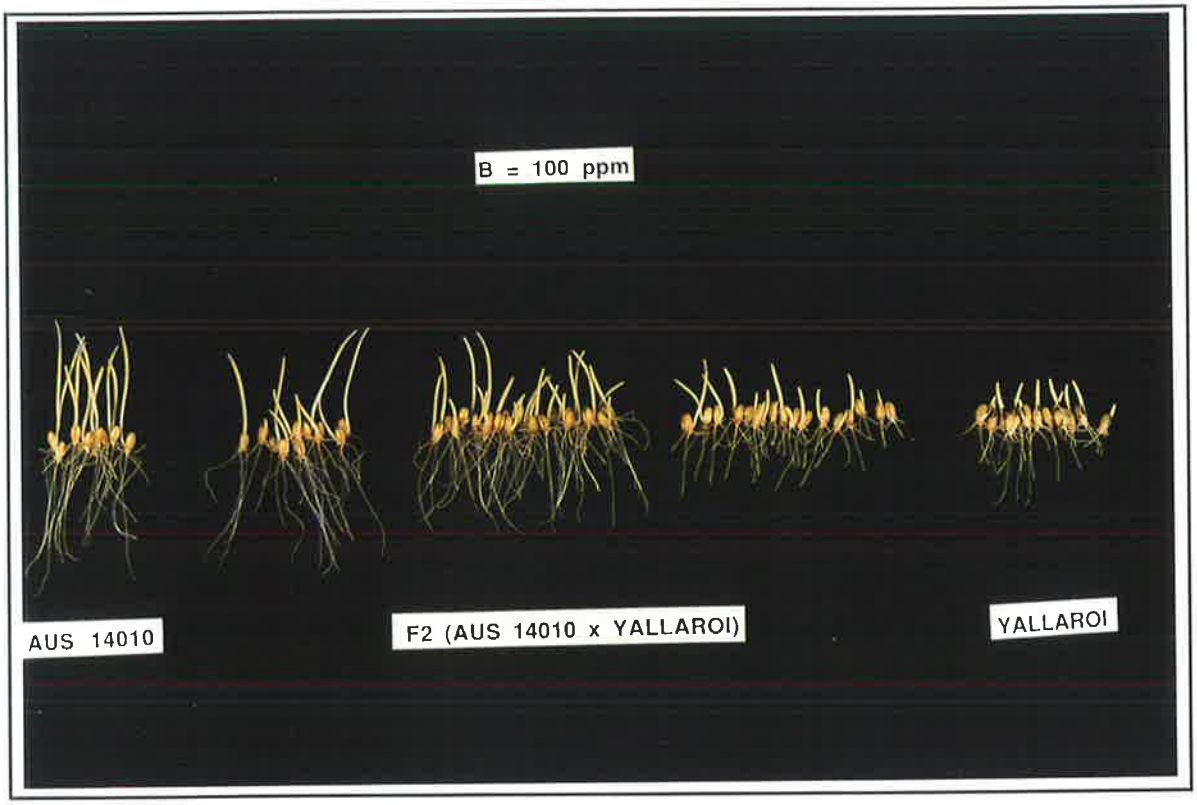
(a) F_2 generation;

From left to right, AUS 14010, F_2 plants which respond as tolerant, intermediate and sensitive, respectively, and Yallaroi.

(b) F_3 generation;

From left to right, AUS 14010, Yallaroi, F_2 derived F_3 families identified as a homozygous tolerant, segregating and a homozygous sensitive, respectively.

(a)


B = 100 ppm
AUS 14010
F2 (AUS 14010 x YALLAROI)
YALLAROI

(b)


B=100PPM
AUS 14010
YALLAROI

Plate 4.2 Example of response of F_2 and F_2 derived F_3 families of AUS 10110 x Yallaroi, tested at B100.

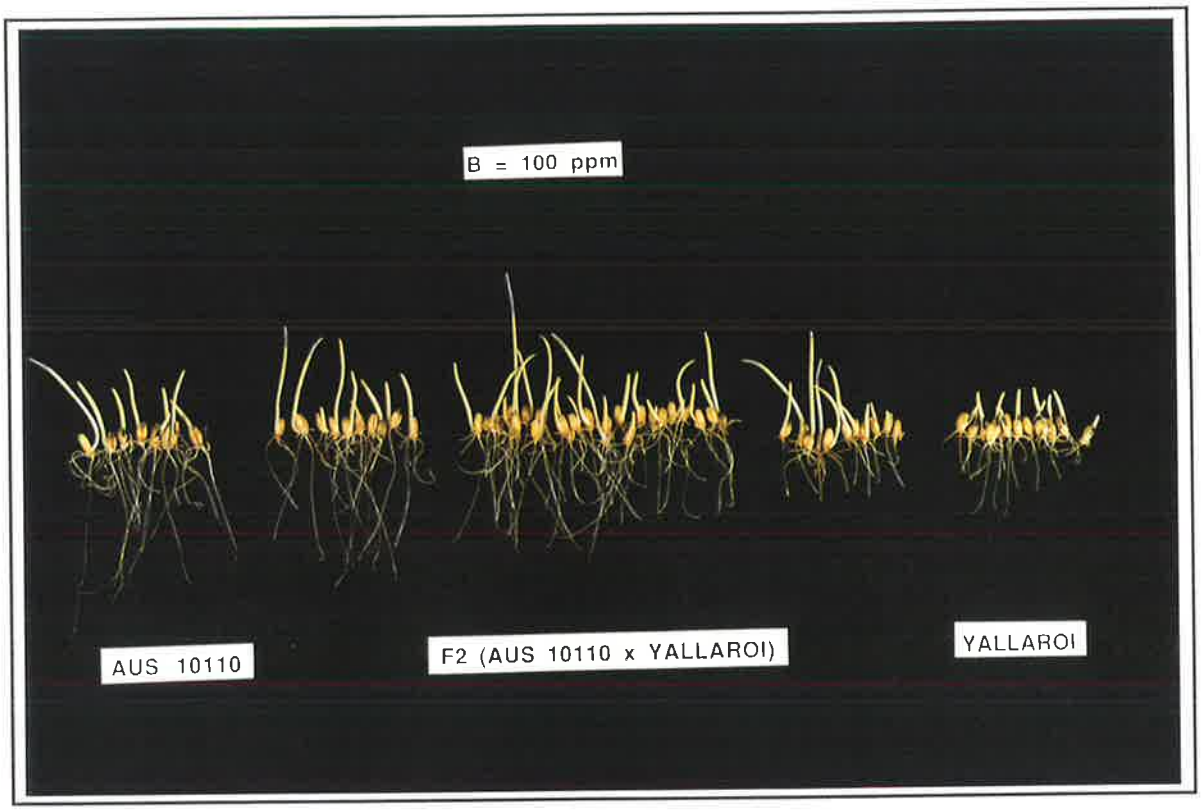
(a) F_2 generation;

From left to right, AUS 10110, F_2 plants which respond as tolerant, intermediate and sensitive, respectively, and Yallaroi.

(b) F_3 generation;

From left to right, AUS 14010, Yallaroi, F_2 derived F_3 families identified as homozygous tolerant, two homozygous intermediate and a homozygous sensitive, respectively.

(a)


B = 100 ppm
AUS 10110
F2 (AUS 10110 x YALLAROI)
YALLAROI

(b)


B=100PPM
AUS 10110
YALLAROI

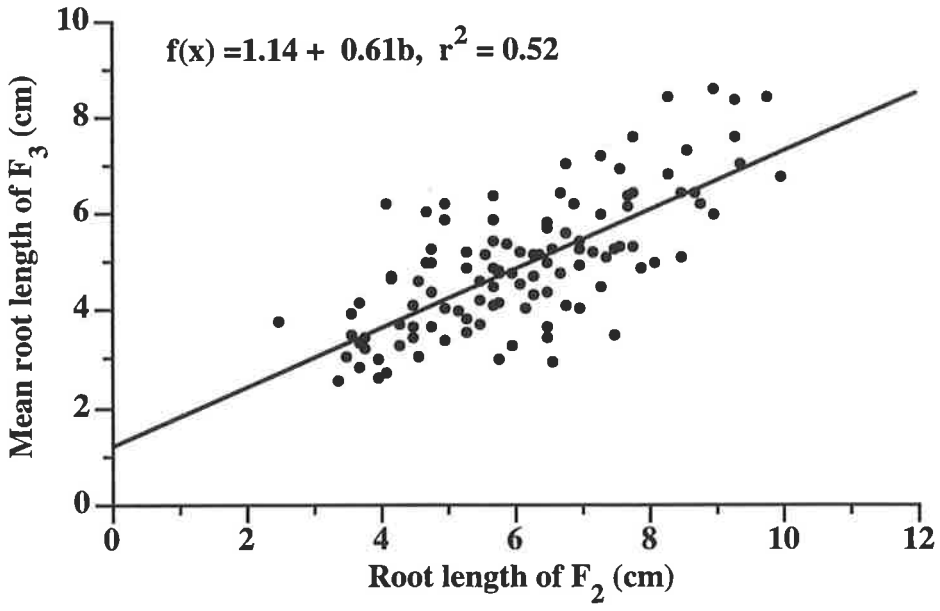
Table 4.4.7 Regression analysis of the response of F_2 plants and their F_3 progeny to high concentrations of B.

Cross		Regression parameter				
P_1	P_2	r	r^2	b	SE[a]	CI[a]
AUS 10105 (T)	AUS 10344 (MT)	0.41	0.17	0.30**	0.07	0.16-0.44
AUS 10110 (T)	AUS 10344 (MT)	0.32	0.10	0.18**	0.16	0.08-0.29
	AUS 14010 (MT)	0.45	0.20	0.36**	0.08	0.21-0.52
	AUS 10348 (MS)	0.72	0.52	0.61**	0.06	0.50-0.72
	Yallaroi (MS)	0.60	0.36	0.76**	0.13	0.51-1.02
AUS 10344 (MT)	AUS 14010 (MT)	0.40	0.16	0.36**	0.09	0.20-0.54
	AUS 10348 (MS)	0.61	0.37	0.47**	0.06	0.35-0.59
	Yallaroi (MS)	0.55	0.30	0.36**	0.06	0.24-0.48
AUS 14010 (MT)	AUS 10348 (MS)	0.55	0.30	0.43**	0.06	0.32-0.55
	Yallaroi (MS)	0.50	0.25	0.49**	0.09	0.30-0.68

[a] Standard error (SE) and confidence interval (CI) of slope (b)

** significant at $P<0.01$

(a) AUS 10110 x AUS 10348



(b) AUS 10344 x AUS 10348

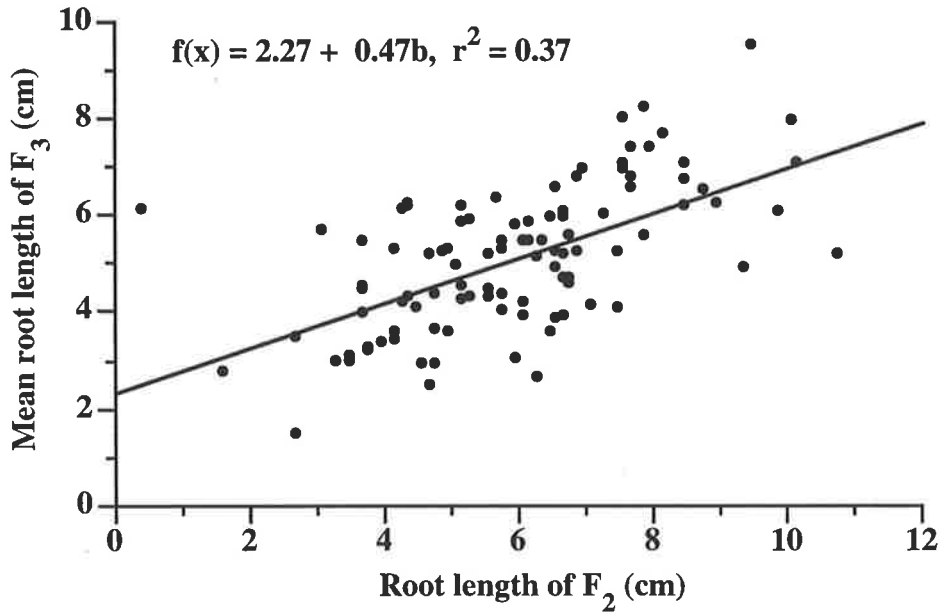
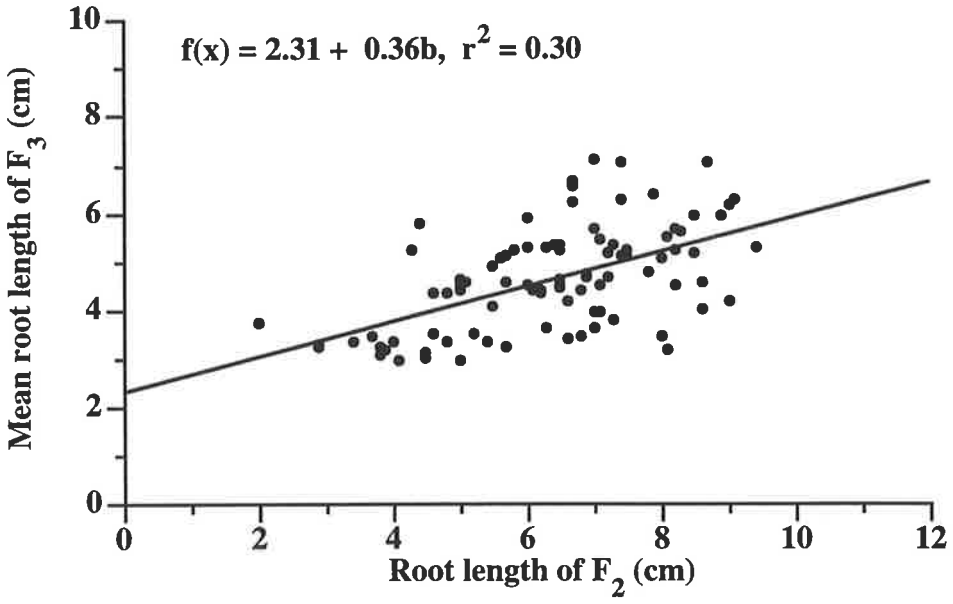


Figure 4.9 Relationships between root length of F_2 parents and mean root length of F_3 families grown at a high concentration of B for the crosses;

(a) AUS 10110 (T) x AUS 10348 (MS) **(b)** AUS 10344 (MT) x AUS 10348 (MS)

(c) AUS 10344 x Yallaroi



(d) AUS 10110 x Yallaroi

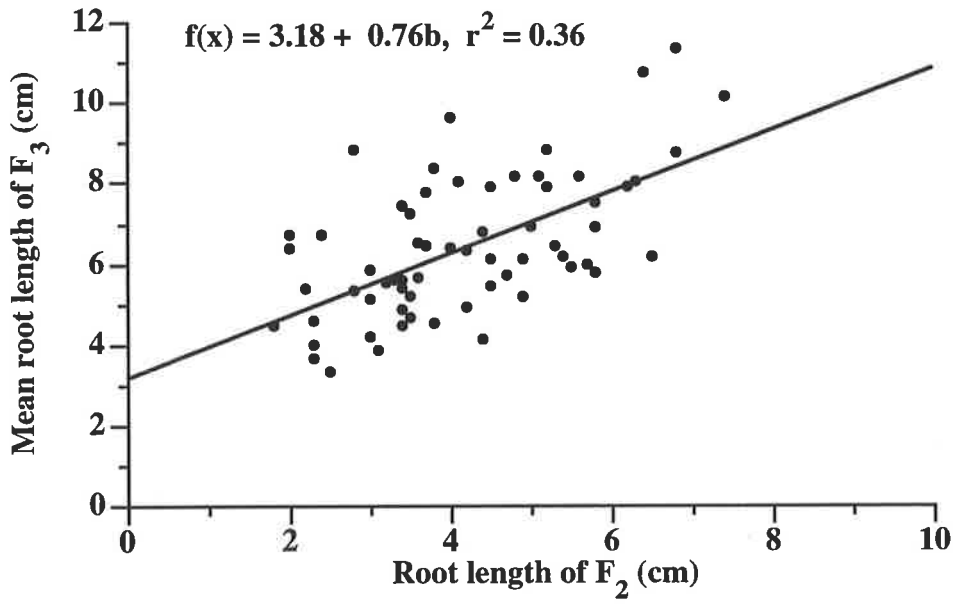


Figure 4.9 (continued) Relationships between root length of F_2 parents and mean root length of F_3 families grown at a high concentration of B for the crosses;

(c) AUS 10344 (MT) x Yallaroi (MS) **(d)** AUS 10110 (T) x Yallaroi (MS)

4.4.4 Discussion

Phenotypic variation for the response of F_2 and F_3 generations to high concentrations of B existed when measured as a quantitative character, namely, root length of seedlings grown in filter paper soaked with high concentrations of B. The environmental variance was relatively low compared to the phenotypic variance and, therefore, most of the variation of segregating populations resulted from genetic variance. With the exception of several crosses where transgressive segregation was observed, the number of genes controlling B tolerance was found to reflect the difference in response between parents. For example, the d value (the departure of one of a pair of corresponding homozygotes from their mid-parent value) for the crosses segregating at a single, two and more than two genes ranged between 1.2-1.7, 2.0-2.7 and 3.1-3.3, respectively (Table 4.4.3).

The measurement of B response of F_2 derived F_3 families gave more precise results than the response of F_2 plants. This was to be expected since the value of each F_2 individual was the expression of the phenotype, which included both genotypic and environmental effects, whereas the response of F_2 derived F_3 families allowed the genotype of the F_2 parent to be identified. The screening of F_2 derived F_3 families was able to distinguish homozygous from heterozygous families by comparing a family's variance to the LSD of the parental variances. This method was more accurate for the crosses differing at one gene than crosses involving two genes. Families were assigned to three categories (homozygous tolerant, segregating and homozygous sensitive) for the one gene model and four categories (homozygous tolerant, homozygous intermediate, segregating and homozygous sensitive) for the two genes model. In the two gene model, the intermediate homozygous category consisted of two genotypes (AAbb and aaBB) and the segregating category consisted of five genotypes (AABb, AaBB, AaBb, Aabb and aaBb).

An example of misclassification with the two gene model was shown in the cross AUS 10110 (T) x AUS 10348 (MS) (Table 4.4.6), where the numbers of families classified

as homozygous tolerant and homozygous intermediate was over-estimated whereas the number of families classified as segregating were under-estimated. This was likely to result from some of the AABb, AaBB Aabb or aaBb families being misclassified into the homozygous categories (homozygous tolerant or intermediate). The excessive number of homozygous tolerant families may have resulted from the low confidence interval of the mean of AUS 10110 (T) (mean 6-9 cm compared to 8-11 cm when tested in other crosses). This, together with some of the AABb, AaBB, Aabb or aaBb families producing, by chance, a large number of seedlings with a uniform root length within a family, causing an intermediate mean and low variance within the family, resulted in misclassification into the homozygous category.

The results from the crosses expected to segregate at two genes in general showed a lower probability of fit to the expected ratio than one gene model (P ranged 0.07-0.62 for two genes compared to 0.36-0.97 for one gene, Table 4.4.6 v Table 4.4.5). While it is possible that this was the result of there being a smaller number of families tested than expected due to some of F_2 plants failing to produce F_3 seed during multiplication in the glasshouse, the comparison of observed to expected variances of F_2 derived F_3 families showed that these crosses fitted with the two gene model with a high level of probability (Table 4.4.3). It must be concluded that these crosses produced fewer homozygous tolerant and sensitive families than expected for single gene segregation (5 to 9 families observed, compared to more than 20 expected for a single gene), indicating that these crosses were segregating for more than one gene. Furthermore, the results from this study showed single gene difference between parents having a close level of response to B, and were found from the crosses between T x MT (AUS 10110 (T) and AUS 10105 (T) x AUS 10344 (MT) and AUS 14010 (MT)), MT x MS (AUS 10344 (MT) x Yallaroi (MS) and AUS 14010 (MT) x AUS 10348 (MS)), and MS x S (AUS 10348 (MS) x AUS 13244 (S)) lines, whereas all of the cross that segregated in a pattern consistent with the two gene model occurred between parents which differed more in response to B. Despite the low number of families tested, it can be concluded that crosses between T x MS (AUS 10110 and AUS 10105 (T) x AUS 10348 and

Yallaroi (MS)) and MT x S (AUS 10344 and AUS 14010 (MT) x AUS 13244 (S)) differed at two genes with respect to response to B.

The response of F_2 and F_2 derived F_3 families was consistent with B tolerance in durum being governed by a series of major genes acting additively. No segregation was observed between the most B tolerant lines (AUS 10110 (T) and AUS 10105 (T)) and the same segregation ratios occurred when AUS 10110 (T) and AUS 10105 (T) were crossed to the other parents. Thus, these two lines possess the same genes for B tolerance. Digenic segregation ratios were found for the T x MS and MT x S combinations. This implies that the greatest range of distribution, found in the F_2 from the T x S crosses (AUS 10105 (T) x AUS 13244 (S) and AUS 10110 (T) x AUS 13244 (S), Figure 4.3a and 4.4a, respectively), resulted from segregation at three loci.

A positive linear relationship between root length of F_2 plants and the mean root length of F_2 derived F_3 families existed for most of the crosses tested in this study. The regression coefficients (b) of most crosses involving one gene segregation were lower than crosses with two genes segregation (Table 4.4.7). The slope of the regression line reflects the genetic covariance of offspring on mid-parent, and is equal to half of the additive variance (Falconer, 1989). The additive variance is a function of d (Mather and Jinks, 1977) and the results from this study demonstrated that d of the crosses segregating for two genes is always higher than d from that of single gene segregation. The evidence of additive effects and relatively high regression coefficients suggested that B tolerance in durum can be selected at an early generation in a breeding program.

The relationships between F_2 and F_3 for the crosses between T x MT lines were relatively low (r^2 = 0.17-0.20, Table 4.4.7), compared to the other crosses. The concentrations of B used for testing these is considered to have played a major role in this result. The root lengths of F_2 plants were measured at B100, instead of B150. Initially, the F_2 was tested at B150 but attempts to transfer F_2 plants screened in B150 to potting mix failed because the sensitive F_2 plants developed very short roots and were unable to establish in the soil. Thus, the screening was repeated and the B

treatment was reduced to B100 in order to avoid this damage. Screening the F_2 from these crosses at the comparatively low level of B100 may have induced expression of dominant gene action as found in the response of F_1 hybrids (Section 4.3) with the heterozygous F_2 plants likely to have root lengths similar to the more tolerant parent. To overcome this problem, the F_2 should be screened in the B150 treatment, measured for root length and the seedlings transferred into non-B nutrient solution or water for at least 48 h before transplanting, to remove excess B from the plants (Y. Chantachume pers comm.).

4.5 Confirmation of transgressive segregation for response to high concentrations of B in AUS 10344 (MT) x AUS 14010 (MT) and AUS 10344 (MT) x AUS 10348 (MS)

4.5.1 Introduction

In the previous section, transgressive segregants were detected in crosses between moderately tolerant (MT) x moderately tolerant (MT) (AUS 10344 x AUS 14010), moderately tolerant (MT) x moderately sensitive (MS) (AUS 10344 x AUS 10348) and moderately sensitive (MS) x moderately sensitive (MS) (AUS 10348 x Yallaroi) lines. Transgressive segregation among the progeny of genotypes which possess the same level of tolerance (i.e. MT x MT or MS x MS) indicated that the response to B of the parents is controlled by different genes. To verify the genotypes of plants which transgressed beyond the range of parents, families from the first two crosses were selected and backcrossed to their parents and the resulting progeny were tested for response to B.

4.5.2 Materials and methods

Developement of genetic materials

Genetic materials used in the experiments were derived from two crosses (AUS 10344 (MT) x AUS 14010 (MT) and AUS 10344 (MT) x AUS 10348 (MS)). F_3 families tested in Section 4.4 were selected from both crosses. The development of F_3 derived F_4 lines and the production of F_2 populations derived from selected families and parental genotypes are shown in Figures 4.11 and 4.12.

AUS 10344 (MT) x AUS 14010 (MT)

F_3 families in which all plants produced roots longer than AUS 10344 (MT) were classified as tolerant and roots shorter than AUS 14010 (MT) were classified as sensitive. Four tolerant and four sensitive families were selected and 4 to 6 plants were

multiplied per family (Figure 4.11). F_4 seed was harvested from each plant and these represented F_4 lines derived from a F_3 family. Altogether, 44 lines were harvested. Thirteen seeds per line were tested in the B100 treatment. Parental genotypes, AUS 10344 (MT), AUS 14010 (MT), and genotypes which were expected to have the same root length at high B concentration as tolerant families, i.e. AUS 10110 (T), or sensitive families, i.e. Yallaroi (MS), were included as checks.

One tolerant line (237-5-1) and one sensitive line (237-49-1) were selected and crossed with AUS 10344 (MT), AUS 14010 (MT), AUS 10110 (T) and Yallaroi (MS). F_1 hybrids were multiplied in a glasshouse and the F_2 generations were tested for response to B. Crosses, B treatments and number of F_2 plants tested were as follows;

Cross	B treatment (mgB L^{-1})	Number of F_2
237-5-1 x AUS 10344 (MT)	150	120
237-5-1 x AUS 14010 (MT)	150	120
237-5-1 x AUS 10110 (T)	150	120
237-49-1 x AUS 10344 (MT)	100	120
237-49-1 x AUS 14010 (MT)	100	120
237-49-1 x Yallaroi (MS)	100	120

AUS 10344 (MT) x AUS 10348 (MS)

F_3 families in which all plants produced roots longer than AUS 10344 (MT) were classified as tolerant and roots shorter than AUS 10348 (MS) were classified as sensitive. One tolerant family and one sensitive family were selected and 5 plants were multiplied per family (Figure 4.12). Eight F_4 lines were harvested and tested in the B100 treatment. Parental genotypes, AUS 10344 and AUS 10348, and genotypes which were expected to have the same root length at high B concentration as the tolerant families, i.e. AUS 10110 (T), or sensitive families, i.e. AUS 13244 (S), were included as checks.

A tolerant line (393-25-1) and a sensitive line (393-112-1) were crossed to AUS 10344

Cross production AUS 10344 (MT) x AUS 14010 (MT) (Cross no. 237)

F_1: multiplied in non-toxic soil

F_2: tested at B100, individual plants transplanted into non-B toxic soil

Selection

F_3: tested at B100, 4-6 plants of each selected family were transplanted into non-B toxic soil

Tolerant families;	Sensitive families;
237-5, 237-52, 237-96, 237-93	237-49, 237-79, 237-48, 237-107

F_4: tested at B100

Tolerant lines;	Sensitive lines;
237-5-1 to 237-5-5	237-49-1 to 237-49-6
237-52-1 to 237-52-5	237-79-1 to 237-79-4
237-96-1 to 237-96-6	237-48-1 to 237-48-6
237-93-1 to 237-93-6	237-107-1 to 237-107-6

Cross production

AUS 10344 x 237-5-1	AUS 10344 x 237-49-1
AUS 14010 x 237-5-1	AUS 14010 x 237-49-1
AUS 10110 x 237-5-1	Yallaroi x 237-49-1

F_1: multiplied in non-B toxic soil

F_2: tested at either B100 or B150

Figure 4.11 Diagram showing the method of development of lines derived from the F_3 families of the cross AUS 10344 (MT) x AUS 14010 (MT) and the production of the F_2 between transgressive segregants and check genotypes.

Cross production AUS 10344 (MT) x AUS 10348 (MS) (Cross no. 393)

F_1: multiplied in non-B toxic soil

F_2: tested at B100, individual plants transplanted into non-B toxic soil

Selection

F_3: tested at B100, 5 plants of each selected family were transplanted into non-B toxic soil

Tolerant family;	Sensitive family;
393-25	393-112

F_4: tested at B100

Tolerant lines;	Sensitive lines;
393-25-1 to 393-25-5	393-112-1 to 393-112-5

Cross production

AUS 10344 x 393-25-1	AUS 10344 x 393-112-1
AUS 10348 x 393-25-1	AUS 10348 x 393-112-1
AUS 10110 x 393-25-1	AUS 10110 x 393-112-1
AUS 13244 x 393-25-1	AUS 13244 x 393-112-1

393-25-1 x 393-112-1

F_1: multiplied in non-B toxic soil

F_2: tested at either B50, B100 or B150

Figure 4.12 Diagram showing the method of development of lines derived from the F_3 families of the cross AUS 10344 (MT) x AUS 10348 (MS) and the production of the F_2 between transgressive segregants and check genotypes.

(MT), AUS 10348 (MS), AUS 10110 (T) and AUS 13244 (S). F_1 hybrids were multiplied in a glasshouse and the F_2 populations were tested for response to B. Crosses, B treatments and number of F_2 plants tested were as follows;

Cross	B treatment (mgB L^{-1})	Number of F_2
293-25-1 x AUS 10344 (MT)	150	120
293-25-1 x AUS 10348 (MS)	100	120
293-25-1 x AUS 10110 (T)	150	120
293-112-1 x AUS 10344 (MT)	100	120
293-112-1 x AUS 10348 (MS)	50	120
293-112-1 x AUS 13244 (S)	50	120
293-25-1 x 293-112-1	100	120

Statistical analysis

The response of F_4 derived F_3 lines to B was compared to parents by an unpaired t-test. For both crosses, the segregation patterns of the F_2, compared to parental lines, were plotted. The expected and observed variances for segregation at one and two genes were calculated by the methods described in Section 4.2.

4.5.3 Results

AUS 10344 (MT) x AUS 14010 (MT)

Response of selected lines to B

In the B100 treatment, AUS 10344 (MT) and AUS 14010 (MT) had root lengths of 5.9 and 5.0 cm, respectively (Table 4.5.1, Plate 4.3a). Root lengths of thirteen F_4 lines were significantly longer than AUS 10344 (MT) (mean root length 6.8 to 8.9 cm) and similar to AUS 10110 (T) (8.2 cm). Nine F_4 lines had significantly shorter roots than AUS 14010 (MT) (mean root length 3.2 to 4.3 cm) and similar to Yallaroi (MS) (3.6 cm).

Response of F_2 populations

The observed variances of the F_2 populations between the tolerant selection (237-5-1) and the two parents, AUS 10344 (MT) and AUS 14010 (MT) were consistent with the expected variances of a single gene model with d about 1.4-1.6 cm (Table 4.5.2 and Figure 4.13). No segregation was observed (d = -0.4) between the tolerant selection, 237-5-1 and the tolerant check, AUS 10110 (T), and the expected variance of both single gene and two gene models were within the range of the observed variance and very close to the environmental variance.

The observed variances of the F_2 populations between the sensitive selection (237-49-1) and the two parents, AUS 10344 (MT) and AUS 14010 (MT) were consistent with the expected variances of a single gene model with d about -0.9 to -1.2 cm (Table 4.5.2 and Figure 4.14). No segregation was observed (d = -0.2) between the sensitive selection, 237-49-1 and the moderately sensitive check, Yallaroi (MS), and the expected variance of both single gene and two gene models were within the range of the observed variance.

Table 4.5.1 Root length (cm) of selected families and lines from the cross AUS 10344 (MT) x AUS 14010 (MT) tested in 100 mgB L^{-1}.

Family/line	F_3		F_4			
	mean	var	mean	var	diff. from parent	t-test
Tolerant						
237-5	10.2	0.6				
237-5-1			8.9	1.2	3.0	7.05**
237-5-2			5.9	1.4	0.3	0.07
237-5-3			8.7	0.3	2.8	9.84**
237-5-4			7.9	2.2	1.9	3.75**
237-5-5			7.4	0.4	1.5	4.90**
237-52	10.1	0.5				
237-52-1			7.5	0.3	1.6	5.42**
237-52-2			7.1	0.3	1.2	4.17**
237-52-3			7.1	0.3	1.2	4.51**
237-52-4			7.2	0.3	1.3	4.52**
237-52-5			6.2	0.3	0.3	1.02
237-96	9.5	0.4				
237-96-1			6.1	0.4	0.2	0.76
237-96-2			8.2	0.7	2.3	6.24**
237-96-3			6.8	0.8	0.9	2.51*
237-96-4			6.7	1.1	0.7	1.90
237-96-5			6.3	0.3	0.4	1.37
237-96-6			6.3	0.3	0.4	1.41
237-93	9.3	0.5				
237-93-1			6.8	1.1	0.9	2.28*
237-93-2			6.8	0.4	0.9	3.17**
237-93-3			5.6	0.4	-0.3	-1.02
237-93-4			5.7	0.4	-0.2	-0.69
237-93-5			5.7	0.8	-0.2	-0.52
237-93-6			6.6	0.5	0.7	2.33*
Sensitive						
237-49	5.1	0.4				
237-49-1			3.8	0.5	-1.2	-4.82**
237-49-2			4.5	0.7	-0.5	-1.99

Continued next page

*, ** Significantly different at $P<0.05$ and 0.01, respectively.

Table 4.5.1 (continued)

Family/line	F_3		F_4			
	mean	var	mean	var	diff. from parent	t-test
237-49-3			5.5	0.8	0.4	1.47
237-49-4			4.8	1.1	-0.2	-0.52
237-49-5			3.9	0.6	-1.1	-4.11**
237-49-6			4.6	0.2	-0.4	-2.08
237-79	5.5	0.1				
237-79-1			5.0	0.5	0.0	0.05
237-79-2			3.7	0.2	-1.3	-7.14**
237-79-3			3.3	0.2	-1.7	-10.17**
237-79-4			3.8	0.2	-1.2	-6.59**
237-48	5.5	0.6				
237-48-1			4.3	0.7	-0.7	-2.54*
237-48-2			5.0	1.0	0.2	0.07
237-48-3			5.0	0.6	-0.0	-0.12
237-48-4			4.1	0.6	-0.9	-3.56*
237-48-5			3.2	1.1	-1.7	-5.32**
237-48-6			3.4	0.3	-1.6	-7.76**
237-107	5.8	0.2				
237-107-1			5.2	1.1	-0.7	-1.65
237-107-2			6.2	0.5	0.3	0.89
237-107-3			6.3	2.6	0.4	0.80
237-107-4			5.5	1.2	-0.7	-1.63
237-107-5			5.7	1.1	-0.2	-0.53
237-107-6			5.7	0.6	-0.2	-0.48
Check						
AUS 10344 (MT)	8.4	0.3	5.9	0.6		
AUS 14010 (MT)	7.4	0.3	5.0	0.2		
AUS 10110 (T)			8.2	0.3		
Yallaroi (MS)			3.6	0.4		

*, ** Significantly different at P<0.05 and 0.01, respectively.

Plate 4.3 Examples of the response of selected F_3 derived F_4 lines of two crosses, tested at B100.

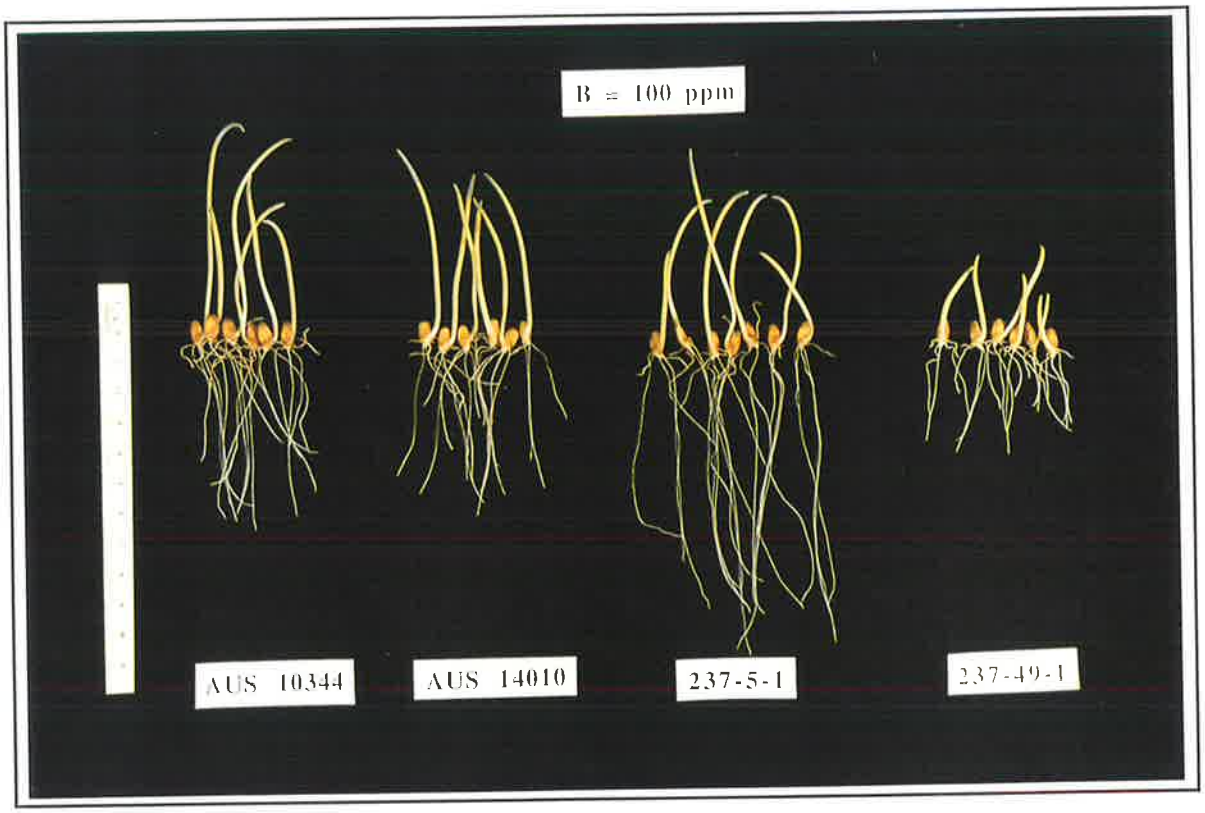
(a) AUS 10344 (MT) x AUS 14010 (MT);

From left to right, AUS 10344 (MT), AUS 14010 (MT), tolerant selection (237-5-1) and sensitive selection (237-49-1).

(b) AUS 10344 (MT) x AUS 10348 (MS);

From left to right, AUS 10344 (MT), AUS 10348 (MS), tolerant selection (393-25-1) and sensitive selection (393-112-1).

(a)


B = 100 ppm
AUS 10344
AUS 14010
237-5-1
237-49-1

(b)


B = 100
AUS 10344
AUS 10348
393-25-1
393-112-1
F4 (AUS 10344 x AUS 10348)

Table 4.5.2 Observed variance of parents and F_2 populations and estimated parameters and expected variances of the F_2 populations for the crosses involving transgressive segregants selected from cross 237 (AUS 10344 x AUS 14010), grown at either B100 or B150 treatments. CI is the confidence interval of the observed variance of F_2.

Cross		Observed variance				Estimated parameters[b]			Expected variance	
P_1[a]	P_2	V_{P1}	V_{P2}	V_{F2}	CI	E	m	d	1 gene	2 genes
B = 150										
237-25-1 (Ts)	AUS 10110 (T)	0.7	1.0	1.1	1.4-0.8	0.9	6.4	-0.4	1.0	0.9
	AUS 10344 (MT)	0.7	0.3	1.3	1.6-1.0	0.5	5.6	1.4	1.5	1.0
	AUS 14010 (MT)	1.0	0.8	2.5	3.2-2.0	0.9	4.6	1.6	2.2	1.5
B = 100										
237-49-1 (Ss)	AUS 10344 (MT)	0.7	0.6	1.0	1.3-0.8	0.6	4.9	-0.9	1.0	0.8
	AUS 14010 (MT)	0.5	0.3	1.3	1.6-1.0	0.4	4.4	-1.2	1.2	0.8
	Yallaroi (MS)	0.7	0.4	0.6	0.8-0.5	0.5	2.9	0.2	0.6	0.6

[a] 237-5-1 = tolerant selection (Ts) and 237-49-1 (Ss) = sensitive selection lines

[b] E = environmental variance, m = mid-point between parents, d = departure of parents from the mid-point

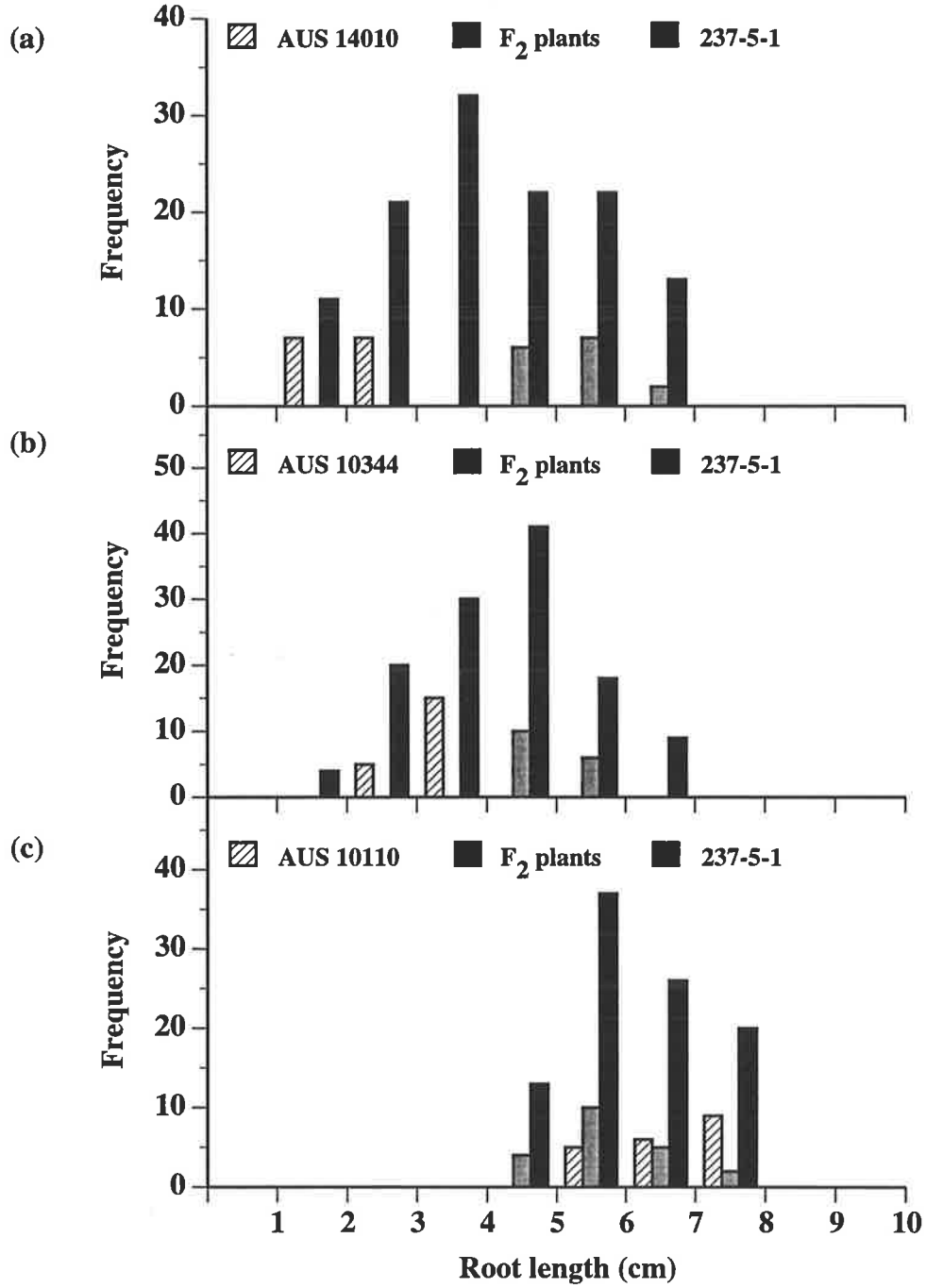


Figure 4.13 Response of parents and F_2 plants grown at a B150 treatment for the crosses involving the tolerant selection (Ts) line 237-5-1,

(a) AUS 14010 (MT) x 237-5-1 (Ts); mean (SD in parentheses) for AUS 14010 and 237-5-1 were 2.9 (0.9) and 6.2 (1.0), respectively.

(b) AUS 10344 (MT) x 237-5-1 (Ts); mean (SD in parentheses) for AUS 10344 and 237-5-1 were 4.2 (0.5), 7.0 (1.0), respectively.

(c) AUS 10110 (T) x 237-5-1 (Ts); mean (SD in parentheses) for AUS 10110 and 237-5-1 were 6.8 (0.4) and 5.7 (0.8), respectively.

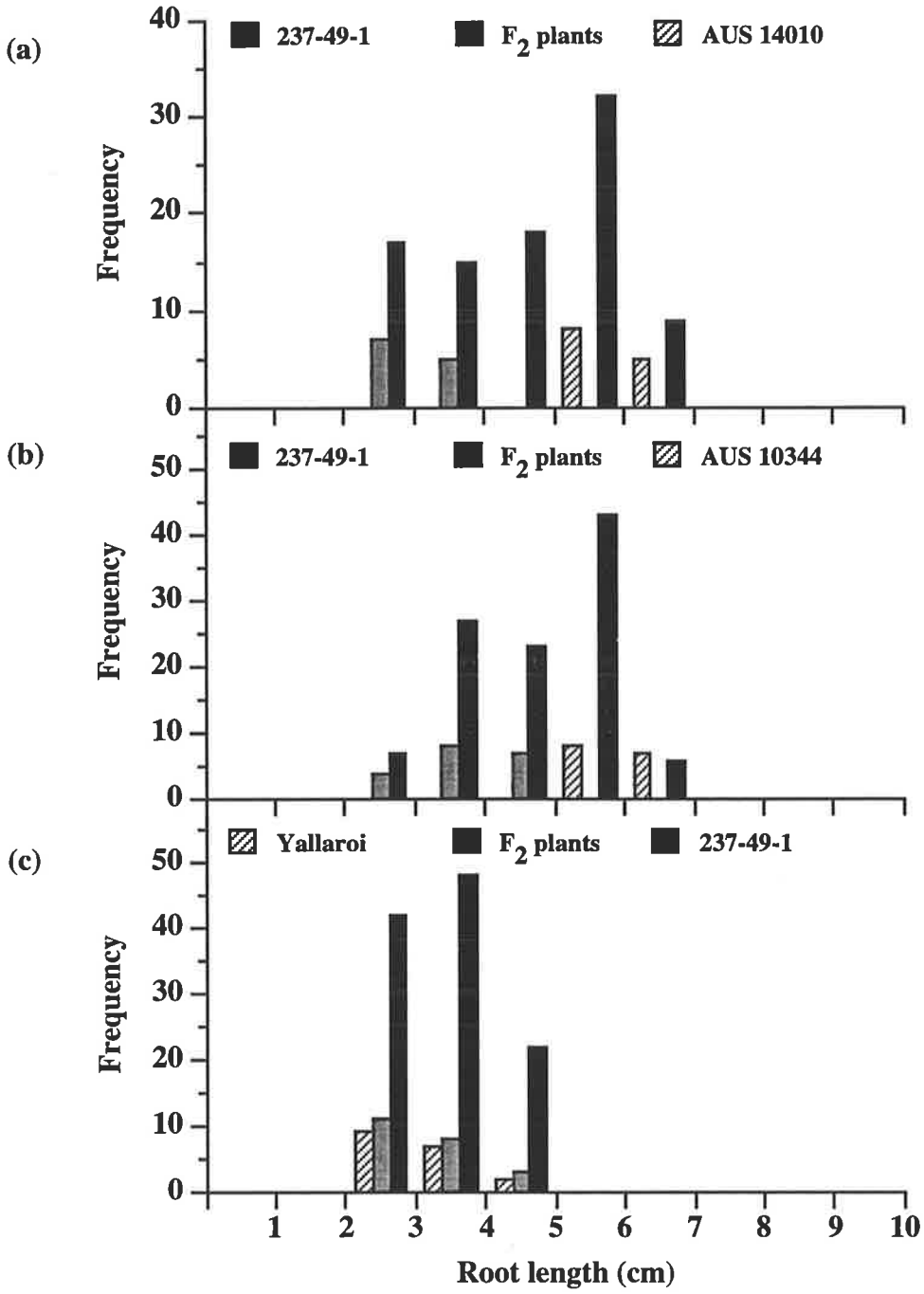


Figure 4.14 Response of parents and F_2 plants grown at a B100 treatment for the crosses involving the sensitive selection (Ss) line 237-49-1,

(a) AUS 14010 (MT) x 237-49-1 (Ss); mean (SD in parentheses) for AUS 14010 and 237-49-1 were 5.7 (0.5) and 3.2 (0.5), respectively.

(b) AUS 10344 (MT) x 237-49-1 (Ss); mean (SD in parentheses) for AUS 10344 and 237-49-1 were 5.5 (0.8) and 3.7 (0.7), respectively.

(c) Yallaroi (MS) x 237-49-1 (Ss); mean (SD in parentheses) for Yallaroi and 237-49-1 were 2.7 (0.8) and 3.0 (0.6), respectively.

AUS 10344 (MT) x AUS 10348 (MS)

Response of selected lines to B

In the B100 treatment, the parental lines AUS 10344 (MT) and AUS 10348 (MS) had root lengths 7.4 and 3.8 cm, respectively (Table 4.5.3, Plate 4.3b). Root lengths of the tolerant F_4 lines were significantly longer than AUS 10344 (MT) (mean root lengths 8.7 to 11.0 cm) and similar to AUS 10110 (T) (10.8 cm). Sensitive F_4 lines were significantly shorter than AUS 10348 (MS) (mean root lengths 2.2 to 2.8 cm) and similar to AUS 13244 (S) (2.1 cm).

Response of F_2 populations

The observed variances of the tolerant selection (393-25-1) x AUS 10110 (T), and AUS 13244 (S) x the sensitive selection (393-112-1) were within the range of expected variance for the one and two gene models and very close to the environmental variance. The non-significant difference between the parents when the tolerant and sensitive selections were crossed to the tolerant (AUS 10110) and sensitive (AUS 13244) checks, respectively (d = -0.4 and 0.1, respectively, Table 4.5.4), and the absence of segregation of the F_2 populations suggested that the tolerant selection (237-25-1) and AUS 10110 (T), and that AUS 13244 (S) and the sensitive selection (393-112-1) have the same genes for B tolerance (Table 4.5.4 , Figure 4.15c and 4.16c).

The distribution of root length and observed variance of the F_2 for the tolerant selection (393-25-1) x AUS 10344 (MT) was consistent with the variance expected for a single gene model (Table 4.5.4 and Figure 4.15b). The range of observed variance of AUS 10348 (MS) x the sensitive selection (393-112-1) was greater than the variances expected for one and two genes, but was closer to a single gene than a two genes model. The segregation of the cross between the tolerant selection (393-25-1) x AUS 10348 (MS) and AUS 10344 (MT) x the sensitive selection (393-112-1) did not deviate from a two genes model (Table 4.5.4, Figures 15a and 16a).

The comparisons of expected and observed variances for the crosses between extreme contrasts in levels of B tolerance (393-25-1 x AUS 13244 (S), 393-25-1 x 393-112-1 and AUS 10110 (T) x 393-112-1) were inconclusive, since the F_2 from these crosses segregated at a larger range than that found for the two genes segregation (d between 3.4-3.8 cm). Therefore, a larger population would be required to compare expected and observed variances for segregation at two and three genes. However, the segregation patterns of these three crosses shown in Figure 4.17 confirmed that the sensitive selection (393-112-1) had the same genotype in respect to B tolerance as AUS 13244 (S) and the tolerant selection (393-25-1) had the same B tolerance genes as AUS 10110 (T). Thus, these two groups were likely to be different at three loci with respect to response to B.

Table 4.5.3 Root length (cm) of selected families from the cross AUS 10344 (MT) x AUS 10348 (MS) tested in filter paper containing 100 mgB L^{-1}.

Family/line	F_3		F_4			
	mean	var	mean	var	diff. from parent	t-test
Tolerant						
393-25	9.5	1.1				
393-25-1			11.0	0.4	3.6	12.42**
393-25-2			9.3	0.6	1.9	5.84**
393-25-3			10.1	0.9	3.7	10.37**
393-25-4			8.7	0.7	1.5	4.51**
393-25-5			11.1	2.5	3.7	4.63**
Sensitive						
393-112	2.5	0.2				
393-112-1			2.2	0.1	-1.6	-3.97**
393-112-2			2.8	1.1	-1.0	-2.24**
393-112-3			2.5	1.0	-1.3	-3.04**
393-112-4			2.5	0.4	-1.3	-3.64**
393-112-5			2.2	0.8	-1.6	-4.01**
Check						
AUS 10344 (MT)	5.9	0.3	7.4	0.5		
AUS 10348 (MS)	3.7	0.4	3.8	0.8		
AUS 10110 (T)			10.8	0.8		
AUS 13244 (S)			2.1	0.9		

** Significantly different at $P<0.01$.

Table 4.5.4 Observed variance of parents and F_2 populations, estimated parameters and expected variances of the F_2 for the crosses involving transgressive segregants selected from cross 393 (AUS 10344 x AUS 10348), grown in filter paper containing 50, 100 or 150 mgB L^{-1}. CI is the confidence interval of the observed variance of F_2.

Cross		Observed variance				Estimated paremeter			Expected variance	
P_1	P_2	V_{P1}	V_{P2}	V_{F2}	CI	E	m	d	1 gene	2 genes
B = 150										
393-25-1 (Ts)[a]	AUS 10110 (T)	0.3	0.4	1.0	1.3-0.8	0.3	7.1	-0.1	0.3	0.3
	AUS 10344 (MT)	0.3	0.2	1.3	1.6-1.0	0.3	6.3	1.5	1.5	0.9
B = 100										
393-25-1 (Ts)[a]	AUS 10348 (MS)	2.1	0.1	3.6	4.6-2.9	1.1	7.1	3.2	6.3	3.7
	AUS 13244 (S)	1.8	0.1	3.9	4.9-3.1	0.9	6.6	3.8	8.2	4.6
	393-112-1 (Ss)[a]	2.0	0.2	2.9	3.7-2.3	1.1	6.5	3.8	8.2	4.7
AUS 10110 (T)	393-112-1 (Ss)[a]	0.8	0.2	3.5	4.4-2.8	0.5	6.1	3.4	6.3	3.4
AUS 10344 (MT)	393-112-1 (Ss)[a]	0.6	0.2	2.8	3.6-2.2	0.4	5.5	2.7	4.0	2.2
B = 50										
AUS 10348 (MS)	393-112-1 (Ss)[a]	0.4	0.6	1.6	2.0-1.2	0.5	8.2	0.9	0.9	0.7
AUS 13244 (S)	393-112-1 (Ss)[a]	0.7	0.6	0.6	0.8-0.5	0.6	7.5	-0.2	0.7	0.7

[a] 237-5-1 = tolerant selection (Ts) and 237-49-1 (Ss) = sensitive selection

[b] E = environmental variance, m = mid-point between parents, d = departure of parents from the mid-point

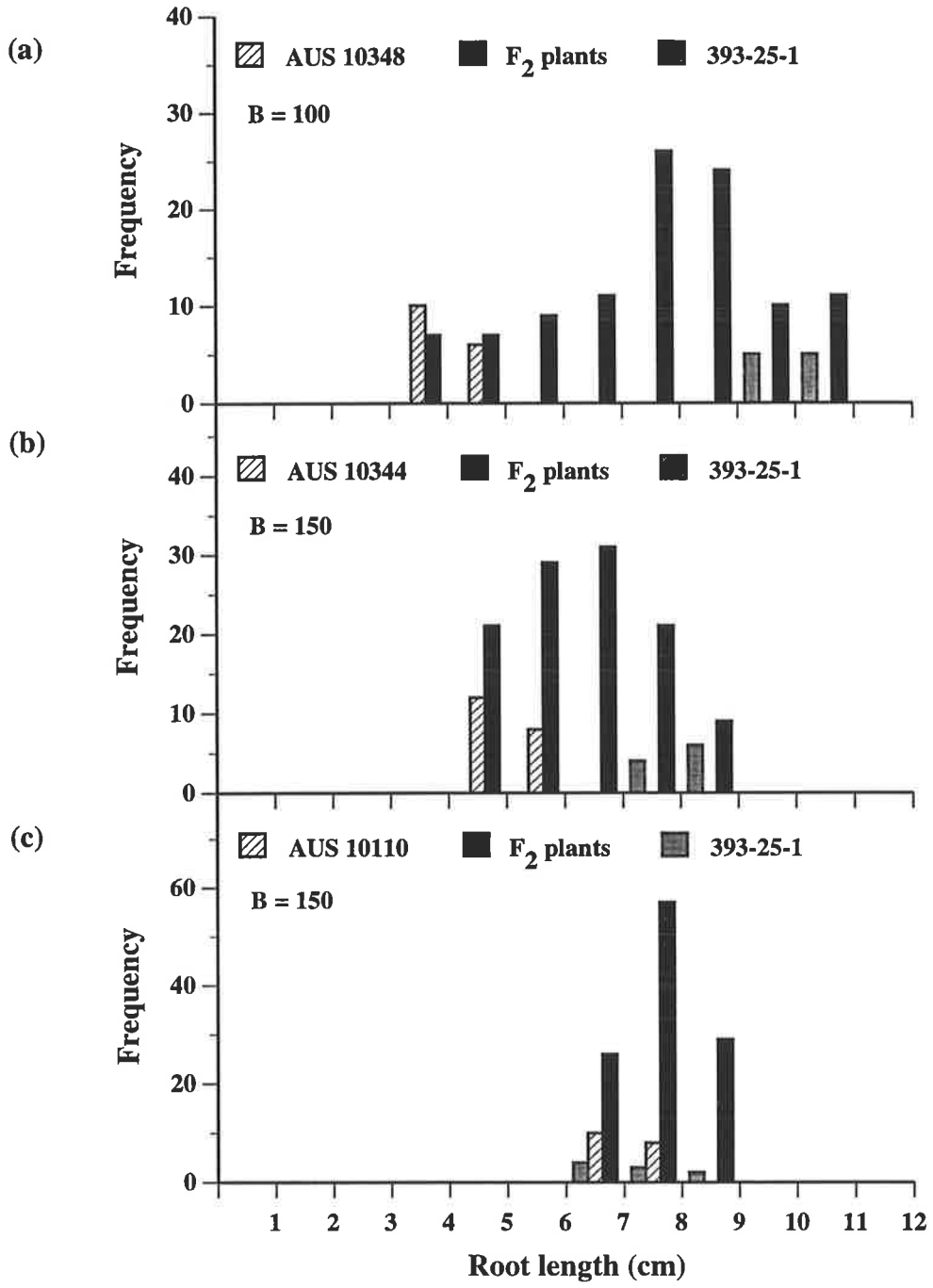


Figure 4.15 Response of parents and F_2 plants grown at B100 and B150 treatments for the crosses involving the tolerant selection (Ts) line 393-25-1,

(a) AUS 10348 (MS) x 393-25-1 (Ts); mean (SD in parentheses) for AUS 10348 and 393-25-1 were 3.8 (0.4) and 10.3 (1.8), respectively.

(b) AUS 10344 (MT) x 393-25-1 (Ts); mean (SD in parentheses) for AUS 10344 and 393-25-1 were 4.8 (0.5) and 7.9 (0.5), respectively.

(c) AUS 10110 (T) x 393-25-1 (Ts); mean (SD in parentheses) for AUS 10110 and 393-25-1 were 7.0 (0.5) and 7.2 (0.6), respectively.

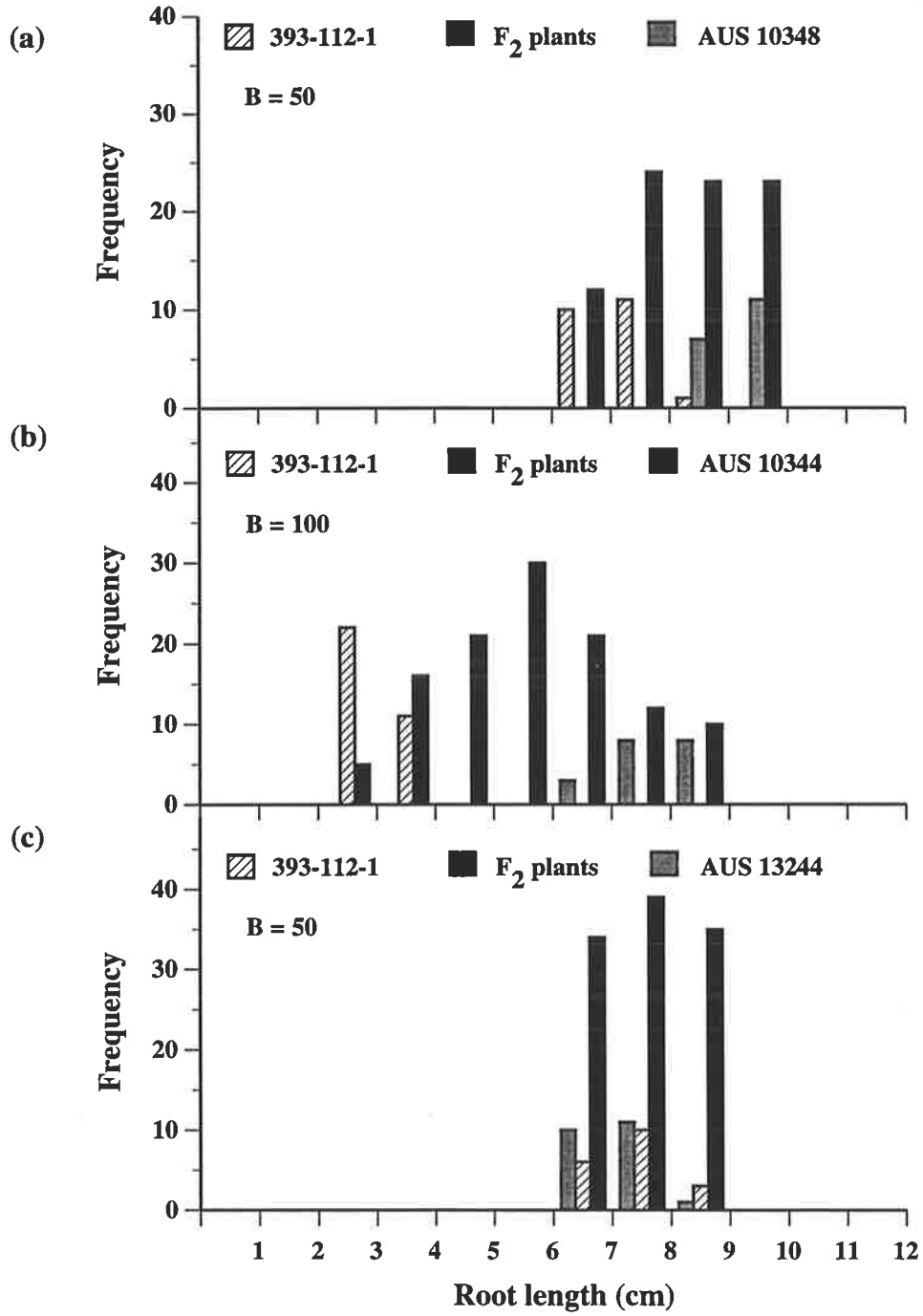


Figure 4.16 Response of parents and F_2 plants grown at B50 and B100 treatments for the crosses involving the sensitive selection (Ss) line 393-112-1,

(a) **AUS 10348 (MS) x 393-112-1 (Ss);** mean (SD in parentheses) for AUS 10348 and 393-112-1 were 9.2 (0.6) and 7.3 (0.8), respectively.

(b) **AUS 10344 (MT) x 393-112-1 (Ss);** mean (SD in parentheses) for AUS 10344 and 393-112-1 were 8.2 (0.8) and 2.8 (0.4), respectively.

(c) **AUS 13244 (S) x 393-112-1 (Ss);** mean (SD in parentheses) for AUS 13244 and 393-112-1 were 7.3 (0.9) and 7.7 (0.8), respectively.

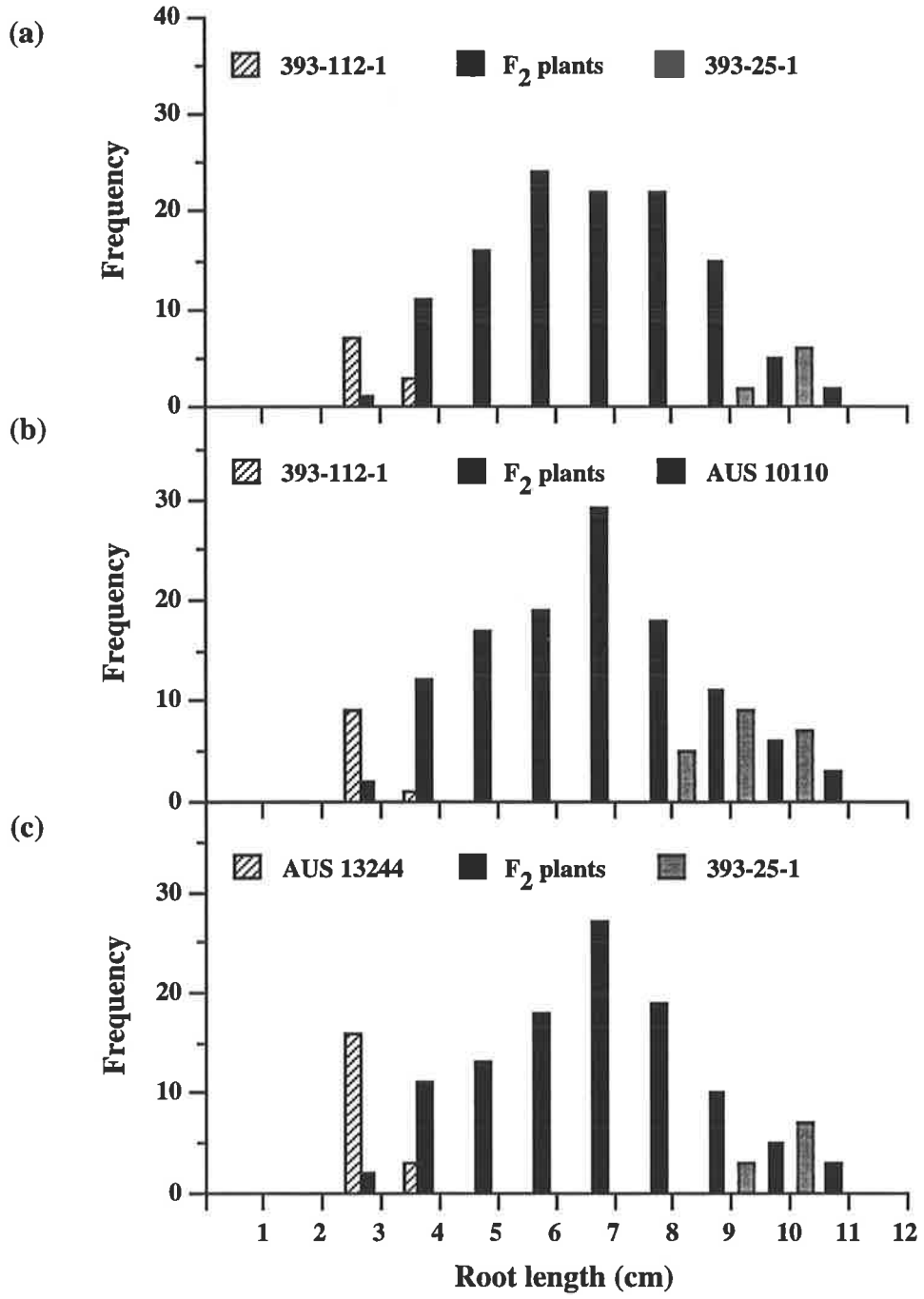


Figure 4.17 Response of parents and F_2 plants grown at B100 treatment for the crosses involving the tolerant (Ts) and sensitive (Ss) selected lines 393-25-1 and 393-112-1, repectively,

(a) **393-25-1 (Ts) x 393-112-1 (Ss)**; mean (SD in parentheses) for 393-25-1 and 393-112-1 were 10.3 (1.4) and 2.7 (0.4), respectively.

(b) **AUS 10110 (T) x 393-112-1 (Ss)**; mean (SD in parentheses) for AUS 10110 and 237-112-1 were 9.5 (0.9) and 2.7 (0.4), respectively.

(c) **393-25-1 (Ts) x AUS 13244 (S)**; mean (SD in parentheses) for 393-25-1 and AUS 13244 were 10.4 (1.3), and 2.7 (0.3), respectively.

4.5.4 Discussion

The segregation ratios measured from F_2 and F_3 populations derived from seven durum genotypes indicated that the parents differed for a number of genes controlling response to high concentrations of B (Section 4.4). The comparison of observed and expected variances and chi-square analyses of segregation ratios demonstrated a single gene difference between the tolerant and moderately tolerant genotypes and two genes difference between tolerant and moderately sensitive genotypes. If the full range in response to B were controlled by three loci and each locus consisted of either one allele conferring tolerance or another allele conferring sensitivity, it can be proposed that adjacent levels of B tolerance could result from allelic variation at one locus.

The number of tolerant genes of the parental lines were expected to be three, two and one for the tolerant (T), moderately tolerant (MT) and moderately sensitive (MS) lines, respectively. The sensitive (S) line was expected to have all sensitive loci, as shown below. The results of Section 4.3 indicated that the response of the F_1 hybrids was intermediate to the parents, therefore it is not possible to classify the alleles conferring tolerance or sensitivity as dominant or recessive. However, the notation proposed by Paull (1990) is followed here whereby the upper case is assigned to the tolerant allele as this is the desired type on B toxic soils.

Parental lines	Response to B	Genotypes
AUS 10105	Tolerant (T)	AABBCC
AUS 10110	Tolerant (T)	AABBCC
AUS 10344	Moderately tolerant (MT)	AABBcc or AAbbCC or aaBBCC
AUS 14010	Moderately tolerant (MT)	AABBcc or AAbbCC or aaBBCC
AUS 10348	Moderately sensitive (MS)	AAbbcc or aaBBcc or aabbCC
Yallaroi	Moderately sensitive (MS)	AAbbcc or aaBBcc or aabbCC
AUS 13244	Sensitive (S)	aabbcc

The number of genes expected to segregate when these genotypes were crossed in all combinations is shown in Table 4.5.5. It would be expected that transgressive segregation might occur within and between the MT and MS genotypes. The results from F_2 and F_3 populations (Section 4.4) showed that transgressive segregation was detected from the crosses AUS 10344 (MT) x AUS 14010 (MT), AUS 10344 (MT) x AUS 10348 (MS) and AUS 10348 (MS) x Yallaroi (MS). Based on the above model, and data presented in Table 4.5.5, the probable genotypes of the parents of crosses producing transgressive segregation are:-

1) if AUS 10344 (MT) = AABBcc, then AUS 14010 (MT) = AAbbCC or aaBBCC
2) if AUS 10344 (MT) = AAbbCC, then AUS 14010 (MT) = aaBBCC or AABBcc
3) if AUS 10344 (MT) = aaBBCC, then AUS 14010 (MT) = AAbbCC or AABBcc
4) if AUS 10348 (MS) = AAbbcc, then Yallaroi (MS) = aaBBcc or aabbCC
5) if AUS 10348 (MS) = aaBBcc, then Yallaroi (MS) = aabbCC or AAbbcc
6) if AUS 10348 (MS) = aabbCC, then Yallaroi (MS) = aaBBcc or AAbbcc

When eight genotypes were crossed in all possible combinations (Table 4.5.5) and compared to the occurrence of transgressive segregation for three crosses initially found in Section 4.4, it can be proposed that AUS 10344 (MT) = AAbbCC, AUS 14010 (MT) = aaBBCC, AUS 10348 (MS) = aaBBcc and Yallaroi (MS) = aabbCC.

Based on the above hypothesis, the cross between AUS 10344 (MT) x AUS 14010 (MT) would produce the most tolerant genotype AABBCC (237-5-1) and moderately sensitive genotype aabbCC (237-49-1). Segregation of the F_2 populations showed that the tolerant selection (237-5-1) and the sensitive selection (237-49-1) were both different from AUS 10344 (MT) and AUS 14010 (MT) at a single locus. There was no segregation in F_2 populations of the crosses AUS 10110 (T) x 237-5-1 and Yallaroi (MS) x 237-49-1. These suggested that the genotypes of the tolerant selection (237-5-1) is the same as AUS 10110 (T) and the sensitive selection (237-49-1) as Yallaroi (MS).

Table 4.5.5 Number of genes at which segregation is expected for crosses among eight homozygous genotypes.

Response	Tolerant	Moderately tolerant			Moderately sensitive			Sens.
Genotype	AABBCC	AABBcc	AAbbCC	aaBBCC	AAbbcc	aaBBcc	aabbCC	aabbcc
AABBCC	**0**	1	1	1	2	2	2	3
AABBcc	1	**0**	2**	2**	1	1	3**	2
AAbbCC	1	2**	**0**	2**	1	3**	1	2
aaBBCC	1	2**	2**	**0**	3**	1	1	2
AAbbcc	2	1	1	3**	**0**	2**	2**	1
aaBBcc	2	1	3**	1	2**	**0**	2**	1
aabbCC	2	3**	1	1	2**	2**	**0**	1
aabbcc	3	2	2	2	1	1	1	**0**

** Transgressive segregation to be expected.

When AUS 10344 (MT) and AUS 10348 (MS) were intercrossed, it would be expected that the extreme level of tolerance of AABBCC and sensitivity of aabbcc would be produced. The results showed that the mean root lengths of the tolerant selection (393-25-1) and the sensitive selection (393-112-1) were significantly different from both parents. Segregation of F_2 populations showed that no segregation was found in the crosses between the tolerant and sensitive check lines and the tolerant and sensitive selections, respectively, namely, AUS 10110 (T) x 393-25-1 and AUS 13244 (S) x 393-112-1. These suggested that the genotypes of the tolerant selection (393-25-1) and sensitive (393-112-1) selections are the same as AUS 10110 (T) and AUS 13244 (S), respectively, confirming the above hypothesis.

In conclusion, the crosses between selected genotypes and the parental lines in this section supported the hypothesis of three genes controlling B tolerance in durum wheat.

For the progeny derived from AUS 10344 (MT) x AUS 14010 (MT), single gene segregation was found with the crosses (tolerant selection x AUS 10344 (MT) or AUS 14010 (MT), sensitive selection x AUS 10344 (MT) or AUS 14010(MT)). For the cross derived from AUS 10344 (MT) x AUS 10348 (MS), single gene segregation in the F_2 population was found for the tolerant selection x AUS 10344 (MT). Two genes segregation was found in the tolerant selection x AUS 10348 (MS) and the sensitive selection x AUS 10344 (MT). These data were supported by the results of the F_2 populations derived from extreme phenotypes (Figure 4.17). These crosses exhibited the same pattern of F_2 distribution, as the largest range of distribution in the previous section (Section 4.4) between AUS 10110 (T) x AUS 13244 (S) and AUS 10105 (T) x AUS 13244 (S) which was hypothesised to be a difference of three genes (Figure 4.3a and 4.4a). Therefore it can be deduced that the genotypes of these lines are as follow;

237-5-1 (Ts), 393-25-1 (Ts), AUS 10110 (T)	AABBCC
AUS 10344 (MT)	AAbbCC
AUS 14010 (MT)	aaBBCC
AUS 10348 (MS)	aaBBcc
237-49-1 (Ss), Yallaroi (MS)	aabbCC
393-112-1 (Ss), AUS 13244 (S)	aabbcc

4.6 Discussion

The response of durum wheat to B was measured as a quantitative character, by the length of roots when seedlings were grown in a high B medium. However, the inheritance of response to B was analysed by both quantitative and qualitative approaches. Determining the number of genes conferring tolerance to B was based on the assumptions of independent assortment, no maternal effect and no multiple alleles.

From the response of F_1 hybrids and segregation in the F_2 and F_3 generations derived from seven parental lines and selected transgressive segregants, it can be concluded that under high B conditions, expression of tolerance in durum wheat, as measured by root length, is determined by at least three nuclear genes, acting additively. However, gene action was influenced by the external B concentrations. Tolerance to B was inherited by additive gene action at the B100 treatment for the T x MS, T x S, MT x MS and MT x S crosses and B150 for the T x MT crosses, but at other treatments, tolerance was expressed as an incompletely dominant trait.

The range of response to B in durum was narrower than that found in bread wheat (Chapter 3). The results from this chapter verify that this might result from durum having fewer loci responsible for B tolerance than bread wheat. Paull et al. (1991a) studied the number of genes conferring B tolerance in F_2 and F_3 populations derived from five bread wheat varieties, ranked between tolerant to very sensitive, and demonstrated that B tolerance in bread wheat was controlled by at least four genes, designated *Bo1* to *Bo4*. The tolerant bread wheat, G61450 was defined as *bo1bo1 Bo2Bo2 Bo3Bo3 Bo4Bo4* and the moderately tolerant, Halberd, was defined as *Bo1Bo1 Bo2Bo2 Bo3Bo3 bo4bo4*. Chantachume (1995) investigated the genetics of B tolerance involving exotic bread wheat lines and found that the very tolerant lines were likely to have another gene, *Bo5,* in addition to those found by Paull et al. (1991a).

Based on the studies for tolerance to high concentration of B in bread wheat, the allele

symbols for B tolerance in durum wheat have been assigned as Bo_T and bo_T, for tolerant and sensitive respectively. The subscript $_T$ have been added into the gene symbol *Bo* assigned in bread wheat by Paull et al. (1991b) to indicate the B tolerance genes that had been identified in tetraploid wheat (R.A. McIntosh pers comm.). The genotypes of lines used in this study are shown in Table 4.6.1.

Table 4.6.1 Proposed genotypes of seven durum lines for genetic control of response to high concentrations of B.

Parental lines	Response to B	Genotypes
AUS 10105	Tolerant (T)	Bo_T1Bo_T1 Bo_T2Bo_T2 Bo_T3Bo_T3
AUS 10110	Tolerant (T)	Bo_T1Bo_T1 Bo_T2Bo_T2 Bo_T3Bo_T3
AUS 10344	Moderately tolerant (MT)	Bo_T1Bo_T1 bo_T2bo_T2 Bo_T3Bo_T3
AUS 14010	Moderately tolerant (MT)	bo_T1bo_T1 Bo_T2Bo_T2 Bo_T3Bo_T3
AUS 10348	Moderately sensitive (MS)	bo_T1bo_T1 Bo_T2Bo_T2 bo_T3bo_T3
Yallaroi	Moderately sensitive (MS)	bo_T1bo_T1 bo_T2bo_T2 Bo_T3Bo_T3
AUS 13244	Sensitive (S)	bo_T1bo_T1 bo_T2bo_T2 bo_T3bo_T3

As B tolerance in durum wheat is controlled by major genes, the backcross method is the most efficient for transferring B tolerance from the tolerant exotic lines into sensitive adapted varieties. This method has been used successfully to create the B tolerant bread wheat, BT-Schomburgk (Moody et al., 1993) with a single gene, *Bo1*, transferred from Halberd to Schomburgk. A yield advantage of BT-Schomburgk over the recurrent parent, was evident throughout areas of South Australia where high concentrations of B are prevalent (Campbell et al., 1994).

B tolerance was not identified among modern varieties of durum wheat, but in the landrace germplasm originating from Asian countries (Chapter 3). A program for breeding B tolerant durum wheat has been initiated at the Waite Institute and AUS 14010 (MT) and AUS 10105 (T) used as donor parents for backcrossing to Yallaroi

(B. Brooks, pers comm).

The transfer of B tolerance gene(s) from the landrace parents into the adapted cultivars may be accompanied by undesirable genes, referred to as linkage drag. Even after many generations of backcrossing and selection, selected genes are often accompanied by linked DNA segments large enough to carry hundreds of genes (Young and Tanksley, 1989) some of these may be undesirable. Therefore, the identification of the chromosomal locations (Chapter 5) and of markers linked to B tolerance gene(s) (Chapter 6) were conducted using both cytogenetics and molecular markers. If linkage between B tolerance and the other markers is identified, it would be useful for breeding through marker assisted selections, and enabling a reduction in the number of generations required to minimize the linkage drag.

Chapter 5

Chromosomal locations of genes conferring B tolerance in durum wheat

5.1 Aneuploid analysis in wheat

5.1.1 Introduction

Bread wheat (*Triticum aestivum* L. em. Thell 2n = 6x = 42, genome AABBDD) and durum wheat (*Triticum turgidum* L. var *durum* 2n = 4x = 28, genome AABB) belong to the genus Triticum of the Triticeae tribe. The wild ancestor of *T. turgidum* is a progenitor of *T. aestivum*. Bread wheat is proposed to have originated from hybridization between one, or a few, genotypes of tetraploid species (AABB) and the diploid *Triticum tauschii* (2n = 2x = 14, genome DD) (McFadden and Sears, 1946). Evidence of homoeology between genomes was first demonstrated by the ability of chromosomes to compensate for one another in nullisomic-tetrasomic combinations (Sears, 1966). Homoeology has also been shown by using telocentric (Riley and Chapman, 1958), morphological, biochemical and molecular markers (Sharp et al., 1989).

Sears (1954) produced a number of aneuploids in the bread wheat cultivar Chinese Spring (CS) including, nullisomics, monosomics, trisomics, tetrasomics, telosomics, isosomics and nullisomic-tetrasomics (Table 5.1.1). These aneuploid lines can be used to locate genes to chromosomes (Sear, 1954; Law et al, 1987). In durum wheat, a number of aneuploids have also been produced but used less extensively, compared to those in bread wheat (Joppa, 1993). The procedure of monosomic analysis in bread wheat is reviewed because aneuploid analysis in durum wheat, using Langdon (LDN) D-genome disomic substitution lines, is a similar procedure.

Table 5.1.1 Nomenclature of various chromosome numbers and pairing in aneuploids of hexaploid and tetraploid wheat (derived from Joppa, 1987; 1993).

Types	Chromosome pairing	
	Hexaploid	Tetraploid
Nullisomic	20^{II}	13^{II}
Monosomic	$20^{II}+1^{I}$	$13^{II}+1^{I}$
Disomic	21^{II}	14^{II}
Trisomic	$20^{II}+1^{III}$	$13^{II}+1^{III}$
Tetrasomic	$20^{II}+1^{IV}$	$13^{II}+1^{IV}$
Nullisomic-tetrasomic	$19^{II}+1^{IV}$	$12^{II}+1^{IV}$
Monotelosomic	$20^{II}+t^{I}$	$13^{II}+t^{I}$
Ditelosomic	$20^{II}+t^{II}$	$13^{II}+t^{II}$
Monotelodisomic	$20^{II}+1t^{II}$	$13^{II}+1t^{II}$
Double monotelosomic	$20^{II}+t^{I}+t^{I}$	$13^{II}+t^{I}+t^{I}$
Double ditelosomic	$20^{II}+t^{II}+t^{II}$	$13^{II}+t^{II}+t^{II}$
Dimonotelosomic	$20^{II}+t^{II}+t^{I}$	$13^{II}+t^{II}+t^{I}$
Monosomic addition	$21^{II}+1^{I}$	$14^{II}+1^{I}$
Disomic addition	$21^{II}+1^{II}$	$14^{II}+1^{II}$
D-genome monosomic substitution	-	$13^{II}+1^{L}+1D^{I}$
D-genome disomic substitution	-	$13^{II}+1D^{II}$

5.1.2 Monosomic analysis

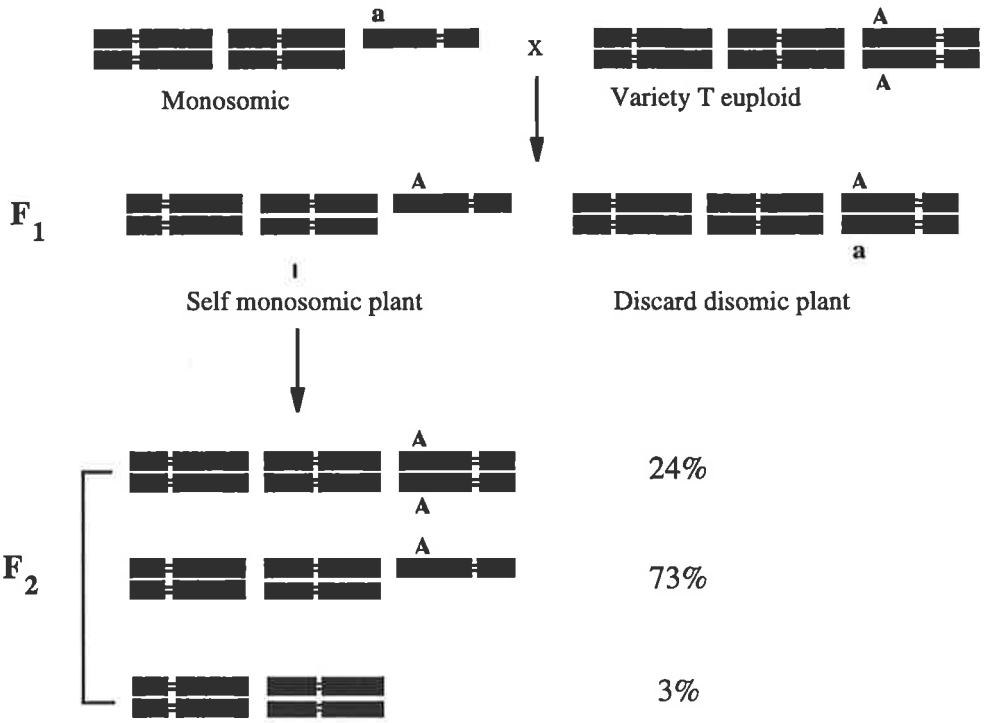
In bread wheat, monosomics can be used to determine the chromosome carrying a particular gene (Law et al., 1987). When a variety for which a set of monosomics is available has a phenotype contrasting with another variety, crosses can be made between the monosomic series as the maternal parent and the tested genotype as a pollinator. The F_1 hybrids will be a mixture of $20^{II}+1^{I}$ and 21^{II}, depending upon the transmission of the monosomic chromosome to the egg (Figure 5.1). In case of a monosomic F_1 hybrid, the hemizygous chromosome is derived from the variety under investigation. F_1 hybrid plants are examined for their chromosome complement and monosomic plants selected. When the gene of interest is dominant, all of the F_1 monosomic hybrids will have the dominant genotype. Variation among the hybrid monosomic families will be expressed in the F_2 generation due to the difference in transmission frequencies between 20^{I} and 21^{I} gametes as either pollen or egg cells. About 4% of pollen cells and 75% of eggs carry 20 chromosomes. Therefore, the F_2 of a selfed monosomic F_1 hybrid could be expected to consist of 24% of disomics (21^{II}), 73% of monosomics ($20^{II}+1^{I}$) and 3% of nullisomics (20^{II}). If a trait under investigation is controlled by a single gene, 20 F_2 populations of the 21 F_2 monosomics series will segregate in the ratio 75% dominant to 25% recessive phenotypes. For the critical cross, all the disomics and monosomics will carry the donor dominant gene and only the nullisomics will lack this gene and have the recessive phenotype, thus the ratio of 97% dominant to 3% recessive is expected (Figure 5.1).

If two genes are involved, monosomic analysis may detect two chromosomes carrying the genes responsible. For example, if the genes are independent, both in their actions and locations, two of the F_2 monosomics families will segregate at an aberrant ratio with none or very few plants with the recessive phenotype being observed and thereby be identified as the critical crosses. The disomic F_2 and the remaining 19 F_2 monosomic families will segregate at the digenic segregation ratio of 15:1 (Law et al., 1987).

Figure 5.1 Diagram showing the procedure of monosomic analysis in bread wheat. A variety 'T' containing the dominant gene AA, is crossed to a monosomic series where plants are of the genotype a or aa, depending upon whether they are monosomic for the critical or background chromosome.

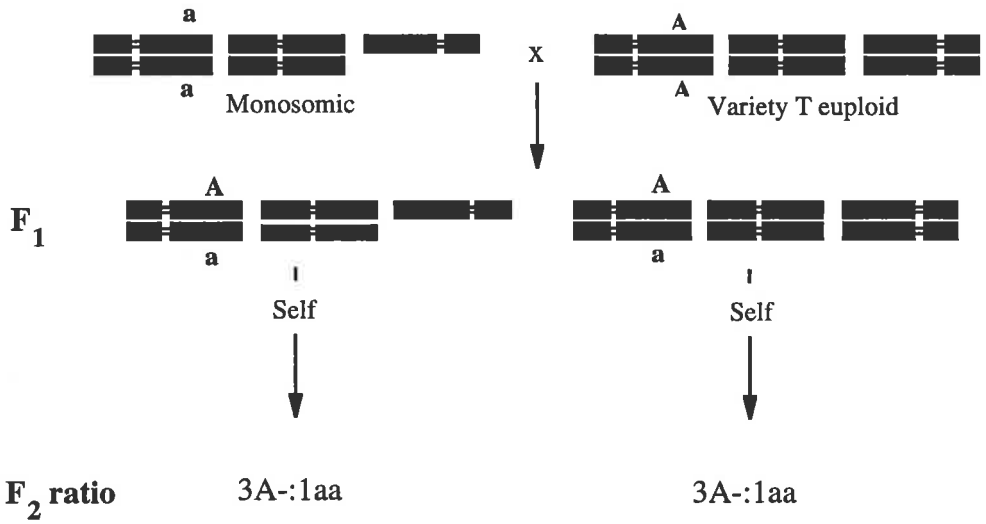
In the F_2, a ratio of 97 : 3 of the dominant phenotype is expected for the critical chromosome but 3 : 1 for the non-critical chromosome.

Critical cross



F_2 ratio 97A-:3--(aa)

Non critical cross



F_2 ratio 3A-:1aa 3A-:1aa

5.1.3 Aneuploid analysis in durum wheat by Langdon (LDN) D-genome disomic substitution lines

The development of several sets of aneuploids in durum wheat has been described by (Giorgi et al., 1978, cited by Joppa, 1987)
Joppa (1987; 1993). These included monosomic, trisomic, D-genome monosomic and disomic substitution, double ditelosomic and dimonotelosomic lines (Table 5.1.1). A complete set of monosomics could not be produced because plants cannot tolerate the deficiency of a whole chromosome so many monosomics had poor vigor and low fertility. Furthermore, the monosomics of tetraploid wheat had very low transmission rates of n-1 gametes (13 chromosomes) through both male and female gametes. Alternatively, whole chromosomes can be added to tetraploid wheat with the full set of trisomics having been produced in the cultivar Cappelli (Joppa, 1987). Other aneuploids currently available in tetraploid wheat are the complete sets of D-genome monosomic and disomic substitution lines, double ditelosomic and a partial set of dimonotelosomics (Joppa, 1993).

The D-genome monosomic substitution lines developed by Joppa and Williams (1977) have been used to locate genes controlling stem rust resistance to chromosomes 2B, 3B and 7A (Salazar and Joppa, 1981). However, the use of monosomic substitution lines in tetraploid wheat is more complicated than that for hexaploid wheat. These lines require cytological examination in each generation for their maintainance and for their use in genetic studies (Joppa and Williams, 1988). Therefore, the D-genome disomic substitution lines have been utilized more extensively than monosomic substitution lines for location of genes to chromosomes of tetraploid wheat. The procedures involved in the development and use of this aneuploid series were demonstrated by Joppa and Williams (1988) and Konzak and Joppa (1988).

Langdon D-genome disomic substitution lines, in which *T. aestivum* cv. Chinese Spring D-genome chromosomes replaced homoeologous A- or B- genome chromosomes of *T. turgidum* cv Langdon, were developed by Joppa and Williams

(1988). The procedure for the use of the LDN disomic substitution lines to determine the chromosomal location of a gene is similar to monosomic analysis in bread wheat. First, contrasting phenotypes between the alternative substitution lines and the genotype of interest must be identified. Then the set of LDN substitution lines are crossed to the tested genotype. The F_1 hybrids will be double monosomics, consisting of thirteen pairs and two univalent chromosomes ($13^{II}+2^{I}$). One of the univalents is an A- or B-genome chromosome from the tested variety and another univalent is the D-genome chromosome of Chinese Spring from the substitution line parent. When selfed, transmission of the monosomes varies from line to line and the male and female gametes have different transmission rates for the monosomes. Joppa and Williams (1988) examined more than 3000 progenies of double monosomics (Table 5.1.2) and calculated the expected frequency of various types of chromosome constitutions of the F_2 progeny (Table 5.1.3). For example, the mean F_2 will consist of 8% monosomics (6% $13^{II}+1^{I}$A/B and 2% $13^{II}+1^{I}$D), 34% double monosomics ($13^{II}+2^{I}$), 36% disomics (30% $13^{II}+1^{II}$A/B and 6% $13^{II}+1^{II}$D), 21% monosomic addition (15% $14^{II}+1^{I}$D and 6% $14^{II}+1^{I}$A/B) and 1% of a disomic addition (15^{II}). If a single dominant gene is located on an A- or B- genome chromosome from the donor tested genotype, 13 of the F_2 populations will segregate as 75% dominant to 25% recessive phenotypes. For the critical cross, most of the monosomic, double monosomic, disomic, monosomic addition and disomic addition plants will carry the dominant gene, but those monosomic and disomic for D-genome chromosomes ($13^{II}+1^{I}$D and $13^{II}+1^{II}$D) will lack this gene and, therefore, have the recessive phenotype (Figure 5.2). From Table 5.1.3, the mean ratio of 92% dominant to 8% recessive is to be expected. The expected ratio calculated by Joppa and Williams (1988) has been used for identifying chromosomal locations of genes controlling a number of characters such as chocolate chaff colour (Konzak and Joppa, 1988) and pest resistance (Amri et al., 1993).

Table 5.1.2 Observed female and male transmission (%) of monosomes in the progeny of double monosomics involving one A- or B-genome chromosome and a homoeologous D-genome chromosome (derived from Joppa and Williams, 1988).

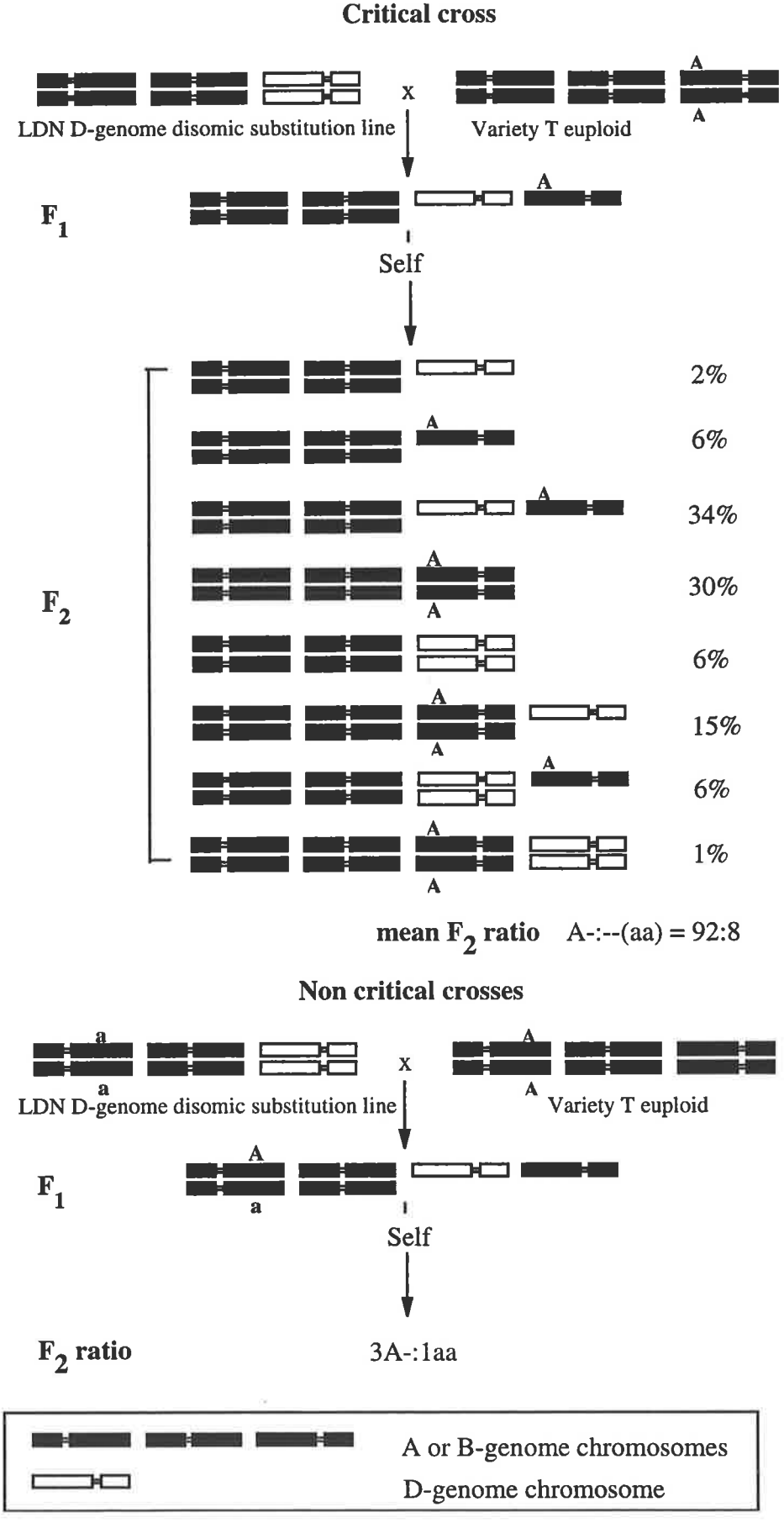
	Female				Male			
Double monosomic	none	D-genome	A- or B-genome	A- or B- and D-genome	none	D-genome	A- or B-genome	A- or B- and D-genome
1A+1D	3	39	44	14	1	26	72	2
1B+1D	1	38	45	15	0	37	60	3
2A+2D	3	41	52	4	0	27	67	7
2B+2D	1	39	38	22	3	26	63	9
3A+3D	7	39	37	18	0	25	72	3
3B+3D	5	36	39	21	0	12	78	1
4A+4D	1	30	45	24	0	3	79	19
4B+4D	2	36	74	18	0	53	41	6
5A+5D	13	30	35	22	0	7	80	14
5B+5D	2	38	43	16	0	10	87	4
6A+6D	38	29	26	7	0	25	69	6
6B+6D	12	24	49	14	1	1	85	13
7A+7D	14	31	39	17	0	15	82	2
7B+7D	2	35	39	24	0	9	81	11
Mean	8	35	40	17	0	19	73	8

Table 5.1.3 Expected frequencies (%) of meiotic chromosome pairing configurations in the zygotes of selfed progeny of double monosomic (derived from Joppa and Williams, 1988). The + and - indicate the presence of a gene located on the critical A- or B-genome chromosome and absent on the D-genome chromosome, respectively.

Double monosomic	13^{II} -	13^{II}+ $1^{I}A/B$ +	13^{II}+ $1^{I}D$ -	13^{II}+ $1^{I}+1^{I}$ +	13^{II}+ $1^{II}A/B$ +	13^{II}+ $1^{II}D$ -	14^{II}+ $1^{I}D$ +	14^{II}+ $1^{I}A/B$ +	15^{II} +	Expected ratio[a] +	-
1A+1D	0	3	1	40	31	10	11	4	0	**89**	**11**
1B+1D	0	1	1	40	27	14	11	7	1	**86**	**14**
2A+2D	0	2	1	42	35	11	6	4	0	**88**	**12**
2B+2D	0	2	1	35	24	10	17	9	2	**89**	**11**
3A+3D	0	5	2	37	27	10	14	6	1	**89**	**11**
3B+3D	0	4	1	33	30	4	20	6	2	**95**	**5**
4A+4D	0	1	0	25	36	1	27	6	4	**99**	**1**
4B+4D	0	1	1	38	18	19	10	12	1	**80**	**20**
5A+5D	0	10	1	28	28	2	22	6	3	**97**	**3**
5B+5D	0	2	0	38	38	4	16	3	1	**96**	**4**
6A+6D	0	27	10	28	18	7	7	4	0	**83**	**17**
6B+6D	0	11	0	23	42	0	18	3	2	**99**	**1**
7A+7D	0	11	2	31	32	5	15	3	0	**93**	**7**
7B+7D	0	1	0	32	31	3	24	6	3	**97**	**3**
Mean	0	6	2	34	30	6	15	6	1	**92**	**8**

[a] the expected ratio of + and - phenotype in F_2 generation.

Figure 5.2 Diagram showing the procedure of LDN D-genome disomic substitution lines analysis in durum wheat. A variety 'T' containing the dominant gene AA, is crossed to each of the 14 substitution lines having a contrasting phenotype to variety T.


Critical cross
A
A
x
LDN D-genome disomic substitution line
Variety T euploid
F1
A
Self
F2
2%
A
6%
A
34%
A
A
30%
6%
A
A
15%
A
A
6%
A
A
1%
mean F2 ratio A-:--(aa) = 92:8
Non critical crosses
a
a
A
A
x
LDN D-genome disomic substitution line
Variety T euploid
F1
A
a
Self
F2 ratio
3A-:1aa
A or B-genome chromosomes
D-genome chromosome

5.1.4 Identification of chromosomes belonging to the different genomes

A number of methods have been used to identify the specific complement of chromosomes present in an aneuploid of wheat. An unknown aneuploid can be crossed with characterized aneuploid stocks followed by an examination of chromosome pairing in the F_1 hybrids at meiosis to identify the critical chromosomes. Konzak and Joppa (1988) were able to identify $13^{II}+1^{II}$ of progeny between a mutant chocolate chaff x LDN 7D(7B) as being either $13^{II}+7B^{II}$ or $13^{II}+7D^{II}$ by backcrossing $13^{II}+1^{II}$ plants to the LDN disomic parent and cytologically examining the progenies. When the unknown plant carried $13^{II}+7B^{II}$ the F_1 hybrid produced 14^{II}, whereas for plants $13^{II}+7D^{II}$, the F_1 hybrid produced $13^{II} + 2^{I}$. Joppa et al. (1987) demonstrated that the *msg* gene found in $13^{II}+1^{I}+1t$ plants was located on chromosome 7A by crossing this phenotype to LDN double ditelosomic 7A lines and observing the chromosome pairing of the progeny.

Chromosomes can be identified if marker stocks with known locations are available. The availability of visual markers such as C-banding, N-banding, isozyme, protein and molecular markers have been used for the identification of individual chromosomes in wheat and its relatives (Gill et al., 1991a; McIntosh, 1987; Sharp et al., 1989). In particular, the recognition in wheat of restriction fragment length polymorphisms (RFLPs) often provides a more powerful implication of a specific chromosome than the use of other markers. More than 300 RFLP loci have been mapped to chromosomes of wheat and its relatives (Hart, 1994).

The applications of genetic markers in the cytogenetic manipulation of the wheat genomes was reviewed by Gale et al. (1989). Sharp et al. (1989) demonstrated the use of probes in the manipulation and analysis of wheat and alien chromosomes within wheat. A set of low copy number wheat RFLP probes was employed to identify homoeologous chromosomes of wheat and alien species. It was found that the number of fragments detected by the probe in the alien species was related to the ploidy level of

the species. For example, three bands were detected in hexaploid wheat, two in tetraploid and a single band was found in some of diploid species.

5.2 General materials and methods

Genotypes

Genotypes used in this chapter consisted of;

(1) *Standard durum genotypes:* AUS 10110 (T), AUS 10344 (MT), AUS 14010 (MT) and Yallaroi (MS) (see Chapters 3 and 4 for descriptions of response to B)

(2) *LDN D-genome disomic substitution lines and their parents, LDN and Chinese Spring:* Seeds of LDN D-genome disomic substitution lines were kindly provided by Dr L.R. Joppa, USDA-ARS and North Dakota State University, Fargo, North Dakota. The fourteen substitution lines were multiplied in standard potting mix in a glasshouse at the Waite Institute.

(3) *Chinese Spring substitution lines:* Substitution lines for homoeologous chromosomes group 7 from the barley cultivar Betzes chromosome 7H introduced into a Chinese Spring background were used. Seeds of Chinese Spring (barley cv. Betzes) lines; 7H(7A), 7H(7B) and 7H(7D) were kindly provided by Drs A.K.M.R. Islam and K.W. Shepherd, Department of Plant Science, Waite Agricultural Research Institute, South Australia.

The chromosome nomenclature of 4A and 4B was reassigned into 4B and 4A, respectively, according to the 7th International Wheat Genetics Symposium. Therefore, the LDN 4D(4A) and LDN 4D(4B) quoted in this study, refer to LDN 4D(4B) and LDN 4D(4A) of Joppa and Williams (1988), respectively.

Screening methods

The response to B was measured by the use of the filter paper method described in Chapter 3. The B treatments used are described in the materials and methods of the indiviual sections in this chapter.

Cytological methods

The chromosome pairing of the LDN disomic substitution lines parents, F_1 hybrids and F_2 plants derived from these parents were determined in pollen mother cells (PMCs) at metaphase I (MI). Spikes at the early boot stage were collected and a single anther from a floret was squashed in aceto-orcein stain and examined microscopically to determine the stage of cell division. When an anther at MI was identified, the remaining two anthers from the floret were fixed in 3 absolute ethanol : 1 glacial acetic acid for 24 h at 4 °C. The anthers were then hydrolyzed in 1N HCl at 60 °C for 10 min and stained with Feulgen stain for 1-2 h and squashed in 45% acetic acid.

RFLP procedures

Genetic materials were tested for restriction fragment length polymorphisms (RFLPs) by DNA probes specific to homoeologous group 7 to classifiy the A- and B- genome chromosomes of B tolerant durum parents and the D-genome chromosome of the substitution line parents. Procedures for DNA extraction, digestion and hybridzation are described fully in Chapter 6. Briefly, DNA was extracted from plants by a mini-prep procedure. DNA was digested with the restriction enzyme, *Dra*I (Promega). Digested DNA was electrophoresed on a 1.0% agarose (Pharmacia) gel in TAE buffer and transferred to Hybond-N^+ (Amersham) nylon membranes. DNA probes, PSR129 (long arm) and CDO595 (short arm), previously mapped to chromosomes of homoeologous group 7 (Chao et al., 1989; Heun et al., 1991) were kindly provided by the Australian Triticeae Mapping Initiative collection at the University of Sydney (Dr P.J. Sharp). PCR amplified inserts were labelled with [^{32}P]dCTP by random primer labelling (Feinberg and Vogelstein, 1983).

5.3 Response of Langdon (LDN) aneuploid parents to high concentrations of B

5.3.1 Introduction

B tolerance in durum wheat was found to be controlled by three major genes, *Bot1*, *Bot2* and *Bot3* (Chapter 4). The genes *Bot1* found in AUS 10344 (MT) and *Bot2* in AUS 14010 (MT) were responsible for the higher level of B tolerance than the Australian variety, Yallaroi (MS). When these two genes were combined together in AUS 10110 (T) and AUS 10105 (T), the highest level of tolerance was observed.

Most agronomically adapted durum varieties were rated as similar to the moderately B sensitive variety, Yallaroi (Chapter 3). To increase B tolerance in adapted varieties, it was proposed to incorporate either *Bot1* or *Bot2* into these varieties by backcrossing. Identification of the chromosomal location of these genes will increase the likelihood of identifying linked markers, which could assist in selection for B tolerance and decrease the number of backcrosses required to produce a tolerant form of the recurrent parent.

Aneuploid analysis with LDN D-genome disomic substitution lines was employed to locate genes *Bot1* and *Bot2*. There are several problems that might hinder determining the location of genes using this aneuploid set. First, the expected segregation ratio of the critical cross in F_2 analysis will depend on the number of genes differing between the substitution lines parents and the B tolerant parents (Table 5.1.3). Moreover, the aneuploid stocks possess a D-genome chromosome which itself might interfere with the gene expression of their progeny if the D-genome chromosome has a gene which is dominant or epistatic to the genes under investigation. Therefore, the initial experiments were undertaken to compare the response of the progeny of the 14 individual LDN disomic substitution lines and the parents, LDN and Chinese Spring, and four standard genotypes. The genetic relationships between LDN and the standard genotypes with respect to B tolerance were also examined.

5.3.2 Materials and methods

Parental screening

Fourteen LDN D-genome disomic substitution lines, LDN and Chinese Spring (CS) were tested for their response to high concentrations of B and compared to four standard B genotypes, AUS 10110 (T), AUS 10344 (MT), AUS 14010 (MT) and Yallaroi (MS). Seven seeds per replicate of each genotype were tested by the filter paper method, described in Chapter 3. The B treatments were arranged at three concentrations of 0, 50 and 100 mgB L^{-1}, designated as B0, B50 and B100, respectively. The B treatments and genotypes were arranged as a split plot design with two replications.

F_2 and F_3 screening

LDN was crossed to AUS 10110 (T), AUS 10344 (MT), AUS 14010 (MT) and Yallaroi (MS). The F_1 generation was grown in a glasshouse. The F_2 generation for each cross was tested in the B100 treatment and seedlings were transferred to normal soil to produce the F_3 generation. The F_3 generation was tested for response to B at the B100 treatment. About 100-130 families of each cross were tested, except AUS 10344 (MT) x LDN where 59 F_3 families were available. The F_3 seeds and parental genotypes were sown with 13 seeds of each F_3 family or parental line per paper. Root length was measured after 12 days.

Statistical analysis

The segregation for B tolerance of F_2 derived F_3 families was determined by using the comparison of expected and observed variances of the F_3 generation (described in Chapter 4). Individual F_2 derived F_3 families were assigned to the categories homozygous tolerant, homozygous intermediate, homozygous sensitive or segregating according to the mean and variance of the family in comparison to the parents. The

goodness of fit of the observed segregation ratio to the segregation expected for particular genetic models was analysed by chi-square analysis.

5.3.3 Results

Response of parents

There was significant variation in response to B among the LDN aneuploids and the standard check genotypes (Figure 5.3a). Although there was significant variation at the control treatment, the relative difference between the longest and shortest root length increased as the concentration of B increased (Figure 5.3b). At the B50 and B100 treatments, three standard genotypes AUS 10110 (T), AUS 10344 (MT) and AUS 14010 (MT), had significantly longer roots than the other genotypes. AUS 10110 (T) had the longest roots at both treatments. The root lengths of Yallaroi (MS), Chinese Spring, LDN and their 14 disomic substitution lines were not significantly different from each other at the two B treatments. These genotypes produced seedlings with root lengths in the range between 5-6 cm in B50 and 3-4 cm at B100 (Figure 5.3a and Plate 5.1).

Response of F_2 derived F_3 families

The comparison between the observed variance and the variances expected for one and two genes showed that the expected variances of the one gene model for AUS 10344 (MT) x LDN and AUS 14010 (MT) x LDN were within the range of the confidence intervals of the observed variance, indicating segregation for a single gene (Table 5.3.1). F_3 families from these crosses were classiffied into three categories, namely, homozygous tolerant, segregating and homozygous sensitive (Figure 5.4b and 5.4c). Chi-square analysis also confirmed segregation in a monogenic ratio for these two crosses (Table 5.3.2).

The expected variances for both the one and two genes models were not in the range of the confidence interval of the observed variances for the crosses AUS 10110 (T) x

LDN and Yallaroi (MS) x LDN. The observed variance of AUS 10110 (T) x LDN was closer to the expected variance of the two genes rather than the one gene model. When the F_3 families were classified into three categories and tested for goodness of fit with the expected ratio of a single gene (Table 5.3.2), a highly significant deviation (χ^2_2 = 52.1) was found. On the other hand, when the F_3 families were classified into four categories, namely, homozygous tolerant, homozygous intermediate, segregating and homozygous sensitive (Figure 5.4a), the observed frequency was consistent with the ratio expected for segregation at two genes (Table 5.3.2).

There was no significant difference between the observed variances of parents and the F_3 generation for the cross Yallaroi x LDN (Table 5.3.1). Also, there was no significant difference between mean root length of Yallaroi and LDN (d = -0.1). Therefore, LDN and Yallaroi are likely to have the same genotype with respect to B tolerance.

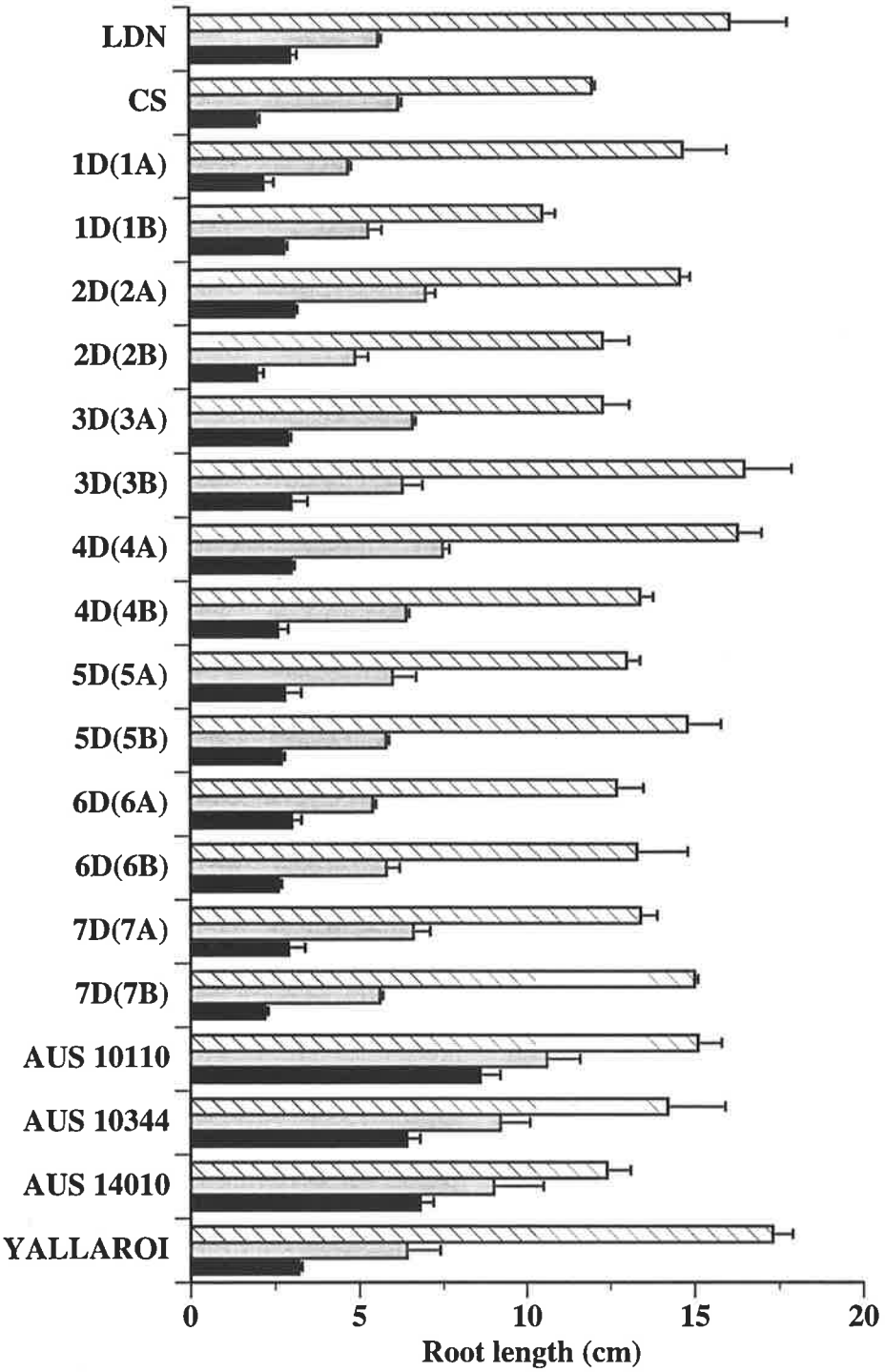


Figure 5.3a Root length (cm) of Langdon (LDN), Chinese Spring (CS), Langdon D-genome disomic substitution lines and check genotypes, grown in filter papers at the B0 (▨), B50 (▩) and B100 (■) treatments. Values are mean ±SE of two replicates.

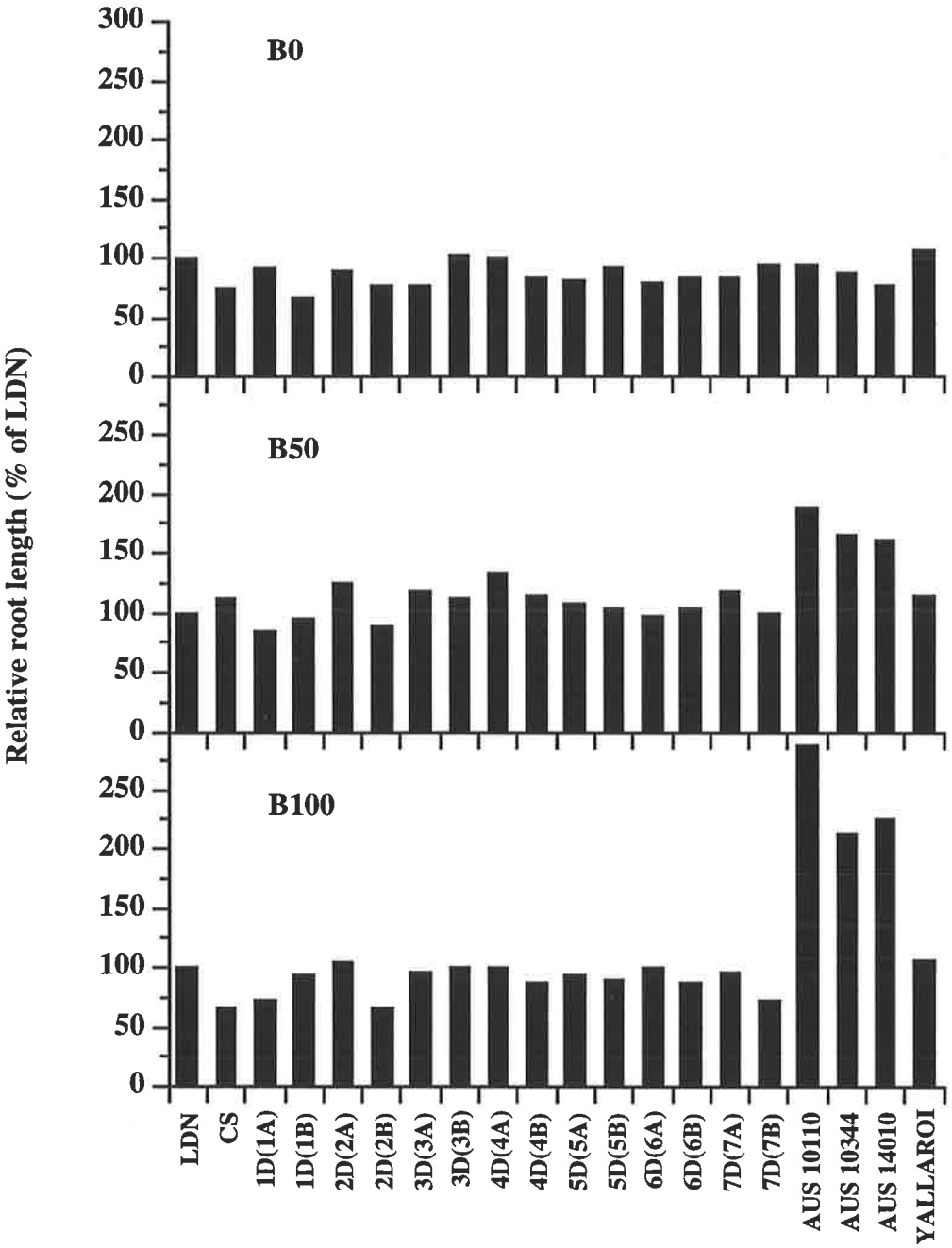


Figure 5.3b Root length (% of LDN) of Langdon (LDN), Chinese Spring (CS), Langdon D-genome disomic substitution lines and check genotypes, grown in filter papers at the B0, B50 and B100 treatments.

Plate 5.1 Respone of LDN, Chinese Spring, LDN D-genome disomic substitution lines and standard durum genotypes, tested at B100 treatment.

(a) Chinese Spring and LDN compared to substitution lines for A-genome chromosomes

From left to right: Chinese Spring, LDN, LDN 1D(1A), LDN 2D(2A), LDN 3D(3A), LDN 4D(4A), LDN 5D(5A), LDN 6D(6A) and LDN 7D(7A)

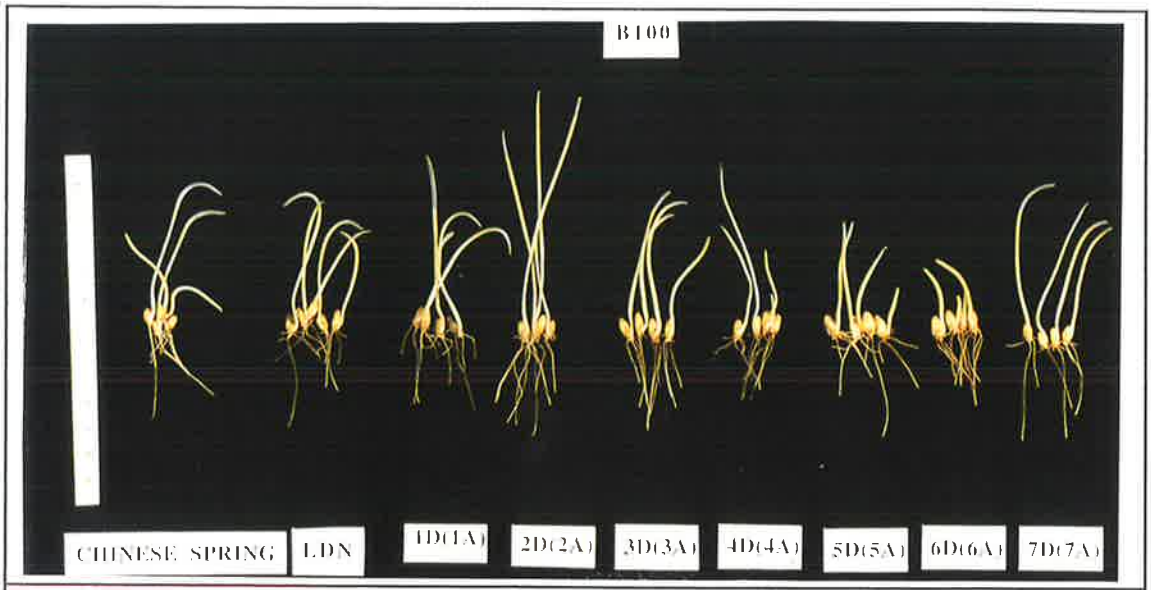
(b) Chinese Spring and LDN compared to substitution lines for B-genome chromosomes

From left to right: Chinese Spring, LDN, LDN 1D(1B), LDN 2D(2B), LDN 3D(3B), LDN 4D(4B), LDN 5D(5B), LDN 6D(6B) and LDN 7D(7B)

(c) Chinese Spring and LDN compared to standard genotypes

From left to right: AUS 10110 (T), AUS 10344 (MT), AUS 14010 (MT), LDN and Chinese Spring

(a)


B100
CHINESE SPRING
LDN
1D(1A)
2D(2A)
3D(3A)
4D(4A)
5D(5A)
6D(6A)
7D(7A)

(b)


B100
CHINESE SPRING
LDN
1D(1B)
2D(2B)
3D(3B)
4D(4B)
5D(5B)
6D(6B)
7D(7B)

(c)


B100
AUS 10110
AUS 10344
AUS 14010
LDN
CHINESE SPRING

Table 5.3.1 Observed variances of parents and F_2 derived F_3 populations, estimated parameters and the expected variance of F_3 tested at the B100 treatment.

Crosses		Observed variances				Estimated parameters[a]			Expected variance	
P_1	P_2	V_{P1}	V_{P2}	V_{F3}	CI[b]	E	m	d	1 gene	2 genes
AUS 10110 (T)	LDN	1.0	0.3	2.4	3.3-2.0	0.7	5.4	3.0	7.1	4.0
AUS 10344 (MT)	LDN	0.5	0.3	1.7	2.3-1.3	0.4	4.4	1.4	1.9	1.1
AUS 14010 (MT)	LDN	0.5	0.3	3.5	5.8-3.2	0.4	4.9	2.1	3.6	2.0
Yallaroi (MS)	LDN	0.5	0.7	0.7	1.0-0.5	0.6	3.9	-0.1	0.6	0.6

[a] E = Environmental variance, m = mid-point between parents, d = departure of parents from the mid-point

[b] CI = Confidence interval of observed F_3 variance

Table 5.3.2 Response of F_3 families from crosses AUS 10110 (T) x LDN, AUS 10344 (MT) x LDN, AUS 14010 (MT) x LDN and Yallaroi (MS) x LDN, tested at the B100 treatment.

Cross	Model	Number of F_3 families				χ^2_n	P
		Tol	Int	Seg	Sens		
AUS 10110 (T) x LDN	Obs	5	20	64	8		
	Exp 1:2:1	24.25	-	48.5	24.25	52.15	<0.01
	Exp 1:2:12:1	6.1	12.1	72.7	6.1	7.02	0.07
AUS 10344 (MT) x LDN	Obs	12	-	31	16		
	Exp 1:2:1	14.75	-	29.5	14.75	0.69	0.71
AUS 14010 (MT) x LDN	Obs	22	-	70	31		
	Exp 1:2:1	30.5	-	61	31	3.70	0.16
Yallaroi (MS) x LDN	Obs	no segregation observed					

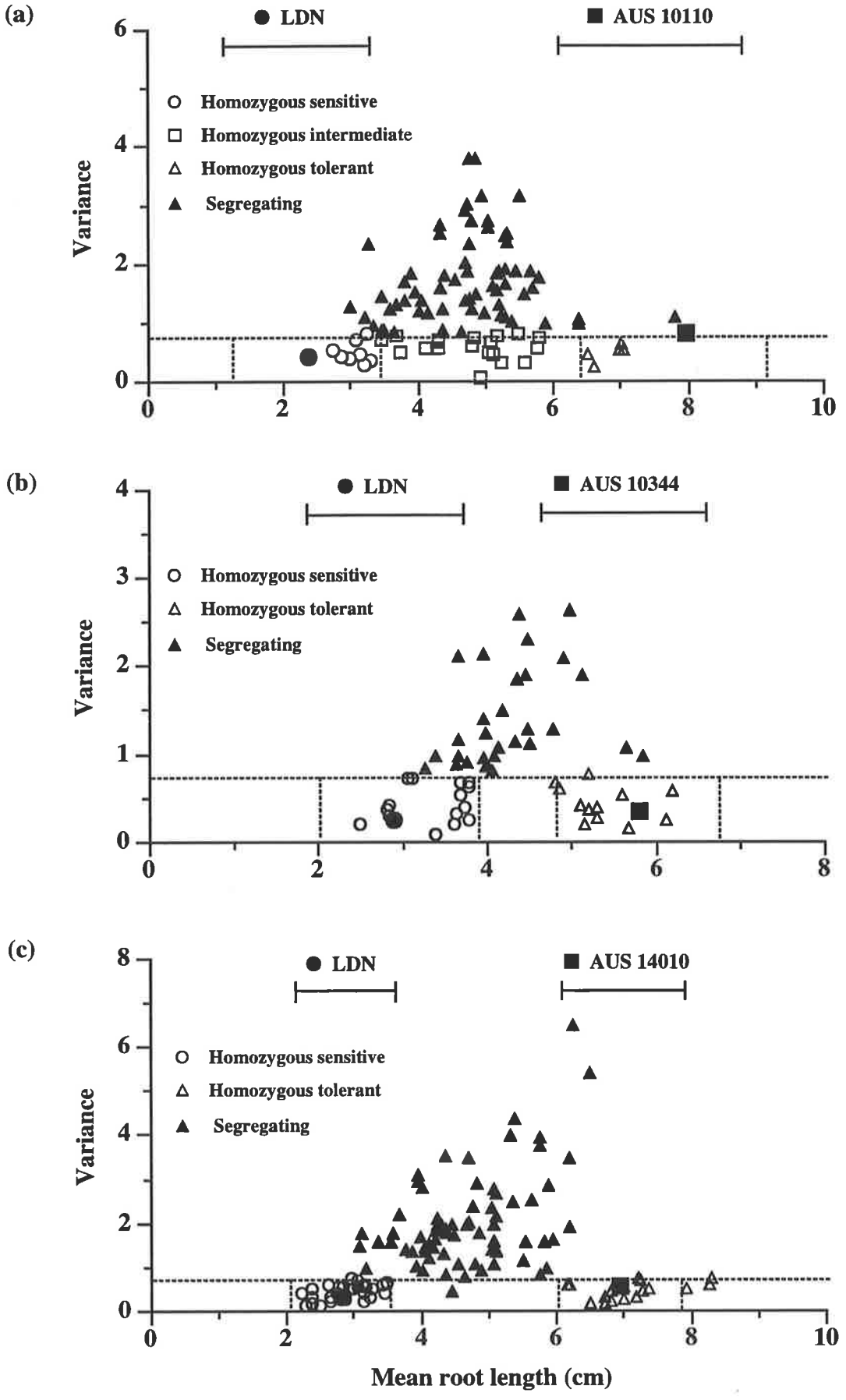
Expected ratios were 1 homozygous tolerant : 2 segregating : 1 homozygous sensitive, χ^2 df = 2 for a single gene and, 1 homozygous tolerant : 2 homozygous intermediate : 12 segregating : 1 homozygous sensitive, χ^2 df = 3 for two genes.

Figure 5.4 Mean root length and variances of parents and F_3 families of

(a) **AUS 10110 x LDN;** the confidence intervals of the mean of AUS 10110 and LDN were 6.4-9.2 and 1.3-3.4 cm, respectively. LSD of parental variances was 0.80, indicated by the horizontal dashed line.

(b) **AUS 10344 x LDN;** the confidence intervals of the mean of AUS 10344 and LDN were 4.8-6.8 and 2.0-3.8 cm, respectively. LSD of parental variances was 0.74, indicated by the horizontal dashed line.

(c) **AUS 14010 x LDN;** The confidence intervals of the mean of AUS 14010 and LDN were 6.0-7.8 and 2.1-3.5 cm, respectively. LSD of parental variances was 0.77, indicated by the horizontal dashed line.


(a)
LDN
AUS 10110
Homozygous sensitive
Homozygous intermediate
Homozygous tolerant
Segregating
Variance
(b)
LDN
AUS 10344
Homozygous sensitive
Homozygous tolerant
Segregating
Variance
(c)
LDN
AUS 14010
Homozygous sensitive
Homozygous tolerant
Segregating
Variance
Mean root length (cm)

5.3.4 Discussion

Root lengths at high concentrations of B of LDN D-genome disomic substitution lines were similar to their parents, LDN and Chinese Spring, and all the lines were classified as moderately sensitive to B as was Yallaroi (MS). These results were consistent with soil screening as in high B soils in a glasshouse experiment, LDN and Yallaroi produced similar dry matter yields, symptoms of B toxicity and shoot B concentrations (Section 3.4). Paull (1990) reported that Chinese Spring was rated as moderately sensitive as was Schomburgk, which was not significantly different from Yallaroi (Section 3.2).

The growth of aneuploid lines might be affected by the aneuploid condition of the chromosomes, with responses such as sterility or inviable seed, this condition might also influence root growth (Murata, 1991). In this study, there was no evidence that the aneuploid condition affected root length when the lines were grown at high concentrations of B. Root lengths of the disomic substitution lines were not significantly different from each other and the parents at all levels of applied B. Maximum root growth of all genotypes was observed at the control treatment (B0), suggesting that the similar reduction in root length of the substitution lines at B50 and B100 was influenced by the sensitive alleles of LDN and/or Chinese Spring, rather than the aneuploid condition. The similarity of response of LDN, Chinese Spring and the substitution lines to high concentrations of B also indicated no effect of the D-genome chromosome on the response to B of the substitution lines. Therefore, a study of the chromosomal location of B tolerance genes in the tolerant lines by crossing to the LDN substitution lines could be undertaken.

The experiment examining the response to B of F_3 populations indicated that the difference between LDN and B tolerant lines was controlled by up to two loci. The lack of segregation in the F_3 generation between LDN and Yallaroi indicated that the same gene(s) were responsible for the response to B tolerance in the two parents.

Moreover, when LDN was crossed to three B tolerant check lines, a single gene segregation was found in AUS 10344 (MT) x LDN and AUS 14010 (MT) x LDN, and two genes in AUS 10110 (MT) x LDN. These ratios were also observed when Yallaroi was crossed to these three lines (Chapter 4). Following the proposed genotypes of Yallaroi and three B tolerant lines shown in Table 4.6.1, the genotype of LDN with respect to B tolerance is *boT1boT1 boT2boT2 BoT3BoT3*. As it was proposed in Chapter 4 that the genotypes of AUS 10344 (MT), AUS 14010 (MT) and AUS 10110 (T) were *BoT1BoT1 boT2boT2 BoT3BoT3*, *boT1boT1 BoT2BoT2 BoT3BoT3* and *BoT1BoT1 BoT2BoT2 BoT3BoT3*, respectively, the chromosomal location of *BoT1* and *BoT2* can be investigated by crossing the LDN D-genome disomic substitution lines to AUS 10344 (MT) and AUS 14010 (MT), respectively.

5.4 Chromosomal locations of genes conditioning tolerance to high concentration of B in three durum genotypes

5.4.1 Introduction

Previous experiments have demonstrated that significant differences exist between the root length of seedlings of the LDN D-genome disomic substitution lines and three B tolerant lines, AUS 10344 (MT), AUS 14010 (MT) and AUS 10110 (T) grown at high concentrations of B. These differences were likely to be the due to *Bo$_T$1* v *bo$_T$1* in AUS 10344 (MT) x LDN, *Bo$_T$2* v *bo$_T$2* in AUS 14010 (MT) x LDN and both genes in AUS 10110 (T) x LDN. Since it was found that the responses to B of LDN D-genome disomic substitution lines were similar to the LDN parent, it might be expected that the same segregation ratios would result when LDN substitution lines were crossed to these B tolerant parents with the exception of the critical cross which would segregate at an aberrant ratio. For example, segregation in the F_2 generation for a non-critical chromosome will result in a ratio of 3 tolerant plus intermediate to 1 sensitive, while the critical chromosome will deviate from a 3 to 1 ratio with an excessive number of tolerant plus intermediate phenotypes. The expected ratio for critical crosses would be expected to be vary with the substitution line parent, with an average of 92 : 8, and the 8% sensitive phenotypes being nullisomic for either an A- or a B- genome chromosome but monosomic or disomic for the homoeologous D-genome chromosome ($13^{II}+1^{I}D/1^{II}D$, Table 5.1.3).

The three B tolerant lines were crossed to the complete set of LDN D-genome disomic substitution lines and aneuploid analysis was employed in the F_2 generation to identify the chromosomal locations of gene(s) responsible for B tolerance. The crosses which deviated from the observed ratio of the control disomic cross were suspected to be the critical crosses. Further analysis for chromosome constitution of the individual F_2 plants of the suspected crosses was undertaken and their response to B was related to their chromosome complement.

5.4.2 Materials and methods

The fourteen LDN D-genome disomic substitution lines were used as females in crosses with B tolerant genotypes, AUS 10344 (MT), AUS 14010 (MT) and AUS 10110 (T). The F_1 hybrid plants were grown in a glasshouse. Pollen mother cells of all plants were examined at metaphase I (MI) of meiosis for their chromosome number. Plants showing 13 pairs plus two univalents ($13^{II}+2^{I}$) were selected to produce the F_2 generation.

The F_2 seeds from LDN D-genome disomic substitution lines x B tolerant lines were tested in filter paper soaked with 100 mgB L^{-1} (B100 treatment). Each paper contained 11 F_2 seeds plus two seeds of LDN and two seeds of the B tolerant parent. About 70-110 F_2 seeds and 14-22 seeds of each parent from each population were tested. The root length of each plant was measured at 12 days after sowing. The F_2 seeds from LDN 3D(3B) x AUS 10344 (MT), LDN 2D(2B) x AUS 10110 (T) and LDN 3D(3B) x AUS 10110 (T) were not available due to sterility of the F_1 plants.

As the response to B between AUS 10344 (MT) or AUS 14010 (MT) and LDN was conferred by a single gene (Section 5.2), the segregation ratio of the critical cross was expected to deviate from normal 3:1 ratio with an excess of tolerant plus intermediate plants. The expected segregation ratio of the critical cross for each substitution line was calculated according to the values derived by Joppa and Williams (1988) as shown in Table 5.1.3.

Response to B between AUS 10110 (T) and LDN was conferred by two genes (Section 5.2). F_2 plants from control and non critical crosses were expected to segregate in a ratio of 15 tolerant plus intermediate to 1 sensitive plant. If the two genes are located on different chromosomes, two critical crosses will be identified and each cross will segregate at a deviation from the digenic ratio with none or a very few sensitive plants. But if the two genes are located on the same chromosome, one critical cross would be identified which would segregate at the ratio of the critical cross calculated for a single

gene, presented in Table 5.1.3. The reason for segregation in a ratio consistent with a single gene is that the critical chromosome is transmitted as a single unit as there is virtually no recombination between the A- or B-genome chromosomes and their homoeologous D-genome chromosomes.

The LDN substitution lines x B tolerant lines were evaluated for response to high concentration of B in two stages. The initial testing included comparing the F_2 populations from the substitution lines to the control disomic (LDN x B tolerant lines) populations. For each cross, the mean and variance of the F_2 was compared to the parents. The F_2 plants were then classified into two categories, namely, tolerant plus intermediate and sensitive, according to their response in comparison to the parental checks, which were included with every cross. F_2 plants which had root lengths less than the mean plus one standard deviation of the sensitive parent, LDN, were initially classified as sensitive. The mean, variance and segregation ratio of the F_2 populations derived from substitution lines crosses were compared with the F_2 derived from the appropriate disomic control cross. The critical cross was expected to have a higher mean root length, lower variance and less sensitive F_2 plants than the control disomic cross.

The F_2 seedlings of the putative critical crosses were transplanted to low B potting mix. About two to three weeks after transplanting, 10 cm of leaf was harvested from each plant for DNA extraction and RFLP analysis to determine the chromosome present. At the early booting stage, pollen mother cells were examined at meiosis for their chromosome number. At maturity, seeds from individual plants were harvested to represent a derived F_3 family. F_2 derived F_3 families were tested for response to B at the B100 treatment. The F_3 seeds and parental genotypes were sown with 13 seeds of each F_3 family or parental line per paper. Root lengths were measured after 12 days.

5.4.3 Results

LDN D-genome substitution lines x AUS 10344 (MT)

Comparison of F_2 populations

The F_2 generation of LDN D-genome disomic substitution lines x AUS 10344 (MT) crosses were assessed for response to a high concentration of B. Mean root length of the parental checks, LDN and AUS 10344 (MT), were significantly different for every cross examined and the mean root length of the F_2s were intermediate to the parents (Table 5.4.1). F_2 plants were then classified into two categories, tolerant plus intermediate and sensitive. The segregation ratios of the control disomic LDN x AUS 10344 (MT) and most of the substitution lines crosses, except 2D(2B), 6D(6B) and 7D(7B), were consistent with a monogenic ratio of 3 tolerant plus intermediate to 1 sensitive (χ^2 = 0.11 to 2.04, Table 5.4.2).

The crosses from 2D(2B) and 6D(6A) deviated from the ratio of 3 tolerant plus intermediate to 1 sensitive (χ^2 = 6.58 and 6.18, respectively), but the fact that both crosses produced an excess of sensitive plants is evidence that the B tolerance gene is not located on either chromosome 2B or 6A. Segregation of the F_2 from the cross including 7D(7B) significantly deviated from the monogenic ratio of 3 tolerant plus intermediate to 1 sensitive (χ^2 = 29.10) and included a low number of sensitive plants. Confirmation that this cross produced the most tolerant plus intermediate plants was shown by the highest mean and mean/variance ratio of all the F_2s (Table 5.4.2). This indicates that the gene for B tolerance was probably located on chromosome 7B of AUS 10344 (MT).

Table 5.4.1 Mean response of tolerant and sensitive check parents and the F_2 populations from crosses between LDN D-genome disomic substitution lines and disomic LDN and a B tolerant line AUS 10344, grown at 100 mgB L^{-1}.

Female parents	AUS 10344 (Tolerant)[a]			LDN (Sensitive)			F_2		
(x AUS 10344)	mean	SD	no	mean	SD	no	mean	SD	no
LDN D-genome disomic substitution lines									
1D(1A)	8.4	1.1	18	4.1	0.6	20	6.9	1.9	110
1D(1B)	8.2	1.0	16	4.2	0.8	20	6.7	2.4	110
2D(2A)	7.8	0.8	19	4.3	0.7	20	6.2	1.7	110
2D(2B)	8.6	1.1	14	4.0	0.9	13	5.6	1.7	77
3D(3A)	7.5	1.0	13	3.5	0.8	13	5.9	1.9	77
4D(4A)	8.4	1.2	20	4.0	0.9	19	6.4	1.8	110
4D(4B)	7.8	1.0	18	3.7	0.8	19	5.8	1.5	110
5D(5A)	8.8	1.0	18	3.5	0.8	20	5.8	2.1	110
5D(5B)	7.8	0.8	20	3.4	0.8	19	6.0	1.7	110
6D(6A)	7.4	1.0	15	3.6	0.7	16	5.3	1.8	99
6D(6B)	6.2	0.4	18	3.3	0.8	20	5.3	1.6	110
7D(7A)	7.7	0.9	12	3.6	0.6	13	5.1	1.6	77
7D(7B)	7.6	0.8	20	3.2	0.6	20	7.1	1.5	110
Disomic LDN									
LDN	8.0	0.7	20	3.7	0.8	19	6.8	1.9	110
Mean	7.9	0.9	17	3.7	0.8	18	6.0	1.8	99

[a] Male parent

Table 5.4.2 Mean root lengths of F_2 populations and segregation for B tolerance from crosses between LDN D-genome disomic substitution lines and disomic LDN and a B tolerant line AUS 10344, grown at 100 mgB L^{-1}.

Female parents	Root length of F_2 (cm)			Observed number of F_2[a]		
(x AUS 10344)	mean	variance	mean/var	Tol + Int	Sensitive	χ^2_1
LDN D-genome disomic substitution lines						
1D(1A)	6.9	3.7	1.9	87 *(82.5)*	23 *(27.5)*	0.59
1D(1B)	6.7	5.9	1.1	78 *(82.5)*	32 *(27.5)*	0.98
2D(2A)	6.2	2.8	2.2	78 *(82.5)*	32 *(27.5)*	0.98
2D(2B)	5.6	2.9	1.9	48 *(57.7)*	29 *(19.3)*	6.58*
3D(3A)	5.9	3.6	1.6	60 *(57.7)*	17 *(19.3)*	0.35
4D(4A)	6.4	3.4	1.9	84 *(82.5)*	26 *(27.5)*	0.11
4D(4B)	5.8	2.3	2.5	80 *(82.5)*	30 *(27.5)*	0.30
5D(5A)	5.8	4.4	1.3	81 *(82.5)*	29 *(27.5)*	0.11
5D(5B)	6.0	2.9	2.1	78 *(82.5)*	32 *(27.5)*	0.98
6D(6A)	5.3	3.3	1.6	64 *(74.2)*	35 *(24.8)*	6.18*
6D(6B)	5.3	2.5	2.1	86 *(82.5)*	24 *(27.5)*	0.59
7D(7A)	5.1	2.5	2.0	53 *(57.7)*	24 *(19.3)*	1.59
7D(7B)	7.1	2.3	3.1	107 *(82.5)*	3 *(27.5)*	29.10**
Disomic LDN						
LDN	6.8	3.9	1.7	89 *(82.5)*	21 *(27.5)*	2.04
Mean	6.0	3.3	2.0	76 *(76.5)*	26 *(25.5)*	0.13

[a] Expected ratios for 3 to 1 are shown in brackets.

* and ** significant deviation from the expected ratio of 3 tolerant plus intermediate to 1 sensitive at $P<0.05$ and $P<0.01$, respectively

Effect of chromosome 7B of AUS 10344 (MT) on the response to B

To verify that 7B was the critical chromosome in AUS 10344, the F_2 plants were examined individually for chromosome number and chromosome pairing configuration.

A single-copy RFLP probe, PSR 129 (Chao et al., 1989), was used to identified chromosomes 7A, 7B and 7D. Hybridization of PSR 129 with DNA from Chinese Spring, the durum parents and the D-genome substitution lines group 7 revealed only one fragment in each chromosome. The single copy in each homoeologous chromosome was assigned to 7A, 7B and 7D by hybridization to the Chinese Spring/barley cv Betzes substitution lines (Figure 5.5a). Chao et al. (1989) reported that PSR 129 was located in the long arm of chromosomes of homoeologous group 7. A second probe, CDO595, previously found to be located on the short arm of homoeologous group 7 (Heun et al., 1991), was also tested (Figure 5.5b). Similar results were obtained for the two probes indicating transmission of the whole aneuploid chromosome. Therefore, this method was appropriate to identify the presence or absence of the chromosomes under investigation, namely, 7B and 7D, in this population.

The chromosome complements of 62 F_2 plants from 7D(7B) x AUS 10344 (MT) were examined cytologically, and combined with the results from RFLPs analysis. The chromosome pairing found in F_2 plants consisted of 14^{II}, $13^{II}+2^{I}$ and $14^{II}+1^{I}$, neither nullisomic (13^{II}) nor monosomic ($13^{II}+1^{I}$) plants were observed (Table 5.4.3). Thirty-two plants were 14^{II} but only two of these had an RFLP pattern indicating 7A7D (presence of both 7A and 7D) being identified as disomic for 7D ($13^{II}+7D^{II}$), while 30 plants had an RFLP pattern of 7A7B (presence of both 7A and 7B) and were identified as disomic for 7B ($13^{II}+7B^{II}$). Plants having $13^{II}+2^{I}$ showed only the pattern of 7A7B7D (presence of 7A, 7B and 7D) and were identified as $13^{II}+7B^{I}+7D^{I}$. Ten plants having $14^{II}+1^{I}$ contained chromosome 7A7B7D, and it was not possible to distinguish if they were $13^{II}+7B^{II}+7D^{I}$ or $13^{II}+7D^{II}+7B^{I}$.

The effect of chromosome 7B on response to B was examined from F_2 plants of LDN 7D(7B) x AUS 10344 (MT) (Figure 5.6). Plants nullisomic for 7B ($13^{II}+7D^{II}$) had the shortest roots, and were similar to LDN. Plants having one dose of 7B and one dose of 7D ($13^{II}+7B^{I}+7D^{I}$) showed an intermediate response. Plants having two doses of 7B ($13^{II}+7B^{II}$) had significantly longer roots than the intermediate type. Plants having $14^{II}+1^{I}$ showed the same root length as $13^{II}+7B^{II}$ type, indicating that most of the plants were probably $13^{II}+7B^{II}+7D^{I}$.

The genotype of individual F_2 plants was confirmed by the response of its F_3 family. Examples of the response of F_2 and F_3 families for the control cross compared to the 7D(7B) cross are shown in Figure 5.6 and Plate 5.2. F_2 derived progenies in the control cross were distributed as 12 homozygous tolerant : 31 segregating : 16 homozygous sensitive ($\chi^2 = 0.69$ and $P = 0.71$, Table 5.3.2). Seed of the $13^{II}+7D^{II}$ F_3 families was not available because these plants produced only one ear which was used for chromosome counting. From Figure 5.6b, it can be seen that the $13^{II}+7B^{II}$ and $13^{II}+7B^{II}+7D^{I}$ families had a low standard deviation and high mean indicating they were homozygous tolerant for response to B, and similar to the tolerant parent AUS 10344 (MT). The mean root length of $13^{II}+7B^{I}+7D^{I}$ F_3 families was also the same as the tolerant parent, indicating that most of the progeny of this genotype were tolerant.

Figure 5.5 *DraI* digested DNA from CS, CS/Betzes substitutions lines, LDN, AUS 10344 (MT), AUS 14010 (MT) and F_2 of LDN 7D(7B) x AUS 10344 (MT), probed with **(a) PSR 129** and **(b) CDO 595**.

Lane 1 Chinese Spring (CS)

Lane 2 Barley cv Betzes

Lane 3 Chinese Spring (Betzes) substitution line 7H(7A)

Lane 4 Chinese Spring (Betzes) substitution line 7H(7B)

Lane 5 Chinese Spring (Betzes) substitution line 7H(7D)

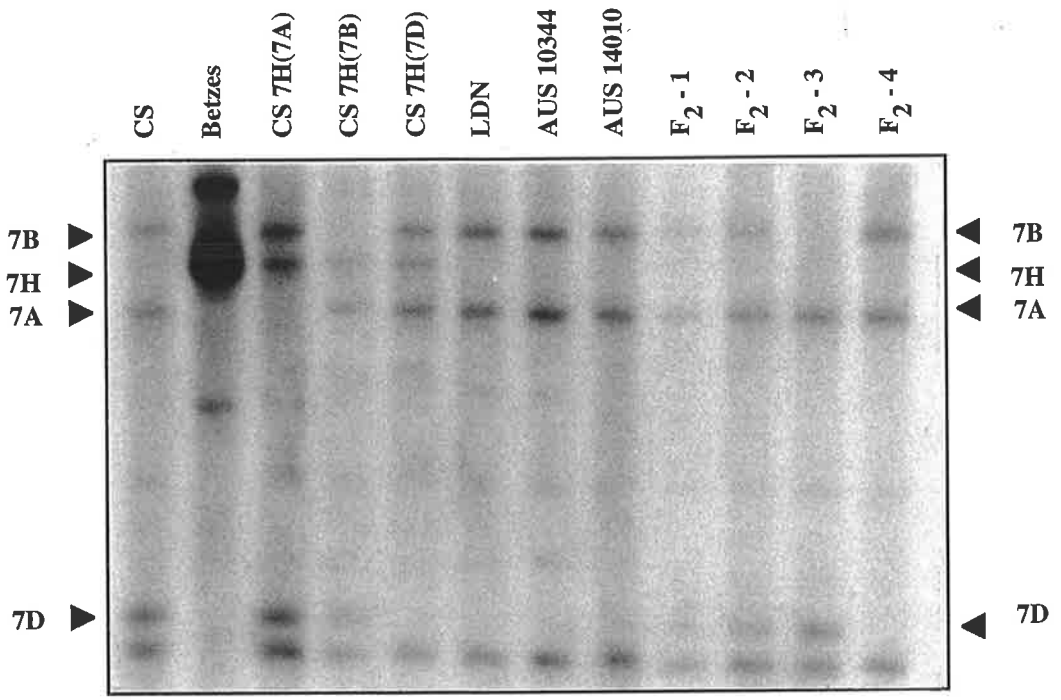
Lane 6 Langdon (LDN)

Lane 7 AUS 10344 (MT)

Lane 8 AUS 14010 (MT)

Lane 9-12 F_2s of LDN 7D(7B) x AUS 10344 (MT)

(a) PSR 129



(b) CDO 595

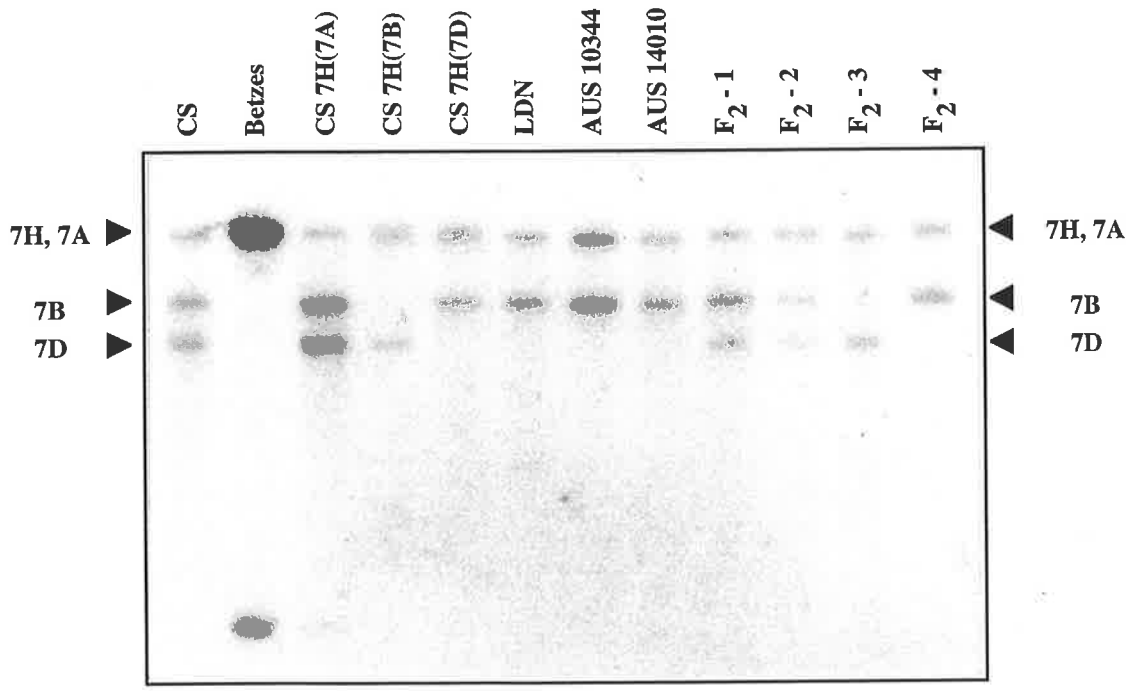


Table 5.4.3 Metaphase I (MI) pairing, RFLPs and proposed chromosome pairing configurations of F_2 progeny of the cross between the 7D(7B) disomic substitution line and the B tolerant line, AUS 10344.

Chromosome pairing at MI	RFLPs						Proposed configuration	No. of plants
	CDO595 (Short arm)			PSR 129 (Long arm)				
	7A	7B	7D	7A	7B	7D		
14^{II}	+	+	-	+	+	-	$13^{II} + 7B^{II}$	30
14^{II}	+	-	+	+	-	+	$13^{II} + 7D^{II}$	2
$13^{II} + 2^{I}$	+	+	+	+	+	+	$13^{II} + 7B^{I} + 7D^{I}$	23
$14^{II} + 1^{I}$	+	+	+	+	+	+	$13^{II} + 7B^{II} + 7D^{I}$ or $13^{II} + 7D^{II} + 7B^{I}$	10

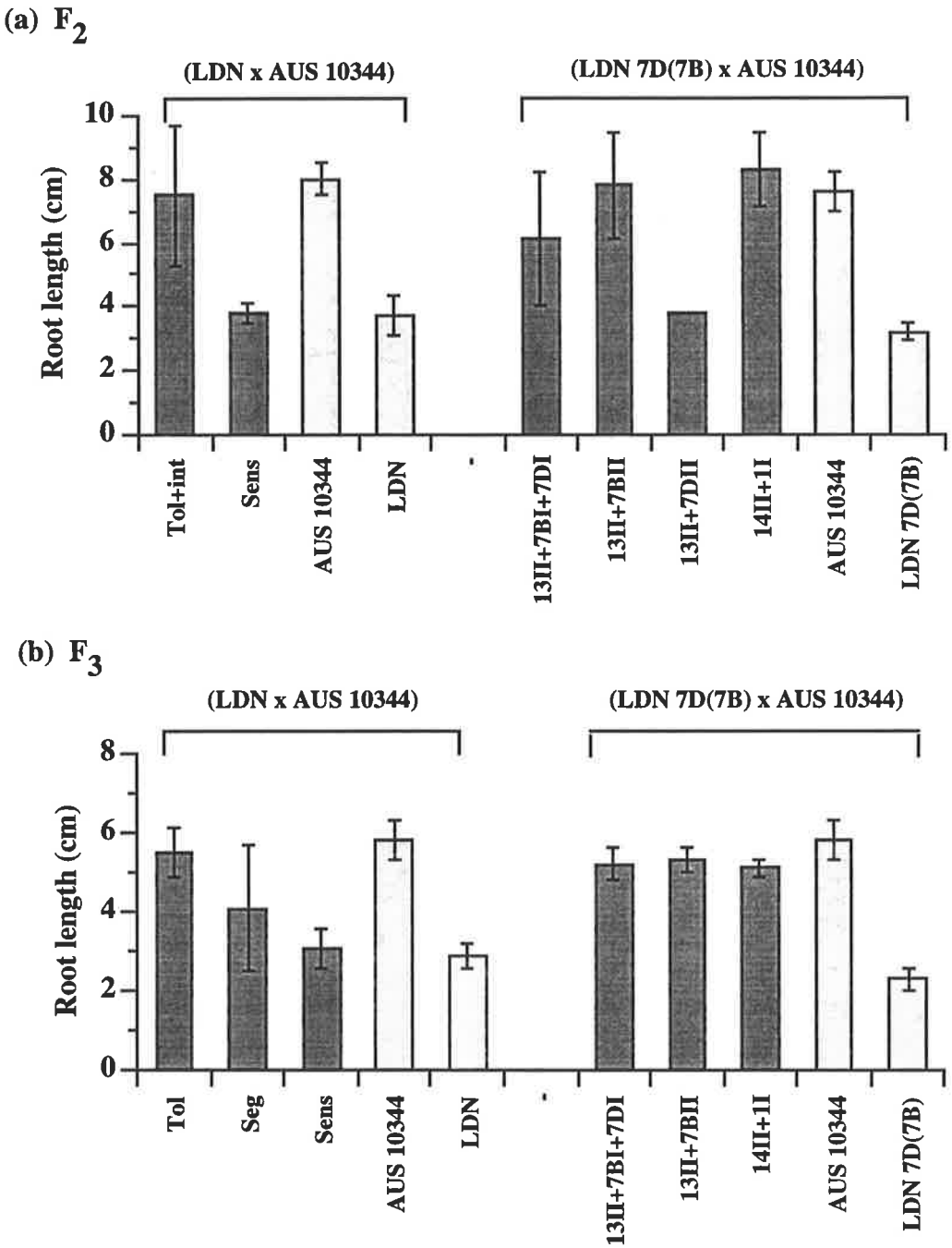


Figure 5.6 Response of F_2 **(a)** and F_3 **(b)** genotypes compared to parental lines for the crosses LDN x AUS 10344 (MT) and LDN 7D(7B) x AUS 10344 (MT) tested at the B100 treatment in filter paper. Vertical bars represent standard deviation within families or parental lines.

Plate 5.2 Respone of F_2 derived F_3 families compared to parental lines, tested at B100 treatment.

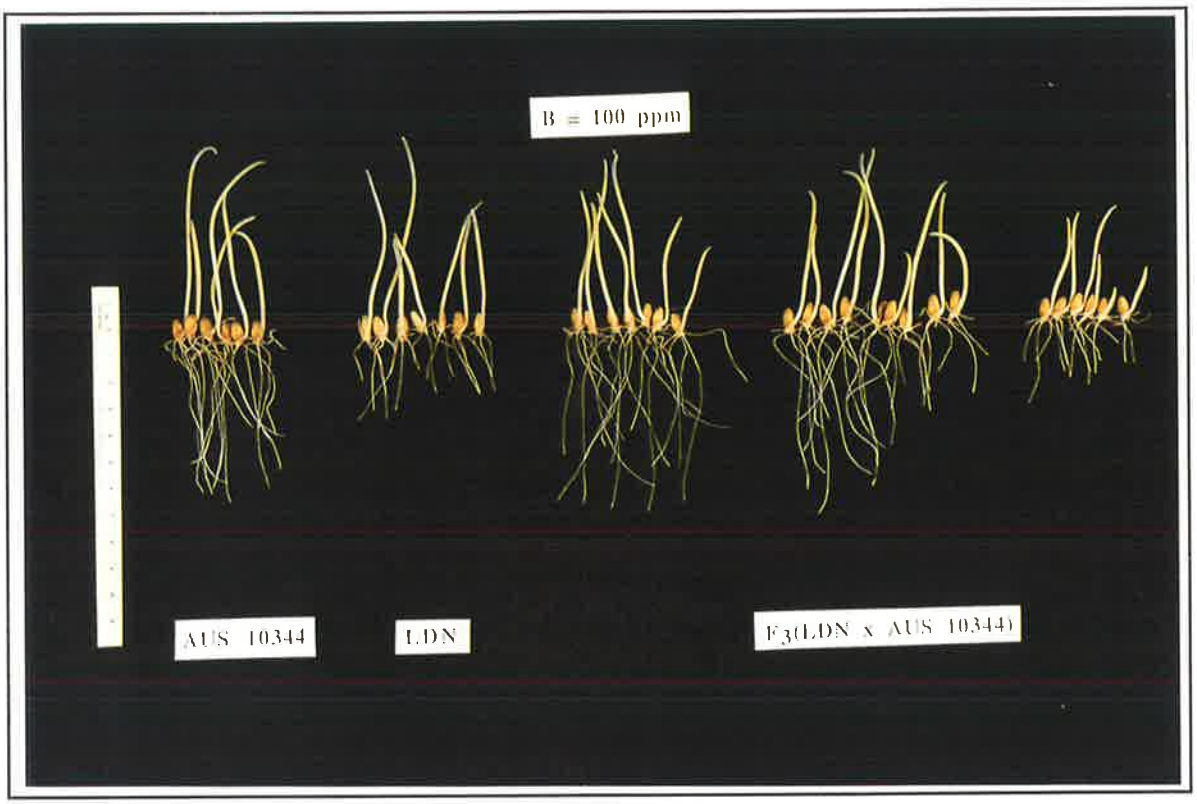
(a) LDN x AUS 10344 (MT)

From left to right: AUS 10344 (MT), LDN, F_2 derived F_3 families classified as homozygous tolerant (AUS 10344 type), segregating and homozygous senstive (LDN type), respectively.

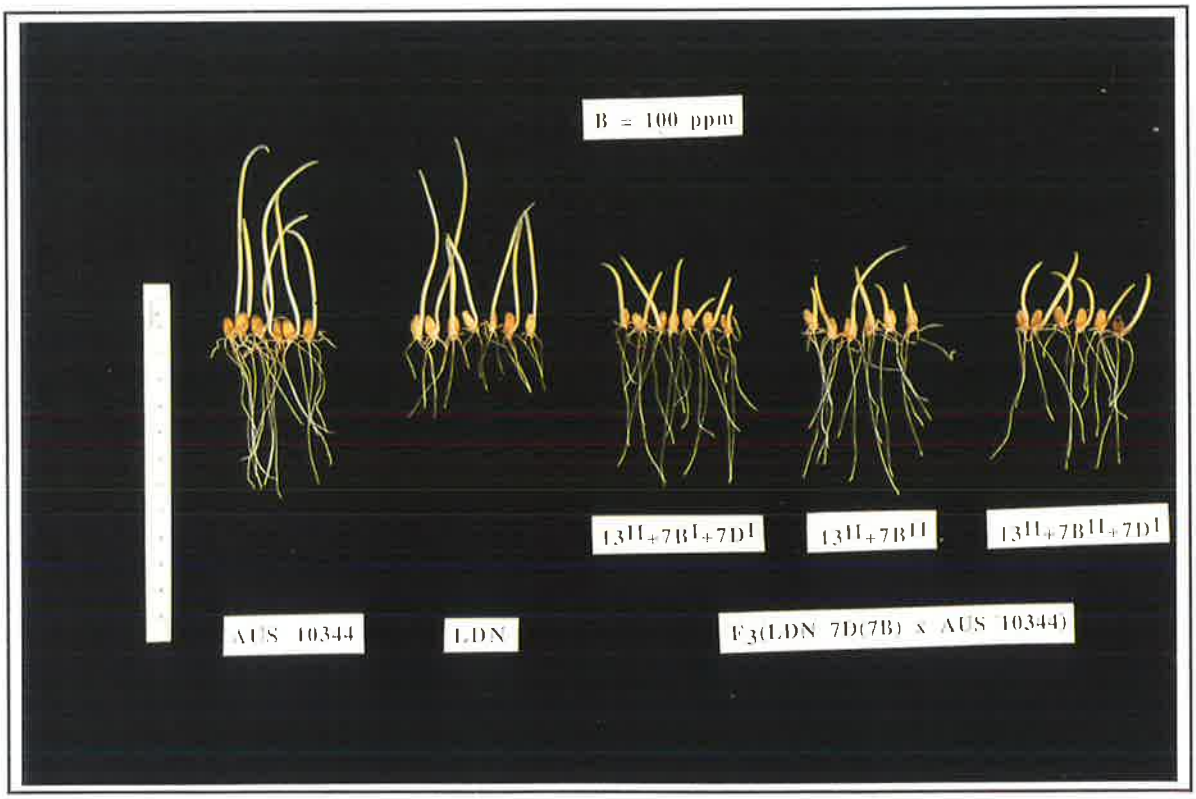
(b) LDN 7D(7B) x AUS 10344 (MT)

From left to right: AUS 10344 (MT), LDN, and three F_3 families derived from F_2 plants having $13^{II} + 7B^{I} + 7D^{I}$, $13^{II} + 7B^{II}$ and $13^{II} + 7B^{II} + 7D^{I}$, respectively.

(a)


B = 100 ppm
AUS 10344
LDN
F3(LDN x AUS 10344)

(b)


B = 100 ppm
$13^{II}+7B^{I}+7D^{I}$
$13^{II}+7B^{II}$
$13^{II}+7B^{II}+7D^{I}$
AUS 10344
LDN
F3(LDN 7D(7B) x AUS 10344)

LDN D-genome substitution lines x AUS 14010 (MT)

Comparison of F_2 populations

The mean root lengths of the parental checks, AUS 14010 (MT) and LDN ranged between 5.9-6.4 and 3.0-3.6 cm, respectively (Table 5.4.4). The mean root lengths of the F_2 population from all crosses were intermediate to the parents (4.8-5.6 cm), except for 3D(3B) and 7D(7B), where high F_2 means were observed (6.5 and 6.4 cm, respectively). Segregation of the F_2 of the control disomic LDN x AUS 14010 (MT) and most of the substitution lines crosses fitted a monogenic ratio of 3 tolerant plus intermediate to 1 sensitive (χ^2 0.11 to 2.18, Table 5.4.5), indicating that the difference in B tolerance between LDN and AUS 14010 (MT) was controlled by one gene.

Significant deviations from a monogenic ratio were found from four crosses including, 2D(2B), 3D(3B), 5D(5A) and 7B(7D). The deviation from the expected ratio for the crosses with 2D(2B) and 5D(5A) was due to an excessive number of sensitive plants (Table 5.4.5). The crosses 3D(3B) and 7D(7B) significantly deviated from the monogenic ratio and both had a low number of sensitive plants. These two crosses were suspected to be the critical crosses with the B tolerance gene located on either 3B or 7B. To verify which was the critical chromosome, the F_2 plants from crosses with 3D(3B) and 7D(7B) were examined individually for chromosome complement and pairing configurations. F_2 derived F_3 families were tested in the B100 treatment.

Table 5.4.4 Mean response of tolerant and sensitive check parents and F_2 populations from crosses between LDN D-genome disomic substitution lines and disomic LDN, and a B tolerant line, AUS 14010, grown in filter paper containing 100 mgB L^{-1}.

Female parents	AUS 14010 (Tolerant)[a]			LDN (Sensitive)			F_2		
(x AUS 14010)	mean	SD	no	mean	SD	no	mean	SD	no
LDN D-genome disomic substitution lines									
1D(1A)	6.1	0.7	18	3.1	0.7	17	4.7	1.4	106
1D(1B)	6.4	0.6	18	3.2	0.6	19	5.4	1.7	110
2D(2A)	5.7	0.8	11	3.6	0.7	18	5.3	1.4	110
2D(2B)	6.0	0.6	18	3.0	0.7	20	4.6	1.6	106
3D(3A)	6.1	0.5	17	3.4	0.9	19	5.1	1.3	108
3D(3B)	6.0	0.7	14	3.2	0.8	20	6.5	1.6	110
4D(4A)	6.2	0.6	18	3.4	0.8	21	5.1	1.5	120
4D(4B)	6.0	0.7	18	3.2	0.8	20	5.6	1.7	120
5D(5A)	6.2	0.7	19	3.6	0.7	18	4.8	1.7	109
5D(5B)	6.3	0.9	16	3.4	0.6	20	5.5	1.6	110
6D(6A)	6.7	0.5	17	3.3	0.6	19	5.1	1.6	105
6D(6B)	5.9	0.8	19	3.4	0.7	18	5.1	1.5	108
7D(7A)	5.9	0.7	16	3.5	0.7	21	5.2	1.7	118
7D(7B)	6.2	0.7	14	3.3	0.6	14	6.4	1.4	73
Disomic LDN									
LDN	6.1	0.8	19	3.5	0.6	20	5.2	1.7	120
Mean	6.1	0.7	17	3.3	0.7	19	5.3	1.6	109

[a] male parent

Table 5.4.5 Mean root lengths of F_2 populations and segregation for response to B for crosses between LDN D-genome disomic substitution lines and disomic LDN, and a B tolerant line AUS 14010 grown at 100 mgB L^{-1}.

Female parents	Root length of F_2 (cm)			Observed number of F_2[a]		
(x AUS 14010)	mean	var	mean/var	Tol + Int	Sensitive	χ^2_1
LDN D-genome disomic substitution lines						
1D(1A)	4.7	1.9	2.5	75 *(79.5)*	31 *(26.5)*	1.02
1D(1B)	5.4	2.9	1.9	83 *(82.5)*	27 *(27.5)*	0.01
2D(2A)	5.3	2.7	2.0	83 *(82.5)*	27 *(27.5)*	0.01
2D(2B)	4.6	2.5	1.8	70 *(79.5)*	36 *(26.5)*	4.54*
3D(3A)	5.1	1.8	2.8	75 *(81.0)*	33 *(27.0)*	1.78
3D(3B)	6.5	2.4	2.7	102 *(87.0)*	8 *(29.0)*	18.43**
4D(4A)	5.1	2.1	2.4	97 *(90.0)*	23 *(30.0)*	2.18
4D(4B)	5.6	2.7	2.1	87 *(90.0)*	33 *(30.0)*	0.40
5D(5A)	4.8	2.8	1.7	72 *(81.0)*	36 *(27.0)*	4.00*
5D(5B)	5.5	2.5	2.2	88 *(81.7)*	21 *(27.3)*	1.91
6D(6A)	5.1	2.4	2.1	77 *(78.7)*	28 *(26.3)*	0.16
6D(6B)	5.1	2.1	2.4	83 *(81.0)*	25 *(27.0)*	0.20
7D(7A)	5.2	2.9	1.8	87 *(88.5)*	31 *(29.5)*	0.10
7D(7B)	6.4	2.1	3.0	68 *(54.7)*	5 *(18.3)*	12.83**
Disomic LDN						
LDN	5.2	3.0	1.7	85 *(90.0)*	35 *(30.0)*	1.11
Mean	5.3	2.4	2.2	82 *(81.7)*	27 *(27.3)*	0.12

[a] Expected ratios for 3 to 1 are shown in brackets.

* and ** significant deviation from the expected ratio of 3 tolerant plus intermediate to 1 sensitive at $P<0.05$ and $P<0.01$, respectively

Effect of 3B and 7B of AUS 14010 (MT) on the response to B of F_2 and F_3 progenies from (LDN 3D(3B) x AUS 14010) and (LDN 7D(7B) x AUS 14010)

The genotypes of individual F_2 plants were identified by testing the F_2 derived F_3 families and compared to the disomic cross. Families were identified as tolerant, segregating or sensitive by the method described in Section 5.2. Chi-square analysis was in agreement with the disomic and 3D(3B) crosses segregating at single locus (Table 5.4.6). In contrast, the cross with 7D(7B) deviated significantly from the expected single gene ratio with only one family being identified as homozygous sensitive.

This was also supported by the chromosome constitutions of the F_2 plants (Table 5.4.7). Chromosome pairing of the 7D(7B) cross was closely related with the response to B (Table 5.4.7). All plants of $13^{II}+2^{I}$ type, which contained one 7B chromosome from the tolerant parent, were identified as heterozygous on the basis of the response of the F_3 progeny, whereas the 3D(3B) derived $13^{II}+2^{I}$ plants included all categories with respect to response to B. For the 7D(7B) cross, ten plants having 14^{II} were identified, with nine being tolerant and one sensitive. Plants having $14^{II}+1^{I}$ contained either one or two 7B chromosomes from AUS 14010 (MT) and were identified as heterozygous or tolerant.

F_2 plants from 7D(7B) x AUS 14010 (MT) were sampled for RFLP analysis, as described for 7D(7B) x AUS 10344 (MT), and progeny tested for response to B. The results (Table 5.4.8, Figure 5.7 and Plate 5.3) were consistent with plants having $13^{II}+7B^{II}$ as being the most tolerant, $13^{II}+7D^{II}$ the most sensitive, while plants having $13^{II}+7B^{I}+7D^{I}$ were intermediate and $14^{II}+1^{I}$ were either tolerant or intermediate. F_2 plants, or F_3 families of the type $13^{II}+7D^{II}$, that is lacking 7B, produced roots of similar length to the sensitive parents (LDN or LDN 7D(7B)), while roots of F_2 plants, or F_3 families with two doses of chromosome 7B were similar in length to the tolerant parent (Figure 5.7). F_2 plants, or F_3 families of the type $13^{II}+7B^{I}+7D^{I}$ and $14^{II}+1^{I}$

produced roots of intermediate length to the parents with the $14^{II}+1^{I}$ type more similar to the tolerant parent.

Table 5.4.6 Classification of response of the F_3 generation from crosses between the LDN, LDN 3D(3B), LDN 7D(7B) and AUS 14010 (MT) when tested in filter paper containing 100 mgB L^{-1}.

Cross		Observed and expected frequencies			χ^2_2
		Tol	Seg	Sens	(1:2:1)
LDN x AUS 14010 (MT)	Obs	25	68	30	
	Exp	30.75	61.5	30.75	1.78
LDN 3D(3B) x AUS 14010 (MT)	Obs	27	41	24	
	Exp	23	46	23	1.28
LDN 7D(7B) x AUS 14010 (MT)	Obs	24	28	1	
	Exp	13.25	26.5	13.25	20.13**

** significant deviation from the expected ratio of 1 tolerant : 2 segregating :1 sensitive at P<0.01

Table 5.4.7 Chromosome pairing at MI and response to B of F_2 derived families from crosses between LDN 3D(3B), LDN 7D(7B) and AUS 14010 (MT) when tested in filter paper containing 100 mgB L^{-1}.

Chromosome pairing of F_2 parents	Observed number of F_2 derived F_3 families							
	LDN 3D(3B)				LDN 7D(7B)			
	Tol	Seg	Sens	χ^2_2	Tol	Seg	Sens	χ^2_2
13^{II} or $13^{II}+1^{I}$	0	0	0	-	0	0	0	-
$13^{II}+2^{I}$	6	6	3	1.8	0	6	0	6.0*
14^{II}	1	4	5	3.6	9	0	1	22.8**
$14^{II}+1^{I}$	2	3	4	1.8	4	2	0	6.0*
Total	*9*	*13*	*12*	*2.4*	*13*	*8*	*1*	*14.7***

** significant deviation from the expected ratio of 1:2:1 at P<0.05 and P<0.01, respectively

Table 5.4.8 Metaphase I (MI) pairing, RFLP patterns, and proposed chromosome pairing configurations of F_2 progeny of the cross between the 7D(7B) disomic substitution line and the B tolerant line, AUS 14010.

Chromosome pairing at MI	RFLPs						Proposed configuration	No. of plants
	CDO595 (Short arm)			PSR 129 (Long arm)				
	7A	7B	7D	7A	7B	7D		
14^{II}	+	+	-	+	+	-	$13^{II} + 7B^{II}$	9
14^{II}	+	-	+	+	-	+	$13^{II} + 7D^{II}$	1
$13^{II} + 2^{I}$	+	+	+	+	+	+	$13^{II} + 7B^{I} + 7D^{I}$	6
$14^{II} + 1^{I}$	+	+	+	+	+	+	$13^{II} + 7B^{II} + 7D^{I}$ or $13^{II} + 7D^{II} + 7B^{I}$	6

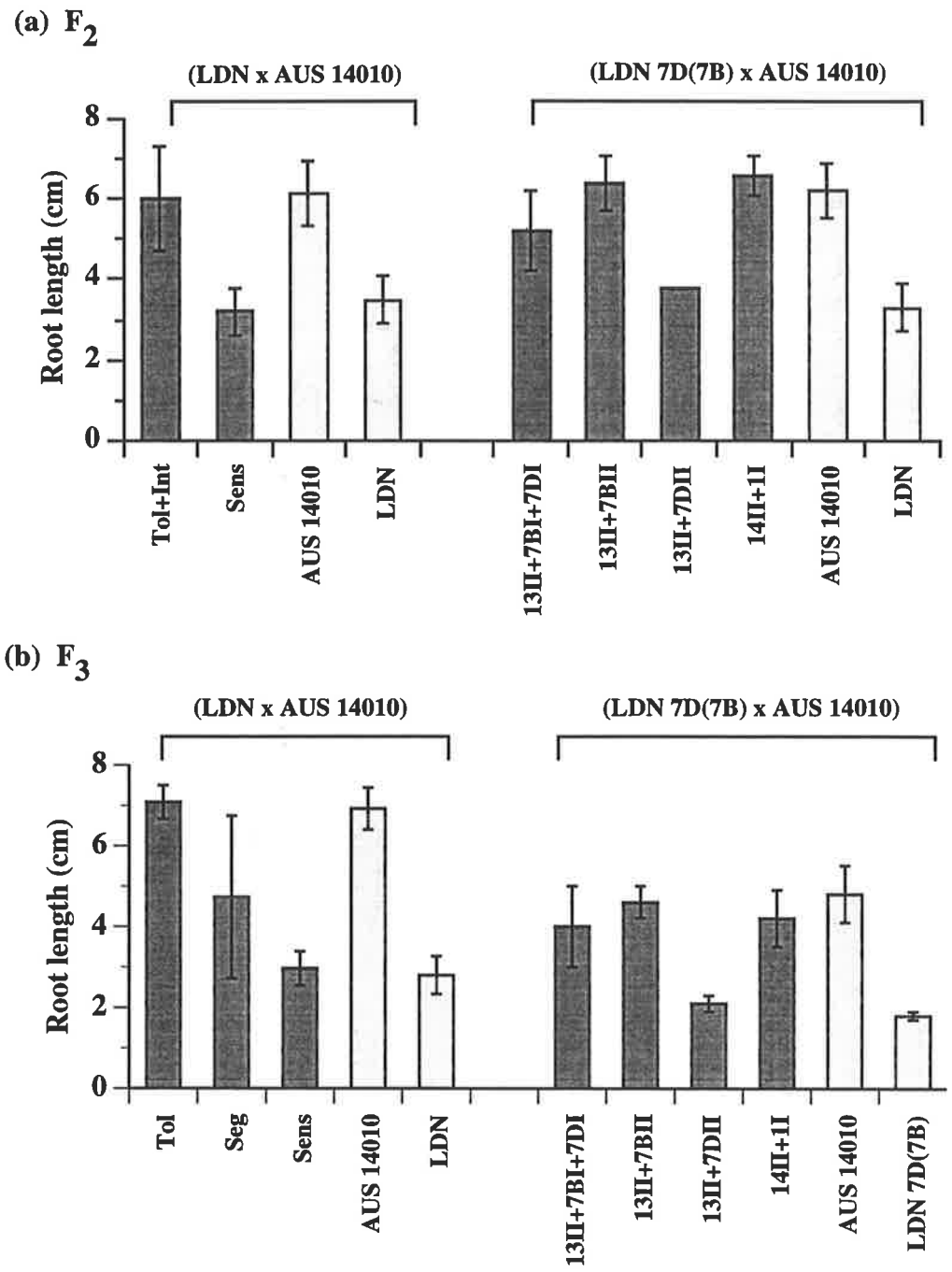


Figure 5.7 Response of **(a)** F_2 and **(b)** F_3 genotypes compared to parental lines for the crosses LDN x AUS 141010 (MT) and LDN 7D(7B) x AUS 14010 (MT) tested in the B100 treatment in filter paper. Vertical bars represent standard deviation within families or parental lines.

Plate 5.3 Respone of F_2 derived F_3 families compared to parental lines, tested at the B100 treatment.

(a) LDN x AUS 14010 (MT)

From left to right: AUS 14010 (MT), LDN, F_2 derived F_3 families classified as homozygous tolerant (AUS 14010 type), segregating and homozygous sensitive (LDN type), respectively.

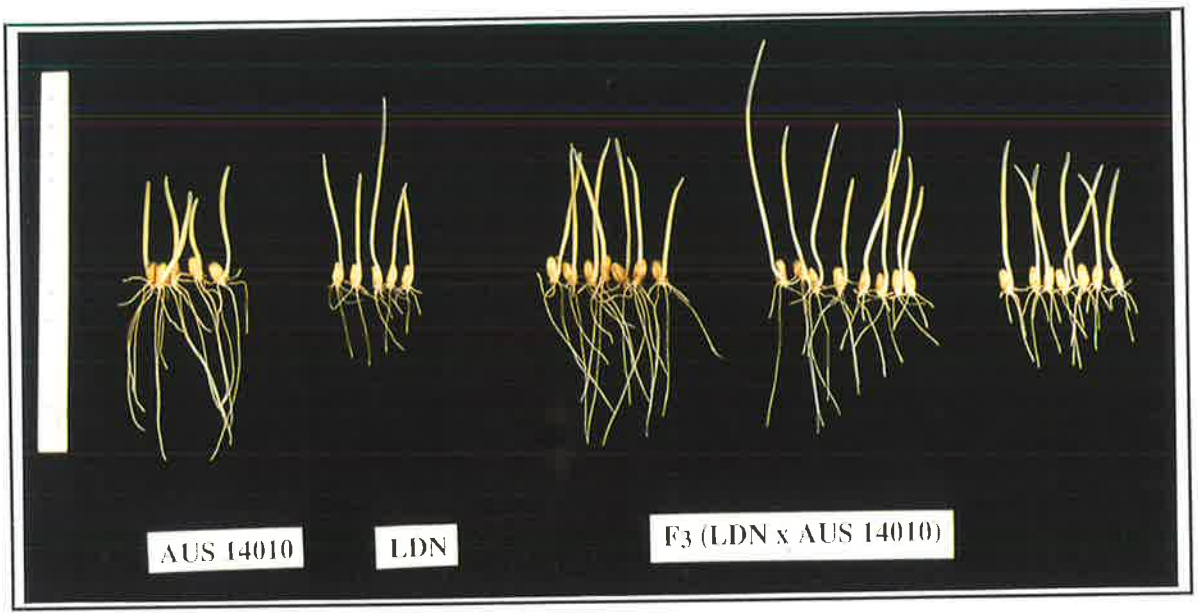
(b) LDN 3D(3B) x AUS 14010 (MT)

From left to right: AUS 14010 (MT), LDN 3D(3B), F_2 derived F_3 families classified as homozygous tolerant (AUS 14010 type), segregating and homozygous sensitive (LDN 3D(3B) type), respectively.

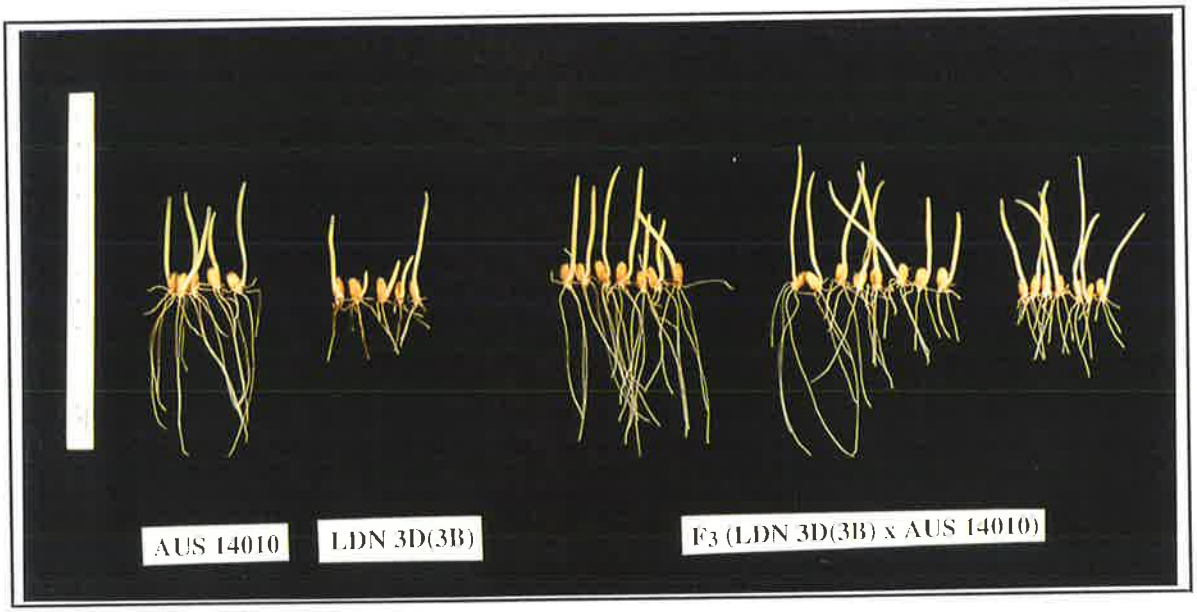
(c) LDN 7D(7B) x AUS 14010 (MT)

From left to right: AUS 14010 (MT), LDN 7D(7B), and four F_3 families derived from F_2 plants having $13^{II} + 7D^{II}$, $13^{II} + 7B^{I} + 7D^{I}$, $13^{II} + 7B^{II}$ and $14^{II} + 1^{I}$, respectively.

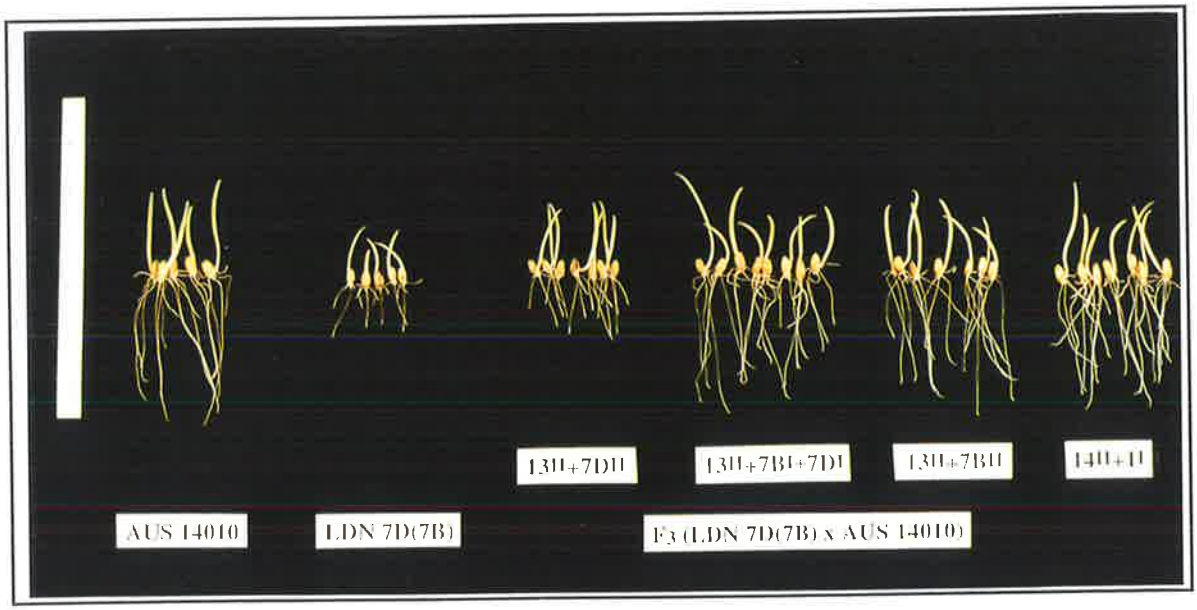
(a)


AUS 14010
LDN
F3 (LDN x AUS 14010)

(b)


AUS 14010
LDN 3D(3B)
F3 (LDN 3D(3B) x AUS 14010)

(c)


AUS 14010
LDN 7D(7B)
13II+7DII
13II+7BI+7DI
13II+7BII
14II+II
F3 (LDN 7D(7B) x AUS 14010)

LDN D-genome substitution lines x AUS 10110 (T)

The segregation of F_2 progeny from disomic LDN and LDN aneuploids, crossed with AUS 10110 (T) significantly deviated from the monogenic ratio of 3 to 1 (Table 5.4.9 and 5.4.10). The results of the analysis of F_2 derived F_3 families from the disomic cross in the previous section (Section 5.3) revealed that LDN and AUS 10110 (T) differ for two genes controlling B tolerance. Therefore, the model of a digenic ratio was tested to identify the crosses which deviated from a 15 to 1 ratio. Six crosses deviated significantly from a digenic ratio and only 7D(7B) had fewer sensitive plants than that expected for a 15:1 ratio and thus was consistent with the response expected for a critical chromosome. The ratio of mean to variance of the 7D(7B) derived F_2 population was high, indicating this cross produced more seedlings with long roots, compared to the other crosses (Table 5.4.10). Therefore, it was suspected that chromosome 7B of AUS 10110 (T) carried gene(s) for B tolerance, but the populations were too small to test for deviation from a 15 : 1 segregation ratio with a high level of confidence.

Response of F_2 derived F_3 families derived from (LDN anueploids x AUS 10110 (T))

Due to an insufficient number of plants in the populations used in these crosses to discriminate between a 15:1 and an anomalous ratio with a high level of confidence, a further experiment was undertaken to examine the response of selected F_2 plants with short roots. If the two genes controlling tolerance to B were located on different chromosomes, then none or very few sensitive F_2 plants will be found in the critical crosses and at least two critical crosses will show segregation ratios differing from 15:1. For example, if the gene *Bo_T1* was located on a non-critical chromosome and segregated at 3*Bo_T1*_:1*bo_T1bo_T1* and *Bo_T2* was located on a critical chromosome (e.g. 7B) and segregated at 97*Bo_T2*_:3*bo_T2bo_T2* (expected segregation ratio for 7B, Table 5.1.3), the two loci would segregate tolerant plus intermediate (*$Bo_T1\_Bo_T2$*_+ *$Bo_T1\_bo_T2bo_T2$* + *$bo_T1bo_T1Bo_T2$*_) to sensitive (*$bo_T1bo_T1bo_T2bo_T2$*) in the ratio of

about 120:1. A similar ratio would be expected for another critical cross. But if the genes were located on the same chromosome, the segregation ratio would be the same as that expected in 7D(7B) x AUS 10344 (MT) and 7D(7B) x AUS 14010 (MT) because the critical chromosome is inherited as a unit due to the absence of pairing and recombination between the A or B and the D-genome chromosomes. Hence, the seven F_2 plants having the shortest roots from each cross were transplanted to potting mix, and F_3 families were tested in the B100 treatment.

In this study, the number of sensitive families for the non-critical crosses was expected to be about 5 to 7 families although less than 5 families were available for some crosses due to sterility of F_2 plants. The results (Figure 5.8) show that for most crosses the selected F_2 plants were rated as either homozygous sensitive (*bo$_T$1bo$_T$1bo$_T$2bo$_T$2*) or segregating at one gene (*bo$_T$1bo$_T$1Bo$_T$2bo$_T$2* or *Bo$_T$1bo$_T$1bo$_T$2bo$_T$2*) when F_3 progeny were tested. The 7D(7B) cross showed only one homozygous sensitive family and most of the families were identified as segregating (*Bo$_T$1bo$_T$1Bo$_T$2bo$_T$2*) or homozygous tolerant (*Bo$_T$1Bo$_T$1Bo$_T$2Bo$_T$2*). Therefore, chromosome 7B was apparently the only critical chromosome for AUS 10110 (T).

Either one or two seedlings from each of the seven selected families of the 7D(7B) cross were transplanted to potting mix for DNA extraction. The chromosome constitution of homoeologous group 7 was examined by RFLP analysis. Only the sensitive family was of the type 7A7D and the rest were either 7A7B or 7A7B7D (Table 5.4.11). This also indicated that the two genes were transmitted through the 7B chromosome of AUS 10110 (T). As the 7D and 7B do not pair in F_1 hybrid plants ($13^{II}+7D^{I}+7B^{I}$), the recombinant types (AB/Ab, AB/aB, Ab/ab and aB/ab) were not expected, but only AB/AB, AB/-- and --/-- were identified as would be expected if 7B was the critical chromosome.

Table 5.4.9 Mean response of tolerant and sensitive check parents and F_2 populations from crosses between LDN D-genome disomic substitution lines, disomic LDN and a B tolerant line, AUS 10110 (T), grown in filter paper containing 100 mgB L^{-1}.

Female parents	AUS 10110 (Tolerant)[a]			LDN (Sensitive)			F_2		
(x AUS 10110)	mean	SD	no	mean	SD	no	mean	SD	no
LDN D-genome disomic substitution lines									
1D(1A)	8.4	1.0	20	2.3	0.6	20	5.0	1.3	105
1D(1B)	7.9	0.8	19	2.5	0.6	20	5.6	1.5	110
2D(2A)	8.2	1.0	20	2.6	0.8	16	4.7	1.5	105
3D(3A)	8.7	0.9	18	2.6	0.5	18	5.1	1.4	108
4D(4A)	7.4	0.6	20	2.9	0.7	18	5.7	1.4	109
4D(4B)	8.0	1.0	18	2.7	0.8	19	4.7	1.3	106
5D(5A)	8.5	1.3	17	2.7	0.7	20	5.3	1.4	110
5D(5B)	7.7	1.2	12	2.7	0.6	14	5.1	1.5	70
6D(6A)	7.1	0.7	19	2.8	0.4	18	5.3	1.5	110
6D(6B)	7.4	1.2	18	2.7	0.4	18	4.9	1.6	108
7D(7A)	7.8	0.7	19	3.0	0.6	19	4.9	1.4	107
7D(7B)	7.6	0.8	16	3.2	0.7	14	5.9	1.3	88
Disomic LDN									
LDN	8.4	0.7	19	3.3	0.6	20	5.7	1.5	109
Mean	7.9	0.9	18	2.8	0.6	18	5.2	1.4	103

[a] male parent

Table 5.4.10 Mean root lengths of F_2 populations and segregation for response to B from crosses between LDN D-genome disomic substitution lines and disomic LDN, and a B tolerant line, AUS 10110, grown in filter paper containing 100 mgB L^{-1}.

Female parents	Root length of F_2 (cm)			Observed number of F_2[a]		χ^2_1	
(x AUS 10110)	mean	var	mean/var	Tol + Int	Sensitive	3:1	15:1
LDN D-genome disomic substitution lines							
1D(1A)	5.0	1.8	2.8	98 *(98.4)*	7 *(6.6)*	18.80**	0.03
1D(1B)	5.6	2.1	2.7	102 *(103.1)*	8 *(6.9)*	18.43**	0.19
2D(2A)	4.7	2.4	2.0	89 *(98.4)*	16 *(6.6)*	5.34*	14.19**
3D(3A)	5.1	1.9	2.7	102 *(103.1)*	8 *(6.9)*	18.43**	0.19
4D(4A)	5.7	2.0	2.9	102 *(103.1)*	8 *(6.9)*	18.43**	0.19
4D(4B)	4.7	1.6	2.9	98 *(103.1)*	12 *(6.9)*	11.65**	4.06*
5D(5A)	5.3	2.1	2.5	100 *(103.1)*	10 *(6.9)*	14.84**	1.51
5D(5B)	5.1	2.2	2.3	61 *(65.6)*	9 *(4.4)*	5.50*	5.20*
6D(6A)	5.3	2.1	2.5	100 *(103.1)*	10 *(6.9)*	14.84**	1.51
6D(6B)	4.9	2.6	1.9	95 *(101 3)*	13 *(6.7)*	9.68**	6.17*
7D(7A)	4.9	2.0	2.5	95 *(103.1)*	15 *(6.9)*	7.57**	10.22**
7D(7B)	5.9	1.6	3.7	87 *(82.5)*	1 *(5.5)*	26.72**	3.93*
Disomic LDN							
LDN	5.7	2.3	2.5	104 *(106.9)*	5 *(7.1)*	24.22**	0.51
Mean	5.2	2.1	2.6	95 *(98.4)*	10 *(6.6)*	13.41**	1.94

[a] Expected ratios for 15 to 1 are shown in brackets.

* and ** significant deviation from the expected ratios at $P<0.05$ and $P<0.01$, respectively

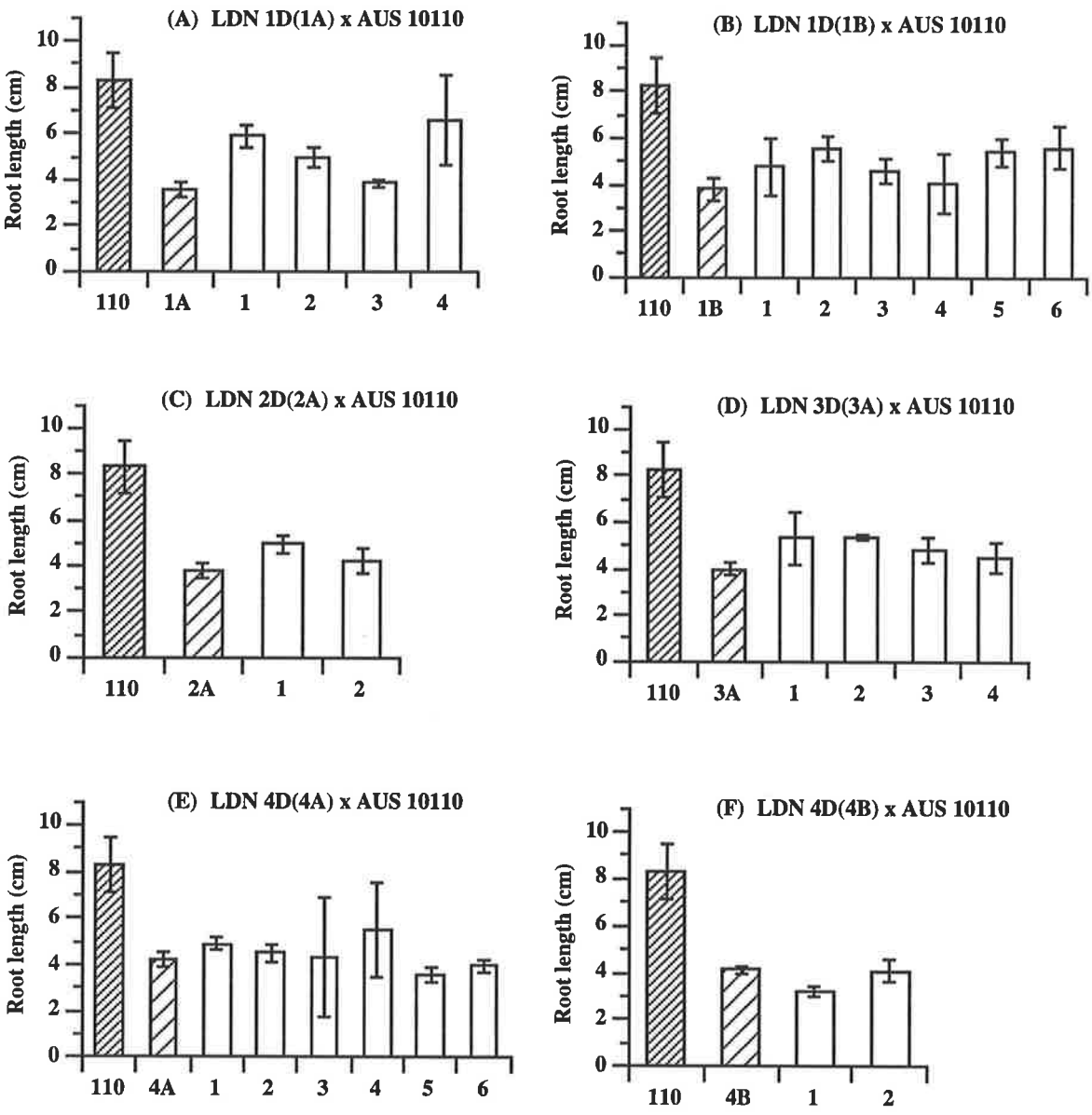


Figure 5.8 Response of AUS 10110 (▨), Langdon (LDN) D-genome disomic substitution lines (▨) and selected F_3 families (□) from the crosses as follow:

(A) LDN 1D(1A) x AUS 10110 (T) **(B)** LDN 1D(1B) x AUS 10110 (T)

(C) LDN 2D(2A) x AUS 10110 (T) **(D)** LDN 3D(3A) x AUS 10110 (T)

(E) LDN 4D(4A) x AUS 10110 (T) **(F)** LDN 4D(4B) x AUS 10110 (T)

tested in filter paper containing 100 mgB L^{-1}. Vertical bars represent standard deviation within families or parental lines, 13 plants per family.

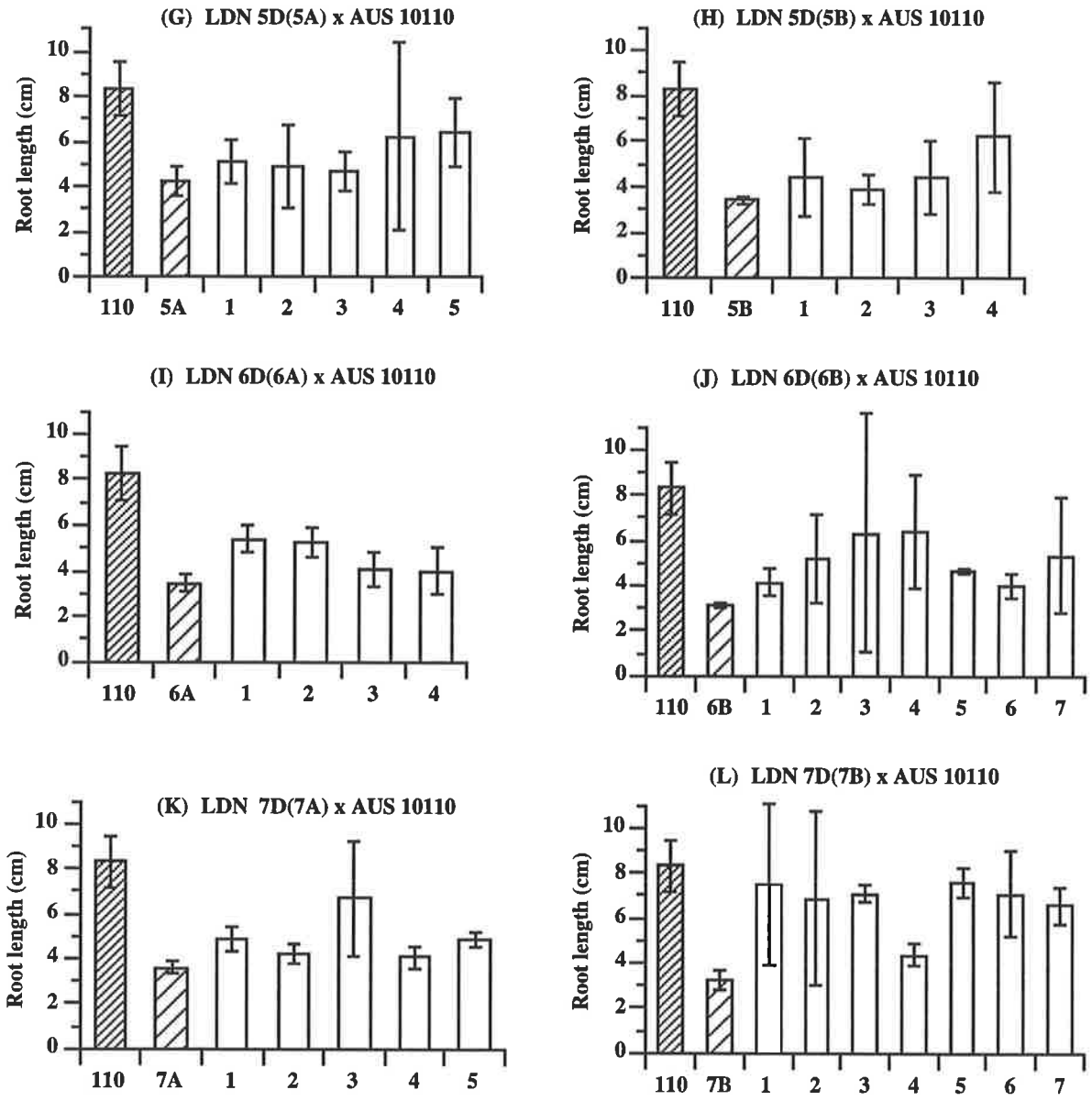


Figure 5.8 (continued) Response of AUS 10110 (▨), Langdon (LDN) D-genome disomic substitution lines (▨) and selected F_3 families (□) from the crosses as follow:

(G) LDN 5D(5A) x AUS 10110 (T)
(H) LDN 5D(5B) x AUS 10110 (T)
(I) LDN 6D(6A) x AUS 10110 (T)
(J) LDN 6D(6B) x AUS 10110 (T)
(K) LDN 7D(7A) x AUS 10110 (T)
(L) LDN 7D(7B) x AUS 10110 (T)

tested in filter paper containing 100 mgB L^{-1}. Vertical bars represent standard deviation within families or parental lines, 13 plants per family.

Table 5.4.11 RFLP patterns and response to B of F_3 families from LDN 7D(7B) x AUS 10110 when tested at B100.

Family and plant	Root length (cm)		RFLPs					
	mean	variance	CDO595 (Short arm)			PSR 129 (Long arm)		
			7A	7B	7D	7A	7B	7D
Family 1	7.5**	3.6						
1-1			+	+	-	+	+	-
1-2			+	+	-	+	+	-
Family 2	6.8**	3.8						
2-1			+	+	-	+	+	-
Family 3	7.1**	0.4						
3-1			+	+	-	+	+	-
Family 4	4.3	0.5						
4-1			+	-	+	+	-	+
4-2			+	-	+	+	-	+
Family 5	7.6**	0.6						
5-1			+	+	-	+	+	-
5-2			+	+	-	+	+	-
Family 6	7.1**	1.9						
6-1			+	+	+	+	+	+
Family 7	6.6**	0.8						
7-1			+	+	-	+	+	-
7-2			+	+	-	+	+	-
Check								
AUS 10110	7.5**	0.6						
LDN 7D(7B)	3.2	0.4						

** significantly different from LDN 7D(7B) at $P<0.01$ by unpaired t-test

5.4.4 Discussion

The differences in response to high concentrations of B between B tolerant lines and LDN and the LDN D-genome substitution lines were under the control of major genes. The F_2 segregation of the control and non-critical crosses demonstrated that the LDN substitution lines and AUS 10344 (MT) differed at the *Bot1* locus and AUS 14010 (MT) at *Bot2*. The response of F_2 and F_3 populations derived from crosses between the B tolerant lines and the LDN 7D(7B) substitution line indicated that chromosome 7B was the most probable location of both loci.

Aneuploid analysis in these studies consisted of the use of LDN D-genome disomic substitution lines crossed to the B tolerant parents with the F_2 generations being screened for response to B and compared with the response of the F_2 derived from the cross between LDN and the B tolerant parent. The analysis of F_2 populations was performed by two methods, namely, the comparision of mean and variance of substitution line crosses with the control cross and by chi-square analysis. Results by both methods were consistent and crosses derived from 7D(7B) always had the highest mean root length and mean/variance ratio compared to the control and the other substitution line crosses. Estimation of the frequency of the two categories (Tol+inter and sensitive) by the use of the mean of LDN plus one standard deviation as a cut-off point to separate groups, followed by chi-square analysis produced results consistent with the first method and also with results of chi-square analysis following progeny testing in the F_3 generation. However, this method has to be used with caution as misclassification of some F_2 plants was found in LDN 3D(3B) x AUS 14010 (MT) (Tables 5.4.5 and 5.4.6). In this cross, the number of sensitive F_2 phenotypes was under-estimated (8 plants in the F_2 but 23 F_2 derived F_3 families) and was likely to have resulted from an underestimation of the correct cut-off point. This might have been caused by a non-uniform distribution of B solution in the filter paper, enabling some sensitive F_2 plants to develop longer roots than the parental check, LDN.

An excessive number of sensitive F_2 phenotypes were observed in some crosses (Tables 5.4.2, 5.4.5 and 5.4.10). This probably resulted from the poor germination or low vigor of some F_2 seeds. Chantachume (1995) also pointed out that differentiating between sensitive, intermediate and tolerant genotypes in the F_2 generation on the basis of mean and standard deviation of the parents is sensitive to the balance between Type I error (under-estimation) and Type II error (over-estimation). This problem can overcome by testing the F_3 generation when the classification was based on mean and within family variance (for example, Figures 5.4, 5.6 and 5.7) compared to the parents.

The use of LDN mean plus one standard deviation as a cut-off point in the F_2 is more sensitive to misclassification than mean plus two standard deviation. The major aim of F_2 screening in this study was to initially identify a cross which was suspected to contain critical chromosome. As the expected number of sensitive plants for critical cross is much less than for the control cross (97:3 for 7B v 75:25 for single gene model, Section 5.2), the difference between these hypotheses is therefore large enough to screen the suspected cross. Cytogenetical analysis and progeny testing of the F_2 plants from crosses suspected to be critical also provided confirmation of the hypothesis.

The cytogenetic confirmation assisted by molecular markers allowed positive identification of the chromosome under investigation and a correlation to the response to B in the segregating population. In the critical crosses, when the background chromosomes were not segregating for response to B, the variable products of the cross between a substitution line and a B tolerant line could only be due to the gene differences between the substituted chromosome and its homoeologue. The chromosome complements of the F_2 plants of the crosses LDN 7D(7B) x AUS 10344 (MT) and LDN 7D(7B) x AUS 14010 (MT) were in agreement with the F_2 plants nullisomic for 7B ($13^{II}+7D^{II}$ type) being as sensitive to B as the LDN or LDN 7D(7B) parents while plants monosomic or disomic for 7B developed significantly longer roots

than the $13^{II}+7D^{II}$ type (Figures 5.6 and 5.7). This suggested that the B tolerant genes identified in AUS 10344 (MT) and AUS 14010 (MT) were both located on chromosome 7B.

In a monosomic analysis of bread wheat, the effect of chromosome dosage can be determined by comparing the response of the character under investigation between substituted F_2 nullisomic, monosomic and disomic plants (Figure 5.1, Law et al., 1987). This method of comparison could not be applied in this study because neither nullisomic nor monosomic plants were observed. The $13^{II}+7B^{I}+7D^{I}$ type showed root lengths intermediate to both parents but the response may be due to the present of the *Bot1* (or *Bot2*) allele on chromosome 7B alone, or in combination with an intolerant allele on the chromosome 7D derived from Chinese Spring. The study of Joppa and Williams (1988) demonstrated that only a low proportion of F_2 progeny (1%) derived from LDN 7D(7B) was monosomic (Table 5.1.3) so the absence of monosomics here was not unexpected. While the absence of monosomics precluded a direct estimation of hemizygous genotype, the intermediate response of the $13^{II}+7B^{I}+7D^{I}$ type suggested that chromosome 7D did not have any epistatic effect on the response to a high concentration of B.

There was no evidence of recombination between homoeologous chromosomes of group 7. Analysis of F_2 plants by molecular markers specific to the long and short arms indicated that the critical chromosomes were inherited intact. For example, all the $13^{II} + 7B^{II}$ plants had both short and long arm bands characteristic of chromosome 7A and 7B and all the $13^{II} + 7D^{II}$ had both short and long arm bands characteristic of chromosome 7A and 7D. This indicates that the response of F_2 plants to B was not due to the interchange of arms of chromosomes of homoeologous group 7.

Digenic segregation for B tolerance was demonstrated for LDN x AUS 10110 (T) and most of the substitution lines crosses except 7B. In the critical cross, the significant difference between the most sensitive and tolerant families was attributed to the absence of chromosome 7B. This was in agreement with AUS 10344 (MT) and AUS 14010

(MT) each being different from AUS 10110 (T) at a single gene (*Bo$_T$2* and *Bo$_T$1*, respectively, Chapter 4) as these two genes were located to chromosome 7B when analysed on seperate aneuploid sets.

The combining of cytogenetic and molecular marker evidence proved to be useful in elucidating the genetic control of B tolerance in durum wheat. This method can also be applied to the production of substitution lines in durum, since the alien chromosome can be easily detected. However, some restrictions should be noted. First, the background genetic effect should be constant or absent, otherwise the response of the substituted chromosome will be confounded with background effects. Second, the detection of homoeologous chromosomes by RFLP probes will be less efficient when the chromosome under investigation is involved in rearrangements. For example, the short arm of chromosome 7B of Triticeae was translocated to 4AL by evolutional translocation (Naranjo et al., 1987; Devos et al., 1994). Marker loci, such as *Xpsr119*, *Xpsr160*, *Xcdo1400*, *Per-4*, *XNra* and *XWx* (Chao et al., 1989; Mickelson-Young et al., 1995; McIntosh, 1988) located on this segment on homoeologous group 7 (7AS and 7DS) were also located on chromosome 4AL but not 7BS. Markers used for applications similar to those in this study should be located on a non-translocated segment. Third, the RFLP maps on which the selection of probes was based, were produced for bread wheat, barley or rye chromosomes. Therefore, the parents should be screened together with the other aneuploid stocks such as nullisomic or alien addition/substitution lines, to confirm the location in durum. Finally, aneuploid analysis assisted by markers applied in this study could not define $14^{II}+1^{I}$ as either $13^{II}+7B^{II}+7D^{I}$ or $13^{II}+7D^{II}+7B^{I}$.

5.5 Discussion

Chromosomes of homoeologous group 7 have been implicated in controlling response to B in bread wheat, durum wheat and *Agropyron elongatum*. A major gene controlling B tolerance in bread wheat was located on chromosome 7B by reciprocal monosomic analysis between Federation and Chinese Spring (Paull, 1990), and by the reciprocal backcross monosomic method between Halberd and monosomic Condor Selection P44 (Chantachume, 1995). A study of the Chinese Spring/*Agropyron elongatum* addition lines identified chromosome 7E as also conferring tolerance to B (Paull et al., 1991). The response to B of AUS 10344 (MT) and AUS 14010 (MT) were similar to Halberd when screened in filter paper (Chapter 3) and in high B soils (A.J. Rathjen, unpublished data). Therefore, it is possible that the B tolerance gene found in Halberd might be the same gene as either that of AUS 10344 (MT) or AUS 14010 (MT), since a gene controlling tolerance to B was located to chromosome 7B for both of these durum wheats. However, to establish that which of the genes on chromosome 7B of durum wheat is the same as in bread wheat, it would be necessary to produce hybrids between the two species and assess their response to B. If all the F_2 progeny react the same as both parents, it can be concluded that the genotypes of the bread wheat and durum are the same with respect to B tolerance.

Bo$_T$1 and *Bo$_T$2* were located on the 7B chromosome of durum wheat and the results from Chapter 4 and Section 5.3 showed that when these genes were present together in AUS 10110 (T) and AUS 10105 (T) (*Bo$_T$1Bo$_T$2Bo$_T$3*), segregation at two loci resulted when these lines were crosses to AUS 10348 (MS) (*bo$_T$1Bo$_T$2bo$_T$3*), Yallaroi (MS) (*bo$_T$1bo$_T$2Bo$_T$3*) and LDN (MS) (*bo$_T$1bo$_T$2Bo$_T$3*), indicating independent segregation of *Bo$_T$1* and *Bo$_T$2*. If these genes were linked to each other, deviation from the digenic ratio would be identified by a higher number of homozygous sensitive and homozygous tolerant plants among the progeny than expected for independent assortment. The evidence of unlinked segregation, identified in Chapter 4, together with the evidence for both genes being located on chromosome 7B (Section 5.4),

suggested that the *Boт1* and *Boт2* loci are located far apart enough on the chromosome to gave a recombination frequency of 50%.

The B tolerance alleles in bread wheat, which were proposed to be responsible for the greater range of response to B than in durum (Chapter 3), were predominately located on chromosome 4A of bread wheat. These included, chromosome 4A of the Chinese Spring/Kenya Farmer substitution lines and 4AL of G61450 x Kenya Farmer (Paull et al., 1993) and 4A of India126 (very tolerant, VT) and Benventuto Inca (VT) x monosomic Condor Selection (MS) (Chantachume, 1995). It can be proposed that the greater range of response of bread wheat to B compared to durum wheat was due to the existence of the other gene(s) on chromosome 4A of bread wheat.

Evidence for the chromosomal translocation between 7B and 4A of wheat has been provided by meiotic pairing and isozyme and RFLP studies. A segment of the original 7BS has been translocated to 4AL and a segment of chromosome 4AL translocated to 5AL (Naranjo et al., 1987; Sharp et al., 1989; Liu et al., 1992; Devos et al., 1994). The 4AL-7BS translocation was proposed to be involved in the development of B tolerant genotypes in bread wheat. A highly significant association between response to B and an RFLP in G61450 x Kenya Farmer was detected by the cDNA probe *XksuG10* located on 4AL (Paull et al., 1993). This location was detected to be close to the break point between 4AL-7BS on the modern 4AL (Devos et al., 1995; Mickelson-Young et al., 1995). Chantachume (1995) proposed that the B tolerance genes in bread wheat probably originated on chromosome 7B. He proposed that the gene controlling B tolerance found on chromosome 4A of G61450 was originally located on chromosome 7B and later translocated to chromosome 4A by the evolutionary translocation. The results from this study also support the hypothesis of Chantachume (1995) since two B tolerant genes were located on chromosome 7B of durum. The reason for the appearance of more than one gene located on 7B was unclear. But compared to the A and D homoeologous chromosomes, it may be explained by the nature of the B-genome chromosome. Chao et al. (1989) observed that RFLP loci on

chromosome 7B were approximately three times as variable as their homoeoloci on 7A and 7D of bread wheat. Joppa et al. (1995) examined the translocation frequency of wild tetraploid wheat chromosomes and found that all of the B-genome chromosomes were involved in translocation at higher frequencies than those of the A-genome chromosomes.

In summary, the location of genes on 7B controlling B tolerance of durum wheat was demonstrated. This finding provides useful information for identifying appropriate markers to aid in selection for tolerance to high concentrations of B in durum wheat. RFLP based genetic markers on the chromosomes of homoeologous group 7 are available (Chao et al., 1989; Heun et al., 1991). In next chapter, linkage analysis using these RFLPs was undertaken in order to identify the locations of *Bo*$_T$*1* and *Bo*$_T$2 on chromosome 7B of AUS 10344 (MT) and AUS 14010 (MT).

Chapter 6

Mapping genes for B tolerance in durum wheat by restriction fragment length polymorphism (RFLP) markers

6.1 Introduction

Linkage maps based on RFLP markers have been constructed for several major crops, including tomato (Bernatzky and Tanksley, 1986), maize (Helentjaris et al., 1986) and rice (McCouch et al., 1988). In Triticeae, linkage maps of wheat and its relatives have been reported and are summarized in Table 2.3(Chapter 2).

The application of RFLP-based maps include the manipulation of plant genomes, gene tagging and quantitative trait loci (QTL) mapping and the their usage in plant breeding have been reviewed (for example, see Anderson et al., 1992; Bartels et al., 1989; Beckmann and Soller, 1983; 1986; Gale et al., 1989). Young and Tanksley (1989) demonstrated the use of RFLPs to detect a "graphical genotype", which reflects the parental origin and allelic composition throughout the genome. This can be applied in plant breeding for detecting undesirable DNA segments, to select the parental donor and calculate the number of backcross generations that should be made to reconstitute the recurrent parent. Tightly linked RFLP markers can be used in plant breeding programs to raise selection efficiency.

The use of RFLPs to identify linkage between a monogenic marker and a quantitative trait locus (QTL), identify allelic variants of QTLs and isolate the QTL were proposed by Beckmann and Soller (1983). Pioneering work on the use of RFLPs to detect QTL was undertaken in tomatoes (Osborn et al., 1987; Tanksley and Hewitt, 1988; Paterson et al., 1991) and barley (Heun, 1992).

deVicente and Tanksley (1993) demonstrated that RFLPs can be used to identify QTLs responsible for transgressive phenotypes in eleven quantitative traits of tomato. RFLP approaches to locate genes or chromosomal regions responsible for transgressive segregation have been reviewed for both diploid and polyploid plant species (Allen, 1993; Sorrells, 1992).

In Chapter 4, transgressive segregation for root growth under high B conditions was observed. The cross between AUS 10344 (MT) and AUS 14010 (MT) produced progeny which transgressed the range of the parents. Test crosses between two extreme progeny suggested that the genotypes of AUS 10344 (MT) and AUS 14010 (MT), with respect to B tolerance, were $Bo_T1Bo_T1bo_T2bo_T2$ and $bo_T1bo_T1Bo_T2Bo_T2$, respectively (Chapter 4, Section 4.5). Aneuploid analysis demonstrated that both Bo_T1 and Bo_T2 genes were located on chromosome 7B (Chapter 5). However, the segregation of progenies when these lines were crossed to each other and to other lines revealed that these two genes were probably independent of each other (Chapter 4).

The F_3 progeny between AUS 10344 (MT) and AUS 14010 (MT) was subjected to linkage analysis using RFLP probes located on homoeologous group 7. The objectives of this study were to identify RFLP markers for B tolerance genes and to identify the specific location of Bo_T1 and Bo_T2 on chromosome 7B. Identifying linked RFLPs on durum wheat will provide improved selection tools for screening B tolerance and other characters in durum wheat breeding program. Identification of markers of known location to Bo_T1 and Bo_T2 would provide confirmation of the hypotheses developed in Chapter 4 and 5 that both Bo_T1 and Bo_T2 are located on chromosome 7B but not linked.

6.2 Materials and methods

Plant materials

Plant materials used in this study consisted of :-

(1) Parental lines and the F_3 population derived from (AUS 10344 (MT) x AUS 14010 (MT)). The method of development of this population and the sampling procedure are shown further in Figure 6.1.

(2) LDN D-genome group 7 disomic substitution lines (LDN 7D(7A) and LDN 7D(7B)),

(3) F_2 plant from the cross (LDN 7D(7B) x AUS 10344 (MT)) identified as moderately tolerant ($13^{II}+7B^{II}$) (Chapter 5), and

(4) F_2 plants from the cross (LDN 7D(7B) x AUS 14010 (MT)) identified as moderately tolerant ($13^{II}+7B^{II}$) and moderately sensitive ($13^{II}+7D^{II}$) (Chapter 5).

The names and responses to B of each genotype are shown in Table 6.1.

B screening

Characterizing of the response to B of AUS 10344 (MT), AUS 14010 (MT) and the mapping populations was undertaken by the filter paper method described in Chapter 3. The B treatment was 100 mgB L^{-1}.

DNA preparation (minipreps)

This procedure followed the protocol described by Langridge et al. (1995b). A piece (approximately 10 cm) of fresh leaf was collected, placed in a 2 ml centrifuge tube (Eppendorf® brand) and frozen in liquid N_2. The leaf material was then ground into a fine powder with a knitting needle. 600 µl of DNA extraction buffer (Appendix 1) was added to the tube and homogenized by a quick vortex. 600 µl of phenol/chloroform/-iso-amyl alcohol (25:24:1) was added and mixed on a rotor for 10 min. The phases were separated by centrifuging at 10,000 rpm for 10 min and the upper phase was transferred to a new 2 ml tube. The phenol/chloroform/iso-amyl alcohol step was

Cross production: **AUS 10344 (MT) x AUS 14010 (MT)**

F_1: (1) multiplied in non-B toxic soils

F_2: (1) tested at B100, 132 plants

(2) individual plants transplanted to non-B toxic soil

(3) harvested plants individually to represent F_2 derived F_3 families

(The initial production of cross, F_1, F_2 and F_3 seed were described in Chapter 4.)

F_3: (1) tested at B100, 13 plants/family, 80[a] F_2 derived F_3 families available

(2) one plant selected at random from each F_3 family transplanted to non-B toxic soil

(3) leaf DNA extracted from each plant for RFLP analysis

(4) plants harvested individually to represent F_3 derived F_4 lines

F_4: (1) tested at B100, 13 plants/family, 55[a] F_3 derived F_4 lines available

Figure 6.1 Diagram showing the method of development of F_2 derived F_3 families and F_3 derived F_4 lines from the cross AUS 10344 (MT) x AUS 14010 (MT).

[a] fewer F_3 families or F_4 lines than F_2 plants were available due to sterility of some plants.

Table 6.1 Lines involved in the screening of AUS 10344 (MT) and AUS 14010 (MT) for RFLP markers linked to the *Bo$_T$1* and *Bo$_T$2* genes. Group 7 chromosomes present and genotypes for B tolerance at the *Bo$_T$1/Bo$_T$2* loci are included.

Line	Group 7 genome	Genotype
AUS 10344 (MT)	7A 7B	*Bo$_T$1Bo$_T$1 bo$_T$2bo$_T$2*
AUS 14010 (MT)	7A 7B	*bo$_T$1bo$_T$1 Bo$_T$2Bo$_T$2*
LDN	7A 7B	*bo$_T$1bo$_T$1 bo$_T$2bo$_T$2*
LDN 7D(7A)	7B 7D	*bo$_T$1bo$_T$1 bo$_T$2bo$_T$2*
LDN 7D(7B)	7A 7D	___ ___ ___ ___[c]
(LDN 7D(7B) x AUS 10344)F_2 plant	7A 7B[a]	*Bo$_T$1Bo$_T$1 bo$_T$2bo$_T$2*
(LDN 7D(7B) x AUS 14010)F_2 plant	7A 7B[a]	*bo$_T$1bo$_T$1 Bo$_T$2Bo$_T$2*
(LDN 7D(7B) x AUS 14010)F_2 plant	7A 7D[b]	___ ___ ___ ___[c]

[a] and [b] plants were identified as $13^{II} + 7B^{II}$ and $13^{II} + 7D^{II}$, respectively (Chapter 5). Leaf DNA from individual plants was collected as described in Section 5.3 (Chapter 5) and stored at -20 °C until used.
[c] these plants lack chromosome 7B and therefore also lack the *Bo$_T$* genes and are moderately sensitive to B (Chapter 5).

repeated and 600 µl of the upper phase was transferred to a new 1.5 ml tube. The DNA was precipitated by adding 60 µl of 3M Na-acetate (pH 4.8) and 600 µl of iso-propanol. The tube was centrifuged at 10,000 rpm for 10 min. The supernatant was removed, and the white DNA pellet was washed twice with 70% ethanol and air-dried for 1 h. The DNA pellet was resuspended in 50 µl R40 (40 µg ml^{-1} RNase A in TE buffer) by placing the tube in a refrigerator overnight.

Restriction enzyme digestion

Approximately 20 µg of extracted DNA was digested with five restriction enzymes (*Eco*RI, *Eco*RV, *Hind*III, *Bam*HI and *Dra*I, Promega). Twenty units of each enzyme were used in a total reaction volume of 10 µl containing 1 µl spermadine, 1 µl BSA, 1.2

µl of 10x reaction buffer (Appendix 1) and made up to the total volume with sterile water. The reactions were incubated at 37 °C for 6 h.

Gel electrophoresis

Digested DNA was loaded onto a 1.0% agarose gel. The gel was electrophoresed in 1x TAE buffer (Appendix 1) overnight at a current of 17 mA. After electrophoresis, gels were stained in 10 µg ml^{-1} ethidium bromide for 20 min and viewed under UV light. Gels were soaked in denaturing solution (Appendix 1) for 20 min and 10x SSC for 2 min. DNA was transferred to a nylon membrane (Hybond-N^+, Amersham) through capillary blotting (Southern, 1975) with 10x SSC for 3-4 h. The membrane containing transferred DNA was rinsed in 5x SSC for 2 min. The DNA on the membrane was fixed by placing the membrane, DNA facing up, on Whatman filter paper (3MM Chr) soaked with 0.4M NaOH, for 20 min. The membrane was neutralized in 50 ml of neutralizing solution (Appendix 1) for 5 min, rinsed in 2x SSC for 2 min and blotted dry on filter paper. The dried membrane was sealed in a plastic bag and kept at 4 °C until used.

RFLP probes

RFLP probes that had previously been mapped to homoeologous group 7 were obtained through the Australian Triticeae Mapping Initiative (Dr P.J. Sharp, The University of Sydney). The names and chromosomal locations of probes tested are shown in Table 6.2.

Probe labelling

Labelling of the probes followed the protocol described by Fienberg and Vogelstein (1983). Approximately 50 ng of probe DNA in 2 µl of nanopure water and 3 µl of random primer solution were mixed in a 1.5 ml tube, denatured by boiling for 5 min and quickly chilled on ice for 5 min. Then 12.5 µl of labelling buffer (Appendix 1), 4 µl of [alpha-^{32}P]dCTP and 1 µl of Klenow enzyme (Promega) were added into the

Table 6.2 Marker loci and chromosomal locations of probes used in this study.

Locus	Probe	Chromosome/arm location[a]	Reference
Xpsr152	PSR 152	7AS 7BS 7DS	Chao et al. (1989)
Xpsr117	PSR 117	7AL 7BL 7DL	Chao et al. (1989)
Xpsr121	PSR 121	7AL 7BL 7DL	Chao et al. (1989)
Xpsr129	PSR 129	7AL 7BL 7DL	Chao et al. (1989)
Xpsr169	PSR 169	7AL 7BL 7DL	Devos et al. (1993)
Xpsr311	PSR 311	7L	Devos et al. (1992)
Xpsr547	PSR 547	7L	Devos et al. (1992)
Xpsr690	PSR 690	7L	Devos et al. (1992)
Xpsr965	PSR 965	7L	Devos et al. (1992)
Xcnl.AWBMA16	AWBMA16	7HS	P.J. Murphy (unpublished)
Xcnl.WG669	WG 669	7HS	Heun et al. (1991)
Xcnl.WG719	WG 719	7HS	Heun et al. (1991)
Xcnl.WG686	WG 686	7HL	Heun et al. (1991)
Xcnl.CDO36	CDO 36	7HS	Heun et al. (1991)
Xcnl.CDO475	CDO 475	7HS	Heun et al. (1991)
Xcnl.CDO595	CDO 595	7HS	Heun et al. (1991)
Xcnl.CDO689	CDO 689	7HS	Heun et al. (1991)
Xcnl.CDO347	CDO 347	7HL	Heun et al. (1991)
Xcnl.BCD98	BCD 98	7HS	Heun et al. (1991)
Xcnl.BCD129	BCD 129	7HS	Heun et al. (1991)

[a] 7A, 7B and 7D of wheat genome and 7H of barley genome

S and L refer to short and long arms of chromosomes, respectively

tube. The tube was incubated at 37 °C in a water bath for 1 h. The radioactively labelled DNA was separated from unincorporated [alpha-^{32}P]dCTP by gel filtration on a Sephadex G-100 column.

Hybridization

The membranes were pre-hybridized at 65 °C for 3-4 h in a hybridization bottle containing 1.8 ml sterile water, 3 ml 5x HSB, 2 ml 10x Denhardts III solution, 3 ml 25% dextran sulphate and 200 µl salmon sperm (5 µg ml^{-1}) (Appendix 1) and placed in a rotating Hybaid® hybridization oven. The labelled probe was mixed with 250 µl of salmon sperm DNA (5 µg ml^{-1}), denatured by boiling for 5 min and chilled on ice for 5 min. The denatured probe was added into a bottle containing a pre-hybridized membrane and hybridized overnight at 65 °C. After hybridization, the membrane was washed with series of solutions (2xSSC + 0.1% SDS; 1xSSC + 0.1% SDS; 0.5xSSC + 0.1% SDS; 0.1xSSC + 0.1% SDS) for 20 min each at 65 °C in a shaking water bath. After the last washing, the membrane was sealed in a plastic bag and exposed to X-ray film (Fuji Medical X-ray Film) in a light proof autoradiograph cassette for 4-5 days at -80 °C. The film was developed using an automatic film developer. After the development of the X-ray film, each allele of the RFLP probes was scored for the substitution lines and parental lines of the mapping population.

RFLP analysis of the F_3 plants derived from AUS 10344 (MT) x AUS 14010 (MT)

DNA from the two parents was digested with five restriction enzymes and RFLP analyses were performed with 20 DNA markers (Table 6.2), previously mapped to homoeologous group 7 of wheat, barley and rye (Chao et al., 1989; Heun et al., 1991; Devos et al., 1993). Aneuploid lines (Table 6.1) were used to locate bands to their respective durum wheat homoeologous group 7 chromosomes.

Thirteen seeds of each of the parents and 80 F_2 derived F_3 families were tested for response to B in filter papers at B100, and root lengths were measured 12 days after

sowing. The development of genetic materials is outlined in Figure 6.1. One seedling of each F_2 derived F_3 family was randomly selected and transplanted to potting mix. A leaf sample was collected from each F_3 plant for DNA extraction and RFLP analysis at four weeks after transplanting. The F_3 population of AUS 10344 (MT) x AUS 14010 (MT) was then tested with enzyme/probe combinations that revealed RFLPs between the parents and were located on chromosome 7B.

Eighty F_3 plants were grown to maturity but only 55 F_3 derived F_4 lines were available for progeny testing for B tolerance due to sterility of some F_3 plants (for the production of lines see Figure 6.1). Plants were harvested individually and 13 seeds from each F_3 derived F_4 line were tested in the B100 treatment to identify the genotypes with respect to B tolerance for the F_3 parents.

Response to B of each F_2 derived F_3 family was compared to the parents by using the mean and variance as described in Section 4.2 (Chapter 4). A family which had a mean outside the confidence interval of the parents was classified as significantly different from the parental mean. A family which had a variance higher than the LSD of the parental variance was classified as segregating. The same criterion was used when comparing F_3 derived F_4 lines and the parents.

Association of B tolerance genes with RFLP markers

The 80 F_3 plants were used to construct a linkage map of chromosome 7B. Linkage analysis was undertaken by the maximum likelihood method using Mapmaker and Mapmaker QTL computer softwares (Macintosh V 2.0 Du Pont). To utilize the Mapmaker program, progenies were scored as "A" allele for homozygous AUS 10344 (MT) type, "B" allele for homozygous AUS 14010 (MT) type and "H" for heterozygous when tested at each marker locus. A LOD score greater than 2.5 was considered to be evidence of the existence of a quantitative trait locus (QTL) (Lander and Botstein, 1989). Of the 80 original F_3 plants, 55 F_3 derived F_4 lines were available for linkage analysis between B tolerance and RFLP markers. The linkage analysis

between response to B of the original F_2 derived F_3 family and markers was also calculated because this generation had a larger population. To verify the association between the B response and markers, the F_2 derived F_3 family and F_3 derived F_4 lines were sorted according to parental alleles for significant markers. The mean root length of alleles was compared by an unpaired t-tested.

6.3 Results

Polymorphisms between parents

AUS 10344 (MT) and AUS 14010 (MT) were compared for RFLPs using 20 probes and five restriction enzymes (Table 6.3). Eight of the 20 probes, and 27% of the probe and enzyme combinations, were polymorphic. Probes that showed polymorphism between parents were detected at five loci on the short arm of chromosome group 7 and included *Xcnl.BCD98*, *Xcnl.BCD129*, *Xcnl.CDO36*, *Xcnl.CDO595* and *Xcnl.WG719*, and three loci on the long arm including *Xcnl.CDO347*, *Xpsr121* and *Xpsr311*. All probes which showed polymorphisms between parents, except PSR311 (which detected *Xpsr311*), were used for further screening. PSR311 was excluded because it gave a high copy number (more than six fragments observed).

The location of RFLPs to chromosome 7B was undertaken by hybridizing the seven selected probes to digested DNA from Chinese Spring, LDN, LDN D-genome disomic substitution lines (LDN 7D(7B) and LDN 7D(7B)), AUS 10344 (MT), AUS 14010 (MT) and the F_2s of LDN 7D(7B) x AUS 10344 (MT) and LDN 7D(7B) x AUS 14010 (MT) which had been classified as $13^{II}+7B^{II}$ in Chapter 5. RFLPs between AUS 10344 (MT) and AUS 14010 (MT), located on chromosome 7B were detected by all seven probes (Figures 6.2 and 6.3 as examples).

Linkage analysis

Linkage analysis calculated from 80 F_3 plants of AUS 10344 (MT) x AUS 14010 (MT) was carried out using the seven probes. Significant associations between markers were detected for two pairs of probes, namely, PSR121-CDO347 and CDO595-WG719. The three remaining probes, BCD98, BCD129 and CDO36, were located on the short arm of 7B (Heun et al., 1992) but segregated independently.

Table 6.3 RFLPs associated with homoeologous group 7 after hybridization of 20 probes to DNA of parental lines, AUS 10344 (MT) and AUS 14010 (MT), digested with five restriction enzymes.

Probe	Restriction enzyme				
	*Eco*RI	*Eco*RV	*Hind*III	*Bam*HI	*Dra*I
Short arm					
AWBMA 16	-	-	-	-	-
PSR 152	-	-	-	-	-
BCD 98	nd	+	+	nd	+
BCD 129	?	+	+	?	+
CDO 36	+	+	?	?	+
CDO 475	-	-	-	-	-
CDO 595	-	-	+	-	-
CDO 689	-	-	-	-	-
WG 669	-	-	-	-	-
WG 719	?	+	+	+	+
Long arm					
PSR 117	-	-	-	-	-
PSR 121	nd	+	+	nd	+
PSR 129	-	-	-	-	-
PSR 169	-	?	?	?	?
PSR 311	+	+	+	+	+
PSR 547	-	-	-	-	-
PSR 690	-	-	-	-	-
PSR 965	?	?	?	?	?
CDO 347	+	+	+	+	+
WG 686	-	-	?	-	-

+, - polymorphisms between parents present or absent, respectively.

? non-digested DNA or unclear bands

nd not determined

Figure 6.2 Hybridization of homoeologous group 7 probe, CDO595, to **(a)** *Eco*RV, **(b)** *Dra*I and **(c)** *Hind*III digested DNA of bread wheat, durum wheat and D-genome disomic substitution lines of group 7. Lines used for all combinations were:-

Lane 1 Chinese Spring (CS)

Lane 2 Langdon (LDN)

Lane 3 LDN 7D(7A)

Lane 4 LDN 7D(7B)

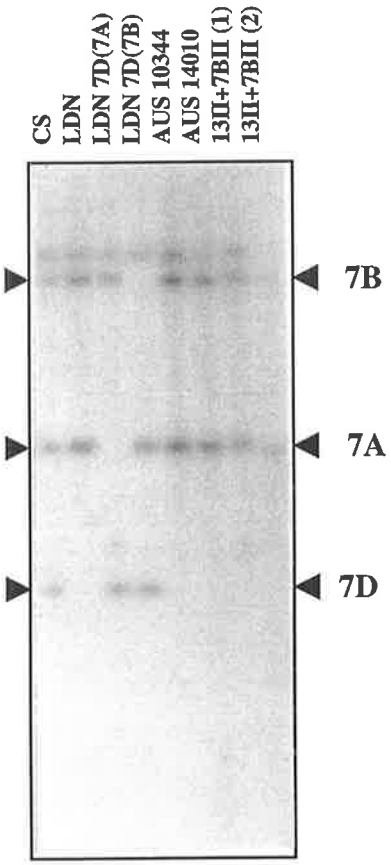
Lane 5 AUS 10344 (MT)

Lane 6 AUS 14010 (MT)

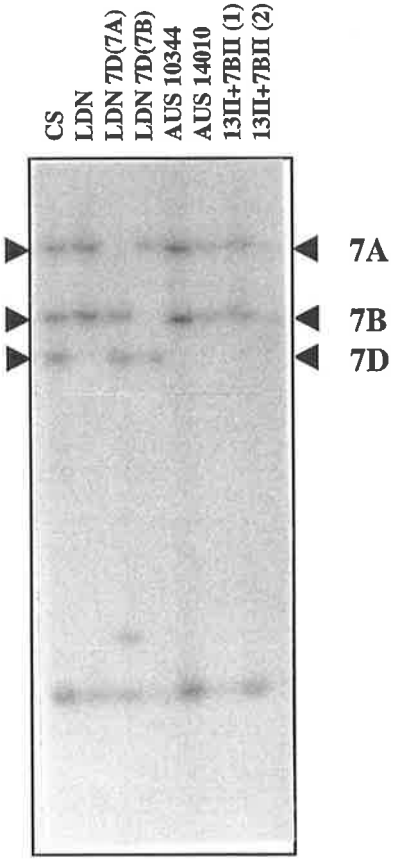
Lane 7 F_2 plant of LDN 7D(7B) x AUS 10344 identified as $13^{II} + 7B^{II}$ (Chapter 5)

Lane 8 F_2 plant of LDN 7D(7B) x AUS 14010 identified as $13^{II} + 7B^{II}$ (Chapter 5)

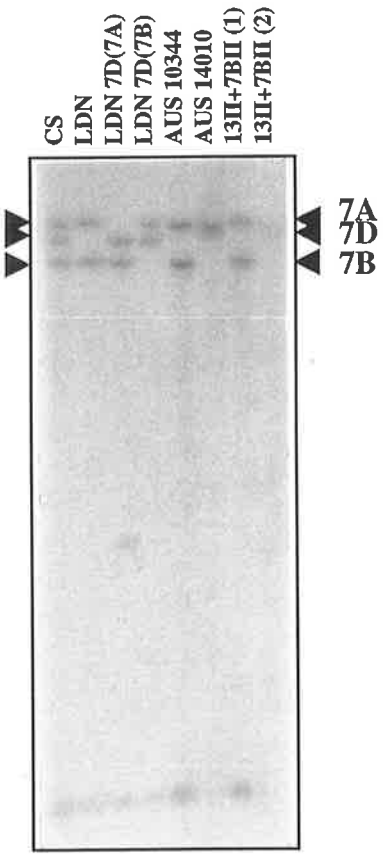
An RFLP between AUS 10344 (MT) and AUS 14010 (MT), located on chromosome 7B, is revealed only by *Hind*III.



(a) *Eco*RV



(b) *Dra*I



(c) *Hind*III

Figure 6.3 Hybridization of homoeologous group 7 probe, WG719, to **(a)** *Dra*I and **(b)** *Hind*III digested DNA of bread wheat, durum wheat and D-genome disomic substitution lines of group 7. Lines used for all combinations are:-

Lane 1 Chinese Spring (CS)

Lane 2 Langdon (LDN)

Lane 3 LDN 7D(7A)

Lane 4 LDN 7D(7B)

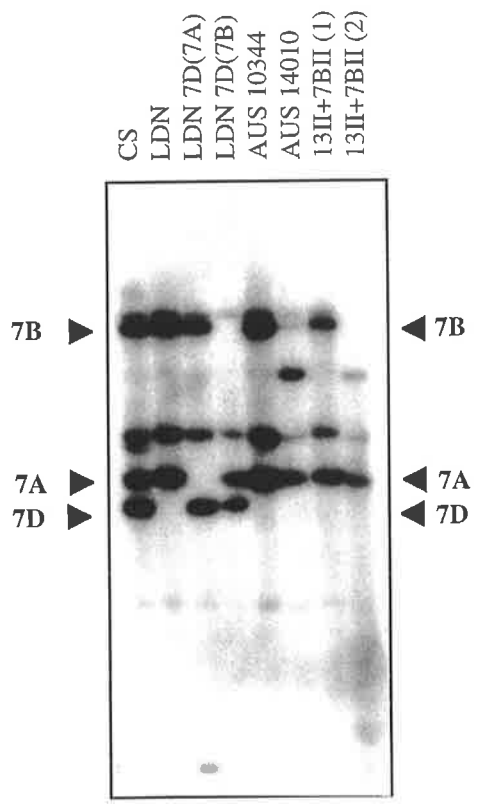
Lane 5 AUS 10344 (MT)

Lane 6 AUS 14010 (MT)

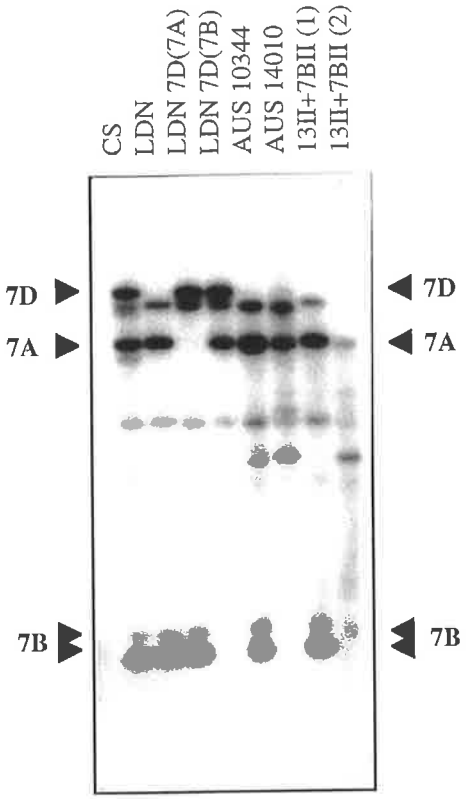
Lane 7 F_2 plant of LDN 7D(7B) x AUS 10344 identified as $13^{II} + 7B^{II}$ (Chapter 5)

Lane 8 F_2 plant of LDN 7D(7B) x AUS 14010 identified as $13^{II} + 7B^{II}$ (Chapter 5)

Both enzymes reveal an RFLP between AUS 10344 (MT) and AUS 14010 (MT), located on chromosome 7B.



(a) *DraI*



(b) *Hind*III

Response to B and RFLP analysis in segregating populations

The responses of the F_3 and F_4 generations were consistent with transgressive segregation as observed in Chapter 4. The overall mean root lengths of the parents (AUS 10344 (MT) and AUS 14010 (MT)) were 8.1 and 7.4 cm, respectively (Figure 6.4a). The confidence intervals of the parental means (at $\alpha = 0.05$) were in the range of 6.3-9.2 cm (AUS 10344 (MT) 7.2-9.2 cm and AUS 14010 (MT) 6.3-8.6 cm). Eleven F_3 families had a mean root length less than 6.3 cm and five families higher than 9.2 cm, with most of them being homozygous (that is the within family variance was less than the LSD of the parental variance, 1.0 cm).

In contrast to the F_3 results, the mean root lengths of most F_4 lines were in the range of the parental lines (Figure 6.4b). There were only eight F_4 lines with a mean root length less than 6.3 cm and six lines greater than 9.2 cm and most of these were segregating (that is the within family variance was more than 1.1 cm). However, not all the F_3 families were represented in the F_4 as many of the families, especially those homozygous sensitive, failed to set seed.

The F_3 population was tested by RFLP analysis using seven probes selected from the screening of the parents. The association between probes and mean root length was determined (Table 6.4). There were significant associations ($P<0.01$) between two of the seven probes, PSR121 and CDO347, and the mean root length of F_3 families (Table 6.4, 6.5 and Figure 6.5). When the families were grouped according to the parental alleles determined by either PSR121 or CDO 347, significantly different root lengths were observed. In both cases, the mean root length of AUS 14010 types (allele "B") was significantly greater than AUS 10344 types (alleles "A") (Table 6.4). These results were in agreement with the distribution of mean root length and parental alleles (Table 6.5), with a high proportion of plants which possessed the allele "A" (AUS 10344 type) having short roots (less than 6 cm). QTL analysis by Mapmaker QTL showed that the B tolerance gene of AUS 14010 was linked to PSR121 and CDO347 with a LOD of 6.2

and 8.2, respectively, with a 95% confidence level. Although a significant association between the marker BCD98 and mean root length of the F_3 was observed when tested by an unpaired t-tested (Table 6.4) but there was no significant relationship between BCD98 and response to B by QTL analysis.

In contrast to the F_3 results, none of the probes was found to be associated with mean root length of the F_4 lines.

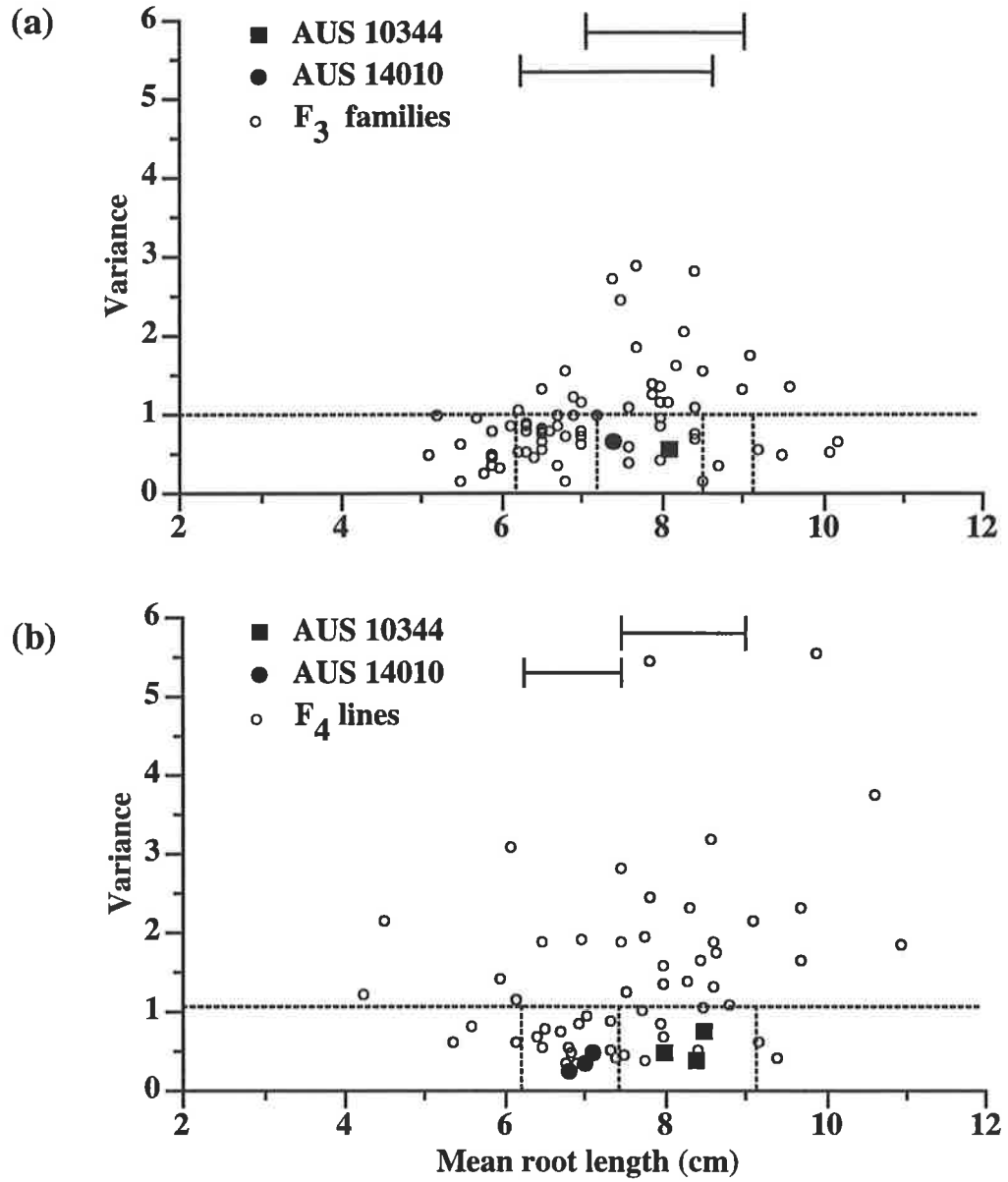


Figure 6.4 Mean root length and variance of parents and F_3 and F_4 progenies grown in filter papers containing 100 mgB L^{-1},

(a) AUS 10344 (MT), AUS 14010 (MT) and F_3 families; confidence intervals of parents were 7.2-9.2 and 6.3-8.6 cm, respectively. LSD of the parents variance was 1.0 cm, indicated by the horizontal dashed line.

(b) AUS 10344 (MT), AUS 14010 (MT) and F_4 lines; confidence intervals of parental lines were 7.4-9.2 and 6.3-7.4 cm, respectively. LSD of the parents variance was 1.1 cm, indicated by the horizontal dashed line.

Table 6.4 Mean root length (cm) of F_3 and F_4 progenies of AUS 10344 (MT) x AUS 14010 (MT) tested at B100 and grouped according to the AUS 10344 "A" allele, AUS 14010 "B" allele and heterozygote "H" when tested with different probe/enzyme combinations.

Probe/enzyme	F_3			F_4		
	mean	var	no.	mean	var	no.
BCD98/*DraI*						
A	7.5	1.4	38	7.6	2.8	37
H	7.0	1.1	10	7.6	2.2	7
B	7.0	1.2	24	7.5	0.6	11
t-test (A v B types)	2.03*			0.10		
BCD129/*DraI*						
A	7.3	0.8	21	7.7	1.9	20
B	7.2	1.8	35	7.5	1.9	22
t-test (A v B types)	-0.26			0.34		
CDO36/*DraI*						
A	7.3	1.1	34	7.4	3.2	31
H	7.3	2.3	21	7.7	0.7	13
B	6.9	1.1	16	7.7	1.5	11
t-test (A v B types)	1.33			-0.50		
CDO347/*DraI*						
A	6.9	1.3	43	7.3	1.9	31
B	7.7	1.1	29	7.7	2.7	23
t-test (A v B types)	-2.89**			-0.82		
CDO595/*HindIII*						
A	7.3	1.6	27	7.5	3.7	22
H	7.3	1.4	17	7.6	1.6	15
B	6.9	1.2	24	7.3	1.0	15
t-test (A v B types)	1.02			0.41		

Continued next page

* and ** indicate a significant difference between A and B alleles at $P<0.05$ and 0.01, respectively

Table 6.4 (continued) Mean root length (cm) of F_3 and F_4 progeny of AUS 10344 (MT) x AUS 14010 (MT) tested at B100 and grouped according to the AUS 10344 "A" allele, AUS 14010 "B" allele and heterozygote "H" when tested with different probe/enzyme combinations.

Probe/enzyme	F_3			F_4		
	mean	var	no.	mean	var	no.
PSR121/*Dra*I						
A	6.6	1.0	29	7.4	1.4	20
B	7.5	1.0	27	7.5	2.2	21
t-test (A v B types)	-3.04**			-0.4		
WG719/*Hind*III						
A	7.4	1.3	39	7.6	2.9	31
B	7.1	1.6	32	7.5	1.2	23
t-test (A v B types)	0.77			0.10		

* and ** indicate a significant difference between A and B alleles at $P<0.05$ and 0.01, respectively

Table 6.5 Mean root length (cm) for the F_3 families of AUS 10344 (MT) x AUS 14010 (MT) tested at B100 treatment and the frequencies of plants homozygous for the AUS 10344 "A" allele and AUS 14010 "B" allele, tested with the probes PSR121 and CDO347.

Root length (cm)	PSR121		CDO347	
	Allele A	Allele B	Allele A	Allele B
≤ 6	9	1	11	0
> 6 - 7	13	11	16	9
> 7 - 8	3	7	8	9
> 8 - 9	3	6	6	7
> 9	1	2	2	5
Total	27	29	43	29

Parental lines	Root length (cm) of parents		
	mean	var	range
AUS 10344 (MT)	8.1	0.3	7.4-8.8
AUS 14010 (MT)	7.4	0.5	6.5-8.4

Figure 6.5 Hybridization of probe/enzyme combinations (a) PSR121/*Hind*III and (b) CDO347/*Dra*I digested DNA of bread wheat, durum wheat and D-genome disomic substitution lines of group 7. Lines used for all combinations were:-

Lane 1 Chinese Spring (CS)

Lane 2 Langdon (LDN)

Lane 3 LDN 7D(7A)

Lane 4 LDN 7D(7B)

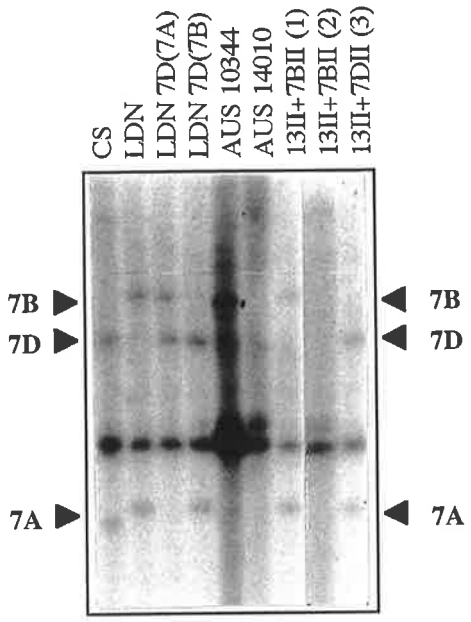
Lane 5 AUS 10344 (MT)

Lane 6 AUS 14010 (MT)

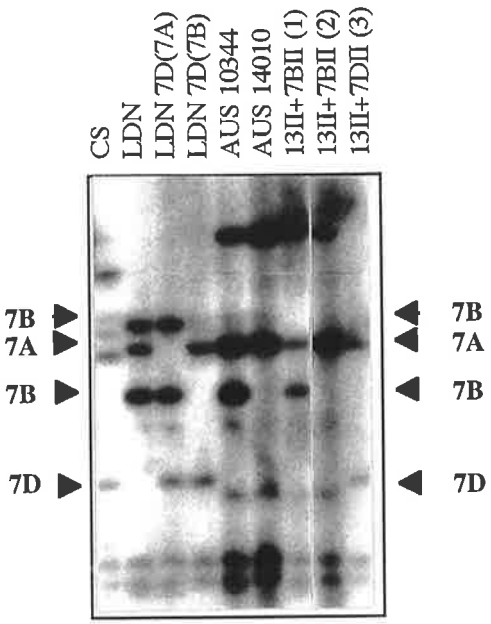
Lane 7 F_2 plant of LDN 7D(7B) x AUS 10344 identified as $13^{II} + 7B^{II}$ (Chapter 5)

Lane 8 F_2 plant of LDN 7D(7B) x AUS 14010 identified as $13^{II} + 7B^{II}$ (Chapter 5)

Lane 9 F_2 plant of LDN 7D(7B) x AUS 14010 identified as $13^{II} + 7D^{II}$ (Chapter 5)



(a) PSR121/*Hind*III



(b) CDO347/*DraI*

6.4 Discussion

Measurement of response to B

The response to B of the population tested in this experiment was measured as a quantitative character, namely, mean root length of F_3 and F_4 families when grown at a high concentration of B. The parents of the mapping population were identified as moderately tolerant to B (Chapter 3) but contained complementary genes responsible for B tolerance, *Boт1* and *Boт2* (Chapter 4), both genes being located on chromosome 7B (Chapter 5). The results from the F_3 and F_4 screening demonstrated that the transgressive segregation observed in the F_2 (Chapter 4) was reproducible in the later generations, confirming that the response to B was subject to small environmental effects and selection for high levels of tolerance can be performed at the early generations.

Due to the transgressive segregation and the overlap of mean root lengths of the parents, AUS 10344 (MT) and AUS 14010 (MT), it was not possible to assign the F_3 families or F_3 derived F_4 lines into discrete genotype categories and the segregation ratios were not determined. In the F_3 generation, the families which had a mean root length in the range of the confidence interval of the parents (6.3-9.2 cm) and a within family variance less than the LSD of the parents were expected to be a mixture of *Boт1Boт1boт2boт2* (AUS 10344 type) and *boт1boт1Boт2Boт2* (AUS 14010 type). The families that showed a variance less than the LSD of the parents but had a mean root length outside 6.3-9.2 cm were classified as either *Boт1Boт1Boт2Boт2* or *boт1boт1boт2boт2*. The families that showed within family variance greater than the LSD of parents were classified as segregating. The same classification would be applied to the F_3 derived F_4 lines.

Transgressive segregation found in the progeny of AUS 10344 (MT) x AUS 14010 (MT) resulted from segregation at two loci (Chapter 4). The reliability of measuring the response to B and association with genetic markers in F_3 and F_4 generations was

dependent on the population size and the number of homozygous families/lines which transgressed beyond the range of parents. The response of the F_4 generation proved to be less reliable than the F_3 because many extremely tolerant or sensitive F_3 plants failed to produce seed. 80 F_3 plants were tested for RFLPs but data on response to B was obtained for only 42-55 F_3 derived F_4 lines (Table 6.4). For example, eleven homozygous sensitive F_3 families were identified but only three of these families were available for progeny testing in F_4 generation due to sterility of the F_3 plants. If sterility of F_3 plants acted as a selection pressure against some genotypes, then it is unlikely that both alleles of each locus were passed on to the progeny at the same frequency. Therefore, 42 to 55 F_4 lines might not have been enough for detecting linkage between markers and the two genes segregating for B tolerance (Sorrells, 1992; Wu et al., 1992).

Polymorphisms of durum parents

RFLP analysis in this study concurred with previous results for bread wheat and barley as probes located to chromosomes of homoeologous group 7 in these species (Chao et al., 1989; Heun et al., 1991; Chen and Gustafson, 1995) were also located to group 7 of durum wheat. Most of polymorphisms in this study occurred on chromosome 7B (Figures 6.2, 6.3 and 6.4). This suggests that the high level of variation found within chromosome 7B, compared to 7A, of bread wheat also exists in the durum wheat lines used in this study. Chao et al. (1989) used 18 probes and 10 restriction enzymes, and reported that RFLPs found in 7B were three times more numerous than those found in 7A and 7D.

The RFLP-based linkage maps of Triticeae (Figure 6.6a) and the availability of LDN aneuploids proved to be useful in identifying RFLPs, located on chromosome 7B, between the durum parents. In this study, only seven RFLPs were identified, including two linked pairs, namely, CDO595-WG719 and PSR121-CDO347. The first group was located on the short arm near the centromere and the latter group near the distal end of the long arm of chromosome 7B (Chao et al., 1989; Heun et al., 1991; Chen and

Gustafson, 1995). The other markers were distributed on the short arm of 7B but were not linked to each other (Figure.6.6b).

Identification of B tolerance related RFLPs

Linkage relationships between RFLPs and genes controlling B tolerance were determined by t-test and Mapmaker QTL analysis. When response to B was analysed at the F_3, significant associations between PSR121 and CDO347 and response to B were observed. These markers were linked to each other at a distance of about 18 cM and located on the long arm of chromosome 7B of the linkage map (Figure 6.6). It is probable that a QTL for B tolerance exists within the region between these two markers as there was a highly significant LOD score for each marker. However, no significant association between B tolerance and markers was observed when the RFLPs were determined at the F_3 and response to B at the F_4 generations.

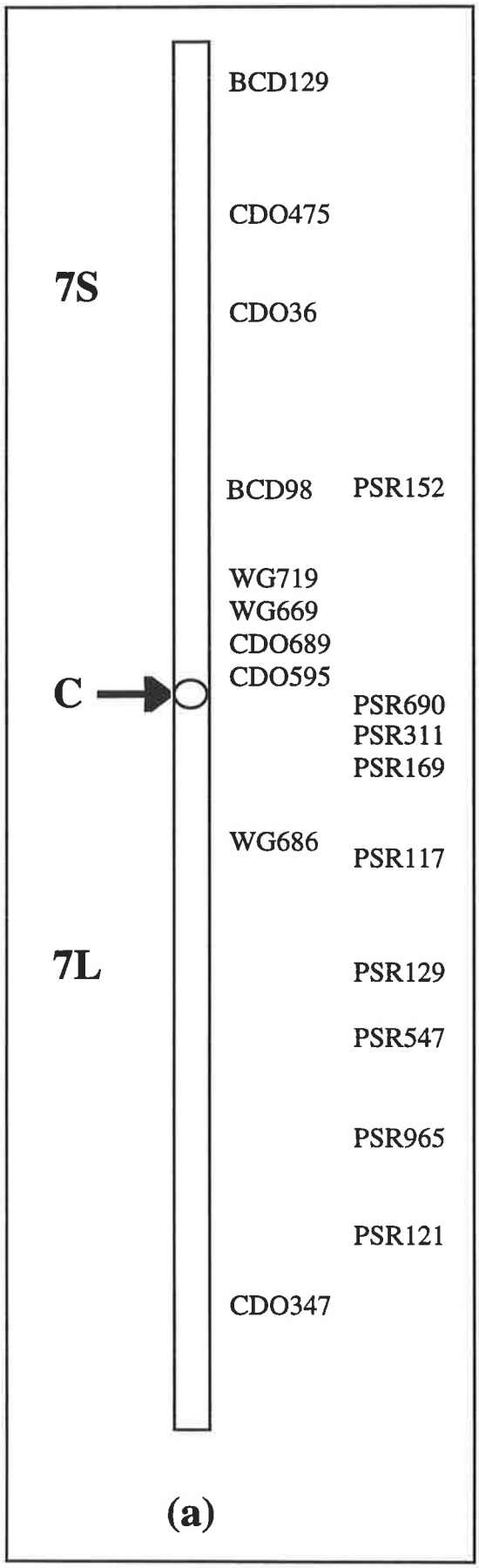
The contrasting results of QTL analysis between the F_3 and F_4 generations when tested with the probes PSR121 and CDO347, may be explained by a distorted segregation of the F_3 derived F_4 lines resulting from the fact that most of the sensitive F_3 plants failed to produce seed and two very tolerant F_3 plants did not flower. QTL analysis used in this study was based on the mean root length of F_2 derived families compared with each allele of each RFLP marker. If one B tolerance gene was linked to the marker, then one homozygotes for this RFLP allele should be associated with a high proportion of tolerant families and, consequently, a higher mean root length than the homozygotes for other allele. As the responses of the two parents were similar, families distributed in the range of the parents could be expected to be a mixture of segregating and homozygous at one locus and parental types for B tolerance (for example, *Bo$_T$1bo$_T$1Bo$_T$2bo$_T$2*, *Bo$_T$1-bo$_T$2bo$_T$2* and *bo$_T$1bo$_T$1Bo$_T$2-*) and the mean root length of these types to be approximately the same.

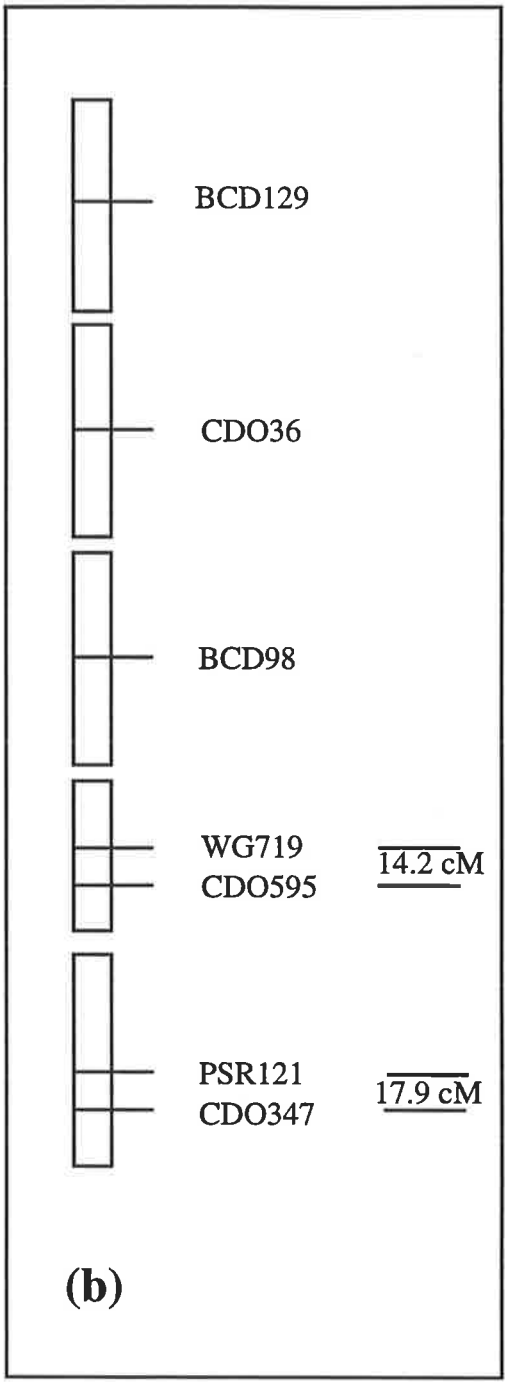
With the failure of many of the extremely sensitive and tolerant lines to reproduce in the F_4, there may have been too few extreme genotypes to detect linkage by QTL analysis.

Figure 6.6 RFLP-based genetic maps of homoeologous group 7 chromosomes;

(a) the approximate locations of BCD, CDO and WG (Heun et al., 1992) and PSR (Chao et al., 1989 and Devos et al., 1993) loci.

(b) the approximate locations of seven loci on chromosome 7B of durum wheat calculated from the 80 F_3 plants from (AUS 10344 x AUS 14010). Loci located on the same segment were significantly associated by linkage analysis.


BCD129
CDO475
7S
CDO36
BCD98
PSR152
WG719
WG669
CDO689
CDO595
C
PSR690
PSR311
PSR169
WG686
PSR117
7L
PSR129
PSR547
PSR965
PSR121
CDO347
(a)


BCD129
CDO36
BCD98
WG719
CDO595
14.2 cM
PSR121
CDO347
17.9 cM
(b)

If a B tolerance gene was linked to PSR121 and CDO347, as found in the F_3, then a significant difference in root length between "A" and "B" alleles at PSR121 and CDO347 might have been observed if a larger number of randomly selected F_4 lines had been obtained.

In analysing the relationship between RFLP genotypes and the mean root length of F_3 families, differences in the mean root length of the F_3 families were observed when tested with the probes PSR121 and CDO347 (Table 6.4). The mean root length of families carrying the "B" allele of AUS 14010 (MT) was significantly greater than the "A" allele of AUS 10344 (MT), for both probes. This suggests that the gene responsible for B tolerance that co-segregated with these markers was likely to be *Bo*$_T$*2* of AUS 14010 (MT) rather than *Bo*$_T$*1* of AUS 10344 (MT). A linkage between *Bo*$_T$*1* and an RFLP marker was not identified. However, the results from previous chapters indicated independent segregation of *Bo*$_T$*1* and *Bo*$_T$*2*. If *Bo*$_T$*2* was located in the region PSR121-CDO347, then *Bo*$_T$*1* may be located far enough from *Bo*$_T$*2* to give a recombination frequency at least 50%, such as on the short arm of 7B. The results in Table 6.4 show that when tested with a probe located on the short arm of 7B, BCD98, the mean root length of allele "A" of AUS 10344 (MT) was significantly higher than "B" allele of AUS 14010 (MT), suggesting the possibility of a relationship between *Bo*$_T$*1* of AUS 10344 (MT) and BCD98. However, this evidence may have occurred by chance because the QTL analysis was not significant. To confirm this hypothesis, further screening should be carried out and more markers close to BCD98 should be identified and tested. Screening of populations segregating for only the *Bo*$_T$*1* gene such as AUS 10344 (MT) x Yallaroi (MS) or AUS 10344 (MT) x LDN (MS) (Chapter 5 and 6) would increase the possibility of detecting linkage between *Bo*$_T$*1* and RFLP markers.

To confirm the finding of a QTL on chromosome 7B, a larger population with a high number of homozygous types should be tested. Later generations of AUS 10344 (MT) x AUS 14010 (MT), such as F_6 or F_7, with a high level of homozygosity would be more appropriate than the F_3 and F_4s. This can be achieved by single seed descent

(Snape, 1988). An alternative study could be undertaken using less complicated crosses such as those involving segregation at a single gene for B tolerance, for example, AUS 14010 (MT) x Yallaroi (MS) and LDN (MS) x AUS 14010 (MT) (Chapters 4 and 5) since polymorphisms between these lines were observed when tested with PSR121 and CDO347 (Figure 6.5).

The results from this study demonstrated the probable location of *Bo$_T$2* on the long arm of 7B but no specific location of *Bo$_T$1* was identified. It was suggested that more markers are required to be tested because most of those included in this study were independent of each other. No polymorphism was detected in several large segments of the chromosome, such as the region between CDO595 and PSR121 on the long arm, BCD129 and CDO36, CDO36 and BCD98, and BCD98-WG719 on the short arm of chromosome 7B (Figure 6.6). In bread wheat, the *Bo1* gene of Halberd was located on chromosome 7B (Chantachume, 1995), however no tight linkage could be detected between *Bo1* and RFLPs markers (J.G. Paull, unpublished data). It is possible that the *Bo1* of Halberd might not be the same gene as *Bo$_T$2* of durum wheat, but is more likely to be similar to, or the same as *Bo$_T$1*.

Chapter 7

General Discussion

Durum wheat genotypes were tested for response to B in soil and filter paper experiments (Chapter 3). When tested in soils, high concentrations of B increased development of leaf symptoms, increased accumulation of B in shoots and reduced dry matter yield. Tolerant genotypes produced less symptoms of B toxicity, lower shoot B concentrations and higher dry matter yields than the sensitive genotypes. When genotypes were grown in filter paper containing high concentrations of B, root lengths of seedlings were reduced and tolerant genotypes produced significantly longer roots than sensitive genotypes. All parameters measured in the soil experiments showed high correlations to each other and with root growth in the filter paper screening method.

Reduction in dry matter yield associated with high concentrations of B have been reported for many crops (Nable, 1988; Paull et al., 1988b; 1992b; Bagheri et al., 1992). The mechanism of tolerance to B, whereby high yield are accompanied by low levels of B in tissue for bread wheat, barley, medicago spp. and peas, was proposed to be due to exclusion of B from the root system (Nable, 1988; Paull et al., 1992b). Although the concentrations of B in roots were not determined, the results of tissue analysis, presented in Chapter 3, suggest that a similar exclusion mechanism operates in durum wheat. The reduction of root growth of seedlings by the filter paper method, and the correlation between genetic variation in response to B in filter paper and soil, were similar to the reaction of bread wheat (Chantachume et al., 1995). A number of studies have shown that root growth was decreased when grown in high B conditions (Morvedt and Osborn, 1965; Holloway and Alston, 1992) and genetic variation in root growth under high B conditions has been reported for bread wheat and barley (Nable

et al., 1990a, Chantachume, 1995). Although there is little information available on the mechanism of tolerance to B, in terms of root growth, it was proposed that it may result from a difference in permeability of root cell membranes (Huang and Graham, 1990).

The ranking of genotypes, when tested in soils and measured 6 to 8 weeks after sowing, was consistent with that when the same genotypes screened in filter paper and measured 12 days after sowing (Chapter 3). This confirmed the finding in bread wheat that B tolerance is expressed at the seedling stage (Paull et al., 1990). The highly significant correlation between root growth at a toxic level of B in filter paper (e.g. root length at B100) and relative root growth (root length at B100/B0) (Chapter 3) suggested that the screening can be undertaken in a single treatment, and assessed for root length relative to genotypes with a known level of tolerance to B (e.g. AUS 10344 (MT) or Yallaroi (MS)). Furthermore, the filter paper method provides a non-destructive measurement, and is time and cost efficient, hence enabling screening of large populations. As assessment is non-destructive and response is expressed during early growth, selected plants may be transplanted to soil and either screened for other desirable characters, before being used as parents for crossing or to enable multiplication of seed for further evaluation.

Limited genotypic variation was found among the varieties of durum wheat agronomically adapted to Australia or from other modern breeding programs. All varieties or advanced lines originating from NSW, Australia or CIMMYT were rated as moderately sensitive or sensitive (Chapter 3). This was in contrast to the findings in bread wheat where higher levels of tolerance were identified within historically important Australian varieties such as those derived from Federation and Currawa, which have been selected and grown in high B areas (Paull et al., 1991b). It is probable that the limited genetic variation in response to B among adapted durum wheat varieties, could be due to:-

- being bred and selected in areas where high concentrations of B do not occur,

- having little genetic variation for B tolerance in the parents used in the breeding programs
- there being a far shorter and less intensive effort in breeding durum wheat, compared to bread wheat, in Australia, thus far less opportunity to develop varieties well adapted to the range of Australian environments.

There was also little variation in response to B among the Mediterranean Basin varieties. This is likely to have resulted from:-

- most of the varieties being grown and selected on deep soils in high rainfall conditions or with supplementary irrigation,
- intense selection for other characters, e.g. quality, having reduced the genetic variation in breeding populations or landraces,
- lack of variation for tolerance to B in the wild progenitor of durum wheat (*T. dicoccoides*) collected from the Israel/Jordan region (A.J. Rathjen, unpublished). Collections of *T. dicoccoides* from further north and east in the fertile crescent have not been tested.

Substantial genetic variation for B tolerance was found in landrace collections of durum wheat originating from India, China and Iraq. This was in agreement with the findings in other crops, such as bread wheat, barley, and peas, in that germplasm originating from these areas provided a high variation for B tolerance (Moody et al., 1988; Bagheri, 1994; Jenkin, 1993; Paull et al., 1992b). There is little information available on the B status of soils in China and Iraq, however high concentrations of B have been described in a number regions of India (Chauhan and Powar, 1978; Chauhan and Asthana, 1981), for example, Uttar Pradesh, Rajasthan, Haryana, Punjab, Gujrat (reviewed by Chauhan and Asthana, 1981). Two of the tolerant accessions (AUS 10110 and AUS 10105) identified in this study were collected from Uttar Pradesh (M. Mackay, pers comm.). It is probable that germplasm of other crops originating from these areas is also likely to provide a high level of B tolerance.

Response to B of F_1 hybrids, F_2 and F_3 populations derived from seven genotypes,

which represented four levels of B tolerance, were used to determine the inheritance of B tolerance (Chapter 4). The results of studies with F_1 hybrids indicated that tolerance was controlled by additive gene action. However, the response of the F_1 hybrids relative to the parents varied with the concentration of B applied. At low B concentrations, the F_1 hybrid was, in general, close to the tolerant parent, but as the concentration of B was increased the response of F_1 hybrids tended more to that of the sensitive parent. A similar pattern of response was reported for bread wheat by Paull (1990). There was no significant difference in the response of pairs of all reciprocal crosses, suggesting no maternal effect in the inheritance of tolerance to B.

Response of F_2 and F_3 generations indicated that B tolerance in durum wheat is controlled by major genes. Three independent loci were proposed, namely, *Bo$_T$1*, *Bo$_T$2* and *Bo$_T$3*. Single gene segregation resulted between T x MT (AUS 10105 (T) and AUS 10110 (T) x AUS 10344 (MT) and AUS 14010 (MT)), MT x MS (AUS 10344 (MT) x Yallaroi (MS), AUS 14010 (MT) x AUS 10348 (MS) and Yallaroi (MS)) and MS x S (AUS 10348 (MS) x AUS 13244 (S)) crosses. Transgressive segregation was observed in three crosses, namely, AUS 10344 (MT) x AUS 14010 (MT), AUS 10344 (MT) x AUS 10348 (MS) and AUS 10348 (MS) x Yallaroi (MS) and this was attributed to the parents having complementary genes controlling response to B.

The genotypes of four tolerant lines were determined as:-

AUS 10110 and AUS 10105 (T, *Bo$_T$1 Bo$_T$2 Bo$_T$3*, originating from India),

AUS 10344 (MT, *Bo$_T$1 bo$_T$2 Bo$_T$3*, from Iraq) and

AUS 14010 (MT, *bo$_T$1 Bo$_T$2 Bo$_T$3*, from China).

The genotypes of moderately sensitive and sensitive lines, in relation to the loci defined for the tolerant lines were:-

AUS 10348 (MS, *bo$_T$1 Bo$_T$2 bo$_T$3*, from Iraq),

Yallaroi (MS, *bo$_T$1 bo$_T$2 Bo$_T$3*, an Australian variety) and

AUS 13244 (S, *bo$_T$1 bo$_T$2 bo$_T$3*, from Afghanistan).

There was no information available of the pedigrees of any of these accessions, except Yallaroi. Therefore, the ancestral relationship of tolerant accessions cannot be traced, nevertheless, two major genes (*Bot1* and *Bot2*) responsible for a higher level of tolerance than that of Yallaroi were identified among genotypes collected in India, Iraq and China. Screening a larger number of landrace accessions of durum wheat distributed in these areas as well as *T. dicoccoides* from Turkey and Iraq may provide more understanding of the adaptation and genetic relationship within the tolerant groups.

Three B tolerant lines, AUS 10344 (MT), AUS 14010 (MT) and AUS 10110 (T), were selected to identify the chromosomal locations of the *Bot1* and *Bot2* genes by crossing these lines to a set of LDN D-genome disomic substitution lines (Chapter 5). Segregation for response to B and chromosome complements of F_2 and F_2 derived F_3 families suggested that the F_2 plants having single or two 7B chromosomes were significantly more tolerant to B than plants nullisomic for 7B. These data suggested that both *Bot1* and *Bot2* are located on chromosome 7B. In bread wheat, Paull (1990) and Chantachume (1995) found that the B tolerant gene *Bo1* was also located on chromosome 7B of Federation and Halberd, respectively.

The method of locating genes of durum wheat to chromosomes by producing crosses between a tester line and a set of LDN D-genome disomic substitution lines has not been used extensively, compared to aneuploid analysis in bread wheat (see Joppa, 1993). However, this method proved to be very efficient in identifying locations of B tolerance genes in durum wheat. Due to the different transmission rates of monosomes or double monosome of the aneuploid plants (in which A and B genome chromosomes a have higher transmission rate than the D-genome chromosome), the segregation of the F_2 derived from the critical LDN aneuploid line and the B tolerant parent produced an excess of tolerant plants. For example, with *Bot1* located on 7B of AUS 10344 (MT), then the F_2 of LDN 7D(7B) x AUS 10344 (MT) included a higher number of tolerant plants than expected for simple Mendelian inheritance

because $13^{II}+2^{I}$ F_1 plants had a higher transmission rate of 7B than 7D chromosomes.

As B tolerance in durum wheat is controlled by major genes, breeding for B tolerance can be undertaken through a backcrossing program. Furthermore, the response of a segregating population measured by root length showed a low environmental variation, suggesting that B tolerance can be selected at an early generation in the seedling stage. However, linkage drag and interaction with other genes often slows the process of introgression of a segment from an exotic accession into the recurrent parent. The location of B tolerance genes to chromosome 7B allows the application of molecular techniques to identify markers which could be used for marker based selection to minimize these negative effects. RFLP markers were used to identify the location of *Bo$_T$1* and *Bo$_T$2* in the progeny of AUS 10344 (MT) x AUS 14010 (MT) (Chapter 6). In this population, transgressive segregation was confirmed in the F_4 progeny, resulting from the parents having complementary genes controlling tolerance to B. The significant association between B tolerance and the RFLP markers, CDO347 and PSR121, indicated that the B tolerance gene (*Bo$_T$2*) in durum line AUS 14010 (MT) is probably located on the long arm of chromosome 7B between these two markers. Testing a larger number in inbred lines, such as F_6 or F_7 lines produced by single seed descent or a double haploid population, or populations involving segregation at a single gene rather than two genes as was the case with the mapping population, would increase the efficiency of the linkage analysis.

B tolerance in bread wheat was available in commercial Australian varieties such as Halberd (Paull et al., 1991b). Therefore, tolerant varieties could be introduced for cultivation in problem areas, and indeed they dominated in these areas prior to the recognition of B toxicity (Paull, 1990). They could also be used as parents for intercrossing or backcrossing to other adapted varieties to integrate B tolerance, as well as other desirable characteristics, into new varieties. In contrast to bread wheat, B tolerance in durum wheat was identified in exotic landraces, which are not well adapted to South Australia. For example, tolerant lines identified in this study

showed poor fertility as the sterility of some accessions and their progenies were evident throughout this study. For example, in the cross AUS 10344 (MT) x AUS 14010 (MT), 132 F_2 plants were tested but only 80 F_2 derived F_3 families were available due to sterility of F_2 plants (Chapter 6). Therefore, overcoming B toxicity in durum wheat cannot be managed by direct introduction of lines to grow in problem areas. Tolerant lines identified in this study are only useful as donor parents in breeding programs. As they are so poorly adapted, many rounds of backcrossing will be required to transfer the gene conferring this character into locally adapted varieites. At the Waite Institute, backcross derived lines are being developed to transfer the *Bo7*2 gene from AUS 14010 (MT) to Yallaroi (MS). Yield comparisons of the backcross derivatives and Yallaroi are being undertaken (B. Brooks, pers comm.).

DNA markers are ideal tools for introgression of desirable characters from exotic germplasm into locally adapted genotypes. The results from this study demonstrated the possibility of mapping the location of the *Bo7*2 gene on chromosome 7B. Once this result is confirmed, flanking markers may be used to retain the *Bo7*2 locus while other markers may be used to identify the genetic background and reduce the number of backcrosses required to reconstitute the recurrent parent. Since B tolerance is controlled by several major genes, this approach can be applied for the rapid development of isogenic lines. The use of isogenic lines will be of importance in many applications, for example, characterization of the physiology and biochemistry of the B tolerance as well as determining the difference in yield between the isogenic lines when grown under "normal" and high B conditions.

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Appendix 1

Solutions used for RFLP analysis in Chapter 5 and 6

DNA extraction buffer	0.1 M Na_2SO_3, 4% Sarkosyl, 0.1 M Tris-HCl, pH 8.0, 10 mM EDTA
10x reaction buffer B	100 mM Tris-HCl, 50 mM $MgCl_2$, 1000 mM $NaCl_2$, 10 nM ß-mercaptoethanol, pH 8.0
1x TAE buffer	40 mM Tris-acetate, 1mM EDTA pH 7.6
20 SSC	for 2 l of distilled water, 350 g 3 M NaCl, 176 g 0.3 M Trisodium citrate
Denaturing solution	for 2 l of distilled water, 175 g 1.5 NaCl, 40 g 0.5 M NaOH
Neutralizing solution	for 2 l of distilled water, 175 g 1.5 NaCl, 121 g 0.5 M Tris-HCl, 0.74 g 0.001 M EDTA Na_2, pH 7.2
Denharts III	2% gelatin, 2% ficoll, 2% Polyvinil-pyrrolidone360, 10% SDS, 5% tetrasodium pyrophosphate, filter at 65 °C
5x HSB	for 1 l of distilled water, 175.3 g 3 NaCl, 30.3 g 100 mM PIPES, 7.5 g 25 mM EDTA Na_2, pH 6.8 with 4 M NaOH
Salmon sperm DNA	add 0.5 g salmon sperm DNA to 100 ml of nanopure H_2O, autoclave
Sephadex G-100	To 300 ml TE buffer add 10 g sephadex G-100, incubate with gentle shaking for 2 h at 65 °C
TE buffer	10 mM Tris-HCl, 1 mM EDTA, pH 8.0