GENETIC STUDIES ON THE TOLERANCE OF WHEAT TO HIGH CONCENTRATIONS OF BORON

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Abstract

Initially, experiments were conducted to prove that a new screening technique, namely the length of seedling roots in filter papers moistened with solutions with high concentrations of boron, for tolerance to high concentrations of boron could be used for distinguishing between tolerant and sensitive genotypes. Seedlings were compared in the filter paper technique with those grown in boron enriched soil to investigate the response of wheat genotypes known to differ in tolerance to high concentrations of boron. Under high boron concentrations in filter papers, the more tolerant genotypes had significantly longer roots than those of the more sensitive genotypes. There was no significant correlations between the root lengths in the control treatment and the other three boron treatments (50, 100, 150 mgB l^{-1}). Thus, the differences in root lengths in the high boron treatments could not be attributed to inherent differences in root growth but to the genetic variation in response to high boron concentrations among varieties. Root lengths in the three boron treatments in filter papers were highly significantly correlated with the three characters routinely determined for plants grown in soil containing high levels of boron, namely the concentration of boron in the shoots, plant dry weight and leaf symptoms, indicating that root length could be used as a selection criterion in genetic studies or breeding programs for boron tolerance.

Genetic control of tolerance to boron was investigated between a moderately tolerant variety Halberd, a tolerant line G61450 and the moderately sensitive varieties Schomburgk and Condor. Two genes, *Bo1* and *Bo4* controlled tolerance to boron in Halberd and G61450, respectively. The genetic control of response to boron was the same for Condor, Schomburgk and a homozygous sensitive line 442S-1 extracted from the cross between G61450 and Halberd.

The chromosomal location of genes controlling tolerance to boron was studied by the use of F_2 monosomic and backcross reciprocal monosomic analysis. The results were consistent for both methods showing that chromosomes 7B and 4A were responsible for tolerance to boron in Halberd and G61450, respectively. Results of the backcross

reciprocal monosomic analysis indicate that chromosome 4A was also the location of genes controlling tolerance to boron in the tolerant exotic lines India 126 and Benventuto Inca.

The genetic relationship, with respect to tolerance to boron, between an Australian moderately tolerant variety BT-Schomburgk and a number of tolerant exotic lines was investigated by testing the F_3 derived F_4 families. Transgressive segregations were observed for the crosses between BT-Schomburgk and Klein Granador and Turkey 1473, indicating at least two different genes controlling response to boron between BT-Schomburgk and these two exotic lines. Monogenic segregations were observed from the crosses between BT-Schomburgk and AUS 4903. The results of the cross between BT-Schomburgk and India 126 were more complicated than those of the other crosses and indicated that more than one gene conferred tolerance to boron in this cross.

This thesis demonstrates that it is possible to breed even more tolerant varieties than Halberd or BT-Schomburgk by transferring boron tolerant genes from tolerant lines including G61450, Turkey 1473, AUS 4903 and Klein Granador into less tolerant but otherwise well adapted varieties.

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(Yodsaporn Chantachume)

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Chapter 1

Introduction

Boron is an essential micronutrient, but has an adverse effect on plant growth when present in high concentrations. Excessive levels of boron in wheat plants result in a yellowing of the leaf tip on the oldest leaves, followed by a non-specific necrosis continuing down the leaf (Paull et al., 1988). High concentrations of boron have been recorded in soils and plant samples obtained from widespread regions of the cereal growing districts of southern Australia (Ralph, 1992) and a yield reduction of up to 11% and 20% in wheat (Moody et al., 1993) and barley (Jenkin, 1993), respectively, could be attributed to boron toxicity. These indicate that boron toxicity is a major problem for cereal production in southern Australia. Soils of South Australia in which boron toxicity has been found are almost invariably sodic and rich in calcium carbonate (Cartwright et al., 1986). This calcium tends to absorb the boron at the top of the B horizon of the soil, thus presenting a layer of high boron to advancing plant roots (Rathjen et al., 1986).

The amelioration of boron toxicity through soil modification (e.g. application of gypsum, leaching with water) is not an economic proposition in southern Australia, therefore the breeding of more tolerant varieties offers the only approach to minimizing yield losses. An understanding of genetics of boron tolerance was a fundamental component necessary for the development of an efficient breeding program for boron tolerance.

Boron tolerant genotypes of wheat and barley (Nable, 1988) and peas and medics (Paull et al., 1992b) maintain lower concentrations of boron in shoots and roots than more sensitive genotypes, and consequently develop less severe symptoms of boron toxicity. This applies for plants grown in both soil and solution culture. A large range in genetic variation in response to boron toxicity has been demonstrated in wheat (Moody et al., 1988).

Tolerance to high concentration of boron in wheat is expressed as a partially dominant character and controlled by major genes which act in an additive manner and have been named *Bo1*, *Bo2* and *Bo3* (Paull, 1990; Paull et al, 1991b). The *Bo1* allele has been transferred from the moderately tolerant variety Halberd to the moderately sensitive variety Schomburgk to produce BT-Schomburgk (Moody et al., 1993) which had a yield advantage up to 11% with the average yield advantage being 3.3% in all trials conducted in a range of soil types across the South Australian cereal belt and at Walpeup in Victoria (Moody et al., 1993).

Aneuploid analysis has been used to identify the chromosomal location of the genes controlling tolerance to boron and the chromosomes of homoeologous groups four and seven were found to be involved in boron tolerance (Paull, 1990). Exotic germplasms more tolerant than Halberd, the most tolerant Australian variety, have been identified (Moody et al., 1988), indicating that a more tolerant variety than Halberd could be bred by transferring the boron tolerance genes from those tolerant exotic lines to Halberd or the other more sensitive but otherwise well adapted varieties. Transgressive segregation was observed from the cross between the moderately tolerant Halberd and a tolerant exotic line G61450 (Paull et al., 1991b). The genetic study of those exotic lines relative to Australian varieties was undertaken here to indicate an appropriate breeding strategy for the transferring boron tolerant genes.

The project reported here comprised two studies, firstly an investigation of screening techniques for boron tolerance and secondly the study of the genetic control of tolerance to boron.

The initial experiments were conducted to establish a new inexpensive, rapid, statistically analyseable, non-destructive screening technique, namely a filter paper technique, which could be used in screening for tolerance to boron as a replacement for screening in boron enriched soil in a glasshouse (Chapter 4). The filter paper technique was then used for the screening of boron tolerance in the genetic studies.

The genetic relationship, with respect to tolerance to boron, was investigated between a moderately tolerant variety Halberd, a tolerant line G61450 and the moderately

sensitive varieties Schomburgk and Condor. A homozygous tolerant and a homozygous sensitive line were selected from (G61450 x Halberd). The crosses between both of these lines and the three varieties G61450, Halberd and Schomburgk and between Schomburgk and Condor were tested for segregation in the F_2 and F_3 generations (Chapter 5).

Since Halberd and G61450 were found to be more tolerant than Condor (Chapter 4) and to differ in their genetic control of boron tolerance (Chapter 5), closer investigation of the genetic control of these varieties was undertaken. Studies on the chromosomal location of genes conferring tolerance in Halberd and G61450 were undertaken by F_2 monosomic analysis with Condor monosomics as aneuploid stocks (Chapter 6).

It was not possible to use the F_2 monosomic analysis when testing six tolerant varieties (India 126, Benventuto Inca, AUS 4041, Lin Calel, Halberd and G61450) (Chapter 7) simultaneously, because of the time required for the cytological examination of the monosomic plants, so the backcross reciprocal monosomic analysis was adopted. The response to boron of only chromosomes of groups four and group seven of the six varieties was examined (Chapter 7) because chromosomes 4A and 7B were responsible for tolerance to boron in G61450 and Halberd, respectively (Chapter 6).

The high level of tolerance to boron identified in exotic accessions could be transferred to the moderately tolerant Australian varieties. To enable an efficient crossing and selection strategy to be devised, the genetic relationship, with respect to tolerance to boron, was investigated between a moderately tolerant Australian variety BT-Schomburgk and a number of exotic tolerant lines (India 126, AUS 4903, Turkey 1473 and Klein Granador) (Chapter 8).

Chapter 2

Literature review

The relationship between boron, plants and soil

2.1 Chemistry

Boron, the only non-metal among the elements of group III in the Periodic Table, has a tendency to form anionic rather than cationic complexes (Keren and Bingham, 1985). In aqueous solutions at pH < 7, boron occurs mainly as undissociated boric acid [$B(OH)_3$], which dissociates to $B(OH)_4^- + H_3O^+$ at higher pH values (Romheld and Marschner, 1991). Thus, in accordance with the electron configuration of boron, boric acid acts as a weak Lewis acid:

$$B(OH)_3 + 2H_2O \Leftrightarrow B(OH)_4^- + H_30^+ \quad pKa = 9.25$$

It has been concluded that boric acid has a trigonal planar structure, whereas the borate ion has a tetrahedral structure in aqueous solution. This difference in structure can lead to differences in the affinity of clay for these two boron species (Keren and Bingham, 1985).

At boron concentration ≤ 0.025 mM, only the monomolecular species $B(OH)_3$ and $B(OH)_4^-$ are usually present in solution (Ingri et al., 1957).

Boric acid forms very stable complexes with organic compounds with a cis-diol configuration. These compounds include sugars, and their derivates are abundant in cell walls. The concentrations of boron in cell walls roughly reflect the differences in boron requirement among plant species (Romheld and Marschner, 1991). For example, boron requirement of cereals was observed to be lower than that of legumes (Bergman, 1984) and the sufficient levels of boron in wheat and barley plants (2.1-10.1 mgB kg^{-1}) (Gupta, 1979) were lower than those in soybean (*Glycine max*) and alfalfa (*Medicago sativa*) (25-60 and 35-80 mgB kg^{-1}, respectively (Bergmann, 1983).

2.2 Source of boron in the environment

Since the distribution of boron in the earth's crust is not uniform, the geochemistry of boron is characterized by an abnormally large range of variation in its concentration in rocks in comparison to those of other elements such as manganese and iron. For example, the concentrations of boron in basic rocks, acid rocks and sedimentary rocks are 1-2, 3 and 100 ppm in comparison to 2000, 1000 and 1000 ppm, respectively, of manganese (Norrish, 1975). The boron content of magmatic rocks increases with the acidity of the rocks, while in sedimentary rock boron is associated with the clay fraction (Kabata-Pendias and Pendias, 1984). Norrish (1975) reported that marine sediments contain more boron than igneous rocks. However, the boron in rocks is not available to plants and most of the plant-available boron in soil comes from the decomposition of soil organic matter and from boron adsorbed and precipitated on to the surfaces of soil particles (Russell, 1973; Bingham, 1973; Bowen, 1977).

In the terrestrial environment, boron is likely to combine with oxygen and is known to form several minerals, mainly hydroxides and silicates, of which the tourmaline group is the most common in soils (Kabata-Pendias and Pendias, 1984). Tourmaline (3-4% boron) is present in soils formed from acid rocks and metamorphosed sediments; however, boron within minerals is not available to plants (Norrish, 1975). Boron can substitute for tetrahedrally coordinated silicon (Si) in some minerals. It is likely that much of the boron in rocks and soils is dispersed in the silicate minerals in this way and would be available only after long periods of weathering (Norrish, 1975).

2.3 Boron in soil

2.3.1 Adsorption of boron

Boron is an essential element in plant nutrition. Low concentrations are required for sufficiency while higher concentrations produce toxicity symptoms and marked yield

reductions. Since the range in concentration between deficiency and toxicity is narrow (Berger, 1949), reactions affecting the availability of boron are of interest for understanding the occurrence of boron toxicity and deficiency of plants.

Boron may be found in three main combinations:

(1) boron in silicate structures;

(2) boron associated with clay minerals, e.g. sesquioxides and iron and aluminium hydroxy compounds; and

(3) organically combined boron.

Boron may enter silicate structures by substituting for Al^{3+} and Si^{4+} ions (Couch and Grim, 1968). Tourmaline, the major mineral of this form, is reported to be the major source of boron in sodic soils (Bhumbla and Chhabra, 1982). However, boron in tourmaline is not available for plant growth. Weathering of boron containing rocks and minerals brings boron into solution, predominantly as $B(OH)_3$.

The adsorption of boron on clay minerals has been studied by many investigators (Couch and Grim, 1968; Hingston, 1964; Keren and Mezuman, 1981; Keren et al., 1981; Keren and O'Connor, 1982; Sims and Bingham, 1967). Increasing pH enhances boron adsorption on montmorillonite, kaolinite and illite clays, showing a maximum in the alkaline pH range. Keren et al. (1981) explained the response of boron adsorption to variations in pH as follows: Below pH 7, $B(OH)_3$ predominated, but because the affinity of the clays for this species was relatively low, the amount of adsorption was small. As the pH increased to about 9, the $B(OH)_4^-$ concentration increased rapidly, as did the adsorption of boron due to the high affinity of clays for $B(OH)_4^-$. Further increase in pH of more than 9 resulted in an enhanced OH^- concentration relative to $B(OH)_4^-$, and boron adsorption decreased rapidly due to the competition of OH^- for the adsorption sites. Although the total surface area of montmorillonite is much greater than that of illite, boron adsorption by illite is much greater than by montmorillonite (Hingston, 1964; Keren and Mazuman, 1981). This is because the adsorption mechanism of boron is a specific type of adsorption. There are two types of surfaces in clay minerals (Van Olphen, 1977): the planar and the

edge surfaces. Boron is adsorbed mainly on the broken edges of the clay platelets, which are found more in illite, rather than on the planar surfaces (Keren and Talpaz, 1984).

Much adsorption of boron by clays is attributed to sesquioxides and iron and aluminium hydroxy compounds present as coatings on the surface of clays (Sims and Bingham, 1968; Ellis and Knezek, 1972). Such adsorption varies with the pH, and boron retention by aluminium hydroxy compounds is far greater than that effected by iron hydroxy compounds (Sims and Bingham, 1968). Beyrouty et al. (1984) determined the strength of interaction between boron and $Al(OH)_3$ surfaces by a combination of infrared and chemical analyses. They suggested that boron replaced or was bonded to surface hydroxide ligands, thereby blocking sites of polymerization. However, these data do not rule out the possibility that precipitation of boron on the surface of $Al(OH)_3$ may also occur.

Boron sorption behaviour in whole soil has indicated an important role for Al and Fe oxides. Bingham et al. (1970) reported a significant correlation between Al_2O_3 content and boron adsorption of four Mexican and six Hawaiian soils. Elrashidi and O'Connor (1982) found that Fe_2O_3, organic C, and cation exchange capacity were the major factors effecting the variance in adsorbed boron of ten soils from Mexico. Boron fixation in Al_2O_3 and Fe_2O_3 is affected by pH, with adsorption peaks at pH 6 to 7 for Al_2O_3 and pH 8 to 9 for Fe_2O_3, followed by a gradual decline at higher pH levels (Scharrer et al., 1956). Sims and Bingham (1968) and McPhail et al. (1972) obtained similar adsorption behaviour using x-ray amorphous hydroxy Fe and Al forms. Boron adsorption was maximum on freshly precipitated materials and decreased with increasing crystallinity resulting from ageing (Sims and Bingham, 1968). Both sets of results also suggested that the mechanism of boron adsorption may be anion exchange with hydroxyl ions. This type of ligand exchange with surface reactive OH^- groups is a mechanism by which anions become specifically adsorbed onto oxide mineral surfaces (McPhail et al., 1972).

Many researchers have suggested that soil organic matter influences extractable boron and the availability of boron to plants. Berger and Truog (1945) found a high positive correlation between available boron and the organic matter content of acid soils,

and that increasing pH had a much greater influence in decreasing the availability of boron in alkaline soils than did organic matter in maintaining availability. Page and Paden (1954) also noted the association between levels of organic matter and available boron in acid soils and postulated that organic matter exerted a greater influence on boron availability than either pH or soil texture. A large part of total boron is held in organic matter in the form of boron-diol complexes (Parks and White, 1952) and the available boron is released by microbial action (Berger, 1962; Berger and Pratt, 1963). Olson and Berger (1946) had previously found that oxidation of soil organic matter resulted in a significant release of boron in forms available for plants and caused a slight decrease in boron fixation.

2.3.2 Interaction of boron with other nutrients

The uptake of boron by plants can be markedly affected by the presence of other plant nutrients in soils. The association between calcium and boron in plant nutrition was first indicated by Brenchley and Warrington (1927) and was studied in depth by Reeve and Shive (1944). It was shown that as the calcium content was increased, more boron was required both to prevent deficiency and to produce toxicity. Eck and Campbell (1962) found that liming decreased boron uptake when soil boron reserves were high. They attributed this effect to a high calcium content. The addition of calcium thus increased the plants' requirement for boron but decreased the ability to absorb it. Tanaka (1967) reported that boron uptake by radish (*Raphanus sativus* L.) was reduced when calcium content of the medium was increased. The effect of calcium on boron uptake may be attributed to the Ca : B ratio in the plant tissue (Marsh and Shive, 1941). The Ca : B ratio has been used to predict boron deficiency; however this ratio should not be given the same importance as levels of the individual elements (Gupta, 1979). Prather (1977) and Takkar (1982) found that at equivalent amounts of calcium, tissue boron concentrations were much higher if $CaSO_4$, rather than $CaNO_3$, was applied to the soil. However, Gupta and MacLeod (1977) reported that increasing soil pH by the addition of lime, rather than the availability of calcium and magnesium, decreased boron uptake in the absence of added boron.

A relationship has been observed between the concentration of potassium in the growth medium and boron nutrition. For example, Reeve and Shive (1944) noted that at low levels of boron supply the effect of potassium was similar to that of calcium, so increasing potassium levels in the nutrient solution accentuated boron deficiency symptoms. At high levels of boron supply, however, increased potassium levels accentuated boron toxicity. Hill and Morill (1975) reported results from field and greenhouse experiments and suggested that there was a significant positive relationship between potassium and boron fertilizer in increasing yields of peanuts, except at the highest boron and potassium levels where yields were reduced. Sinha (1961) attributed the boron deficiency which resulted when potassium was applied to low boron soils to physiological interactions. Patel (1967) showed that boron deficiency symptoms of Bedi Tobacco increased and toxicity symptoms decreased with an increase in Ca : B or K : B ratio. In contrast, Cutcliffe and Gupta (1980) showed that boron concentrations of cauliflower (*Brassica oleracea* L. spp. botrylis) leaf tissues were not greatly affected by phosphorous or potassium treatments, but that applied nitrogen increased the boron content of the tissues.

In many Australian soils, a high concentration of soluble boron and a low level of available zinc may occur simultaneously. The interactions of phosphorous with boron and zinc were studied in barley by Graham et al. (1987). They concluded that both low zinc and high phosphorous supplies increased boron accumulation in barley plants and suggested that fertilizer with available zinc might be applied to reduce boron toxicity. Singh et al. (1990) found that zinc deficiency accentuated boron accumulation to toxic levels in the tops of wheat plants. They also found that boron accumulation in plant tissues increased with the increasing of boron supply more in the absence of zinc than that in its presence. Boron deficiency in maize (*Zea mays* L.) led to the accumulation of physiologically inactive zinc in plants and zinc deficiency symptoms, even though zinc concentration in the plant tissues was not low (Leece, 1980). It is therefore possible that boron may be required for the normal utilization of zinc by plant cells.

Among the macronutrients, nitrogen is the most important in its effect on uptake of boron by plants (Gupta, 1979). Davies (1980) suggested that the availability of boron *per se* was not affected by the application of nitrogen fertilizers, but that, under critical conditions, boron deficiency could be induced by the use of nitrogen. Smithson and Heathcote (1976) found that the application of 250 kg N ha^{-1} depressed cotton yield under boron deficient conditions, but increased yield when boron was applied. The application of boron enhanced the utilization of applied nitrogen in cotton plants by increasing the translocation of nitrogen compounds into the boll (Miley et al., 1969). Davies (1980) described the nitrogen-boron relationship within plants as the inability of boron deficient plants to effect complete protein synthesis. Chapman and Vanselow (1955) found that liberal nitrogen applications were sometimes beneficial in controlling excess boron in citrus. In greenhouse experiments, Gupta et al. (1973) found that the application of nitrogen decreased the severity of boron toxicity symptoms in cereals, but this was not the case in the field experiments (Gupta et al., 1976), where the application of nitrogen was helpful in alleviating boron toxicity on soils low in available nitrogen content.

2.4 Boron uptake by plants

There are arguments about the mechanism of boron uptake by plants between two groups of researchers who support two different theories, namely passive and active mechanisms.

It was first suggested that boron moved to the root surface in the soil solution by mass flow (Oliver and Barber, 1966) and was absorbed as molecular boric acid in a physical, non-metabolic process in response to the boron concentration gradient (Bingham et al., 1970; Oertli and Grgurevic, 1975). Bingham et al. (1970) found that boron absorption by excised barley roots was not affected by the three factors of solution pH (range from 3 to 7), low temperatures and the addition of metabolic inhibitors (KCN, DNP). Hence, they concluded that boron absorption was a physical process, which resulted from the diffusion of undissociated boric acid across the lipid bilayer of the plasma

membrane of root cells. Oertli and Grgurevic (1975) reported that uptake of boron decreased with an increase in pH of the nutrient solution (pH 6 = 100% maximum uptake) and this was consistent with a decrease of undissociated boric acid at more alkaline pH values. Thus, they concluded that the equilibrium between boron in plant tissue and the external solution occurred through the diffusion process, and this equilibrium was controlled by the concentration of undissociated boric acid in the external solution. Tanaka (1967) proposed that in sunflower plants, boron was passively absorbed by excised roots into the free space. He indicated that polysaccharides in the free space compartment complexed with boron in this mode of absorption. However, the conclusion that boron absorption is a physical process may be not true because there is evidence of genetic variation in uptake of boron in many crops including wheat and barley (Nable, 1988).

The concept that boron is passively absorbed by plant roots has been strongly challenged by other investigators. Bowen (1968; 1969) reported that boron uptake by sugarcane leaf tissues, meristematic tissues and excised roots was metabolically regulated and had the characteristic of a carrier mediated reaction. The active uptake could be detected only after boron reversibly accumulated in the free space was washed out by rinsing with 0.5 mM $CaCl_2$ for about thirty minutes (Bowen, 1968; 1969). Bowen (1972) again reported that a component of boron uptake by roots of intact sugarcane plants was under metabolic control, although boron translocation from roots to shoots occurred passively in the transpiration stream. In excised barley roots, active uptake of boron did not occur at 2°C and accumulated boron remained in the free space. Three components of boron in the free space were identified as:

(1) a surface contaminant film of boron on blotted roots,

(2) water free space boron, and

(3) boron reversibly bound in the cell walls (Bowen and Nissen, 1976).

In the presence of boron, a stoichiometic release of H^+ from the roots indicated that boron was bonded by borate complexes with polysaccharides in the cell walls (Bowen and Nissen, 1976). Oliver and Barber (1966) suggested that not all of the boron uptake by plants can be accounted for by transpiratory water uptake at the B : H_20 ratio that occurrs

in soil. They also reported that boron diffusion to the root surface played a minor role in boron supply to plants. This hypothesis is supported by the evidence of differences in water use efficiency among barley varieties differing in tolerance to boron (Walker and Lance, 1991). A difference in stoichiometry of B : H_2O uptake between tolerant and sensitive genotypes was observed, suggesting that the difference in boron uptake between these two genotypes was not simply related to transpiration rate.

Kochian (1991) proposed that the binding of boric acid in intracellular compartments may affect the interpretation of boric acid transport. For example, the appearance of net boron influx into plant cells, where tissue boron concentration exceeds the external boric acid concentration, may not necessarily mean that active transport is occurring. An alternative explanation would be that boric acid complexes with cis-diol groups in the symplasm, which would then allow for more diffusion of free boric acid into the cells. Thellier et al. (1979) also suggested that the greater concentration of boric acid in the symplasm was due to ester formation with cis diols, and not to active transport. Brown and Hu (1993) studied boron uptake in sunflower, squash and cultured tobacco cells with the use of a stable boron isotope and inductively-coupled plasma mass spectrometry (ICP-MS). They found that boron uptake is a non-metabolic process and controlled by the formation of non-exchangeable boron complexes in the cytoplasm and cell wall. The formation of boron complexes varies dependent on temperature, tissue and organelle.

A number of investigators believe that boric acid is transported by a combination of active and passive transports systems. Wildes and Neales (1971) studied storage tissues of discs of carrot (*Daucus carota*). They provided evidence supporting both active transport, probably of $B(OH)_4^-$, and passive transport of $B(OH)_3$. Nissen (1974) also suggested that transport of boric acid could be the combination of both active and passive mechanisms. However, he indicated that active transport of boron predominated at low external boron concentrations, and probably involves $B(OH)_3$ rather than $B(OH)_4^-$, while passive transport of boron may predominate at higher external concentrations. Raven (1980)

proposed that the overall boron distribution between the growing or culture medium and the plant can be interpreted in terms of

(1) passive permeation of boric acid,

(2) cis-diol formation,

(3) active transport of boric acid (or a borate anion) and

(4) the use of total boron (rather than free boric acid) as a sensor for boron regulation.

2.5 Distribution of boron in plants

2.5.1 Boron transport in xylem

Studies of boron transport at the whole plant level reveal a direct, though not stoichiometric, relationship between transpiration rate and boron accumulation by plants. Plants grown in low or high relative humidities differ in their shoot boron concentration according to transpiration rates (Armstrong and Kirkby, 1979; Kohl and Oertli, 1961; Michale and Marschner, 1962; Michael et al., 1969). Boron uptake by sugarcane seedlings grown over a one week period in 30%, 58%, or 95% relative humidity was inversely related to the relative humidity and directly related to transpiration rate (Bowen, 1972). These results were consistent with the work of Nable et al. (1990b) who concluded that the increasing of water use resulted in increased boron accumulation by barley plants. It has generally been assumed that boron moves passively with the transpiration stream, an assumption primarily based on the observed pattern of distribution of boron in leaves (Kohl and Oertli, 1961; Oertli, 1960). Evidence of translocation of boron occurred in the experiment of Oertli (1960) where boron concentrations were highest in the marginal areas of lemon leaves and lowest at the base of the midrib. Kohl and Oertli (1961) reported that boron concentration of Easter lily leaves increased hyperbolically from leaf base to near the tip. They also indicated that boron accumulated in those areas of the leaf where the transpiration stream ends.

Bowen (1972) studied the translocation of boron from root to shoot and concluded that the root-boron content and water flux in the xylem could be used for the prediction of shoot-boron content; after absorption into the root, boron appeared to be passively transported into and through the xylem. Raven (1980) has pointed out that the work of Bowen may be correct at the very low transpiration rates with the relatively high boron concentration in the external solution used during the experiment. However, there was some evidence to support the hypothesis of regulation of boron transport into the xylem by the root. The experiments conducted for barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*) (Nable, 1988) and annual medics (*Medicago spp.*) and peas (*Pisum sativum*) (Paull et al., 1992b) identified an apparently similar mechanism controlling tolerance to boron for these species. The low concentrations of boron in roots indicated that the low concentrations measured in shoots of tolerant lines result from the lower uptake of boron by roots of tolerant lines. The differences between the calculated boron concentrations of the transpiration stream of boron-sufficient C_3 plants (1-65 mmol m^{-3}) (Gauch, 1972) and the normal root boron concentrations on a fresh weight basis (100-1000 mmol m^{-3}) suggested that the shoot transpiration stream does not directly reflect root boron concentrations (Brown and Jones, 1971; Mengel and Kirkby, 1982).

Brown and Jones (1971) investigated boron transport in boron-efficient and boron-inefficient varieties of tomato. When the tomato varieties were grown in boron levels which induced boron deficiency, the root boron concentrations were similar in the efficient and inefficient varieties but the boron concentrations in the xylem sap and shoot were much lower in the inefficient varieties. These results indicate that boron transport in xylem is not merely passive diffusion related to mass flow of water. Halbrook et al. (1986) reported that in table beet (*Beta valgaris* L. cv. Red Ace), for plants studied under a controlled environment, boron translocation to shoots was controlled by dry matter accumulation during early stages of plant development. They also concluded that boron movement in the xylem to shoots was not affected by transpiration rates.

2.5.2 Boron transport in phloem

Boron is generally considered to be an immobile element and that after it is deposited in a leaf it is not removed and retranslocated to other organs such as new leaves or developing fruits. This immobility was explained by some form of fixation (Eaton, 1944). Epstein (1973) attempted to explained this immobility by an inability of boron to enter the phloem. On the other hand, Oertli and Richardson (1970) postulated that boron is able to penetrate and be translocated in the phloem, but then re-entered the xylem of the leaf or petiole and moved back into the leaf via the transpiration stream.

This area is marked by confusion and controversy. Tammes and Van Die (1966) compared the boron content of phloem exudate of Yacca, either obtained from the severed inflorescence stalk (peduncle) (1 mol m^{-3}) or with that in the inflorescence supplied by the phloem (2 mol m^{-3}) and that in the leaves which are the source for the phloem fluid (34 mol m^{-3}). From this study, it appears that boron does enter the phloem.

In several studies, the stable boron isotope (^{10}B) was used as a stable tracer for the ^{11}B isotope. In order to monitor the translocation of boron out of the leaves, the ^{10}B isotope was applied to leaves in white clover (*Trifolium repens* L.) (Martini and Thellier, 1980) and radish (*Raphanus sativa*s L.) (Chamel el al., 1981). Chamel et al. (1981) concluded that boron applied to leaves of radish penetrated the epidermis and was translocated to other parts of the plants. However, the largest fraction was retained in the treated leaf. Boron distribution within the treated leaf was homogeneous, leading the authors to suggest that the low rate of boron translocation from the treated leaf was a result of it being partly bound as a borax polysaccharide complex (Mengel and Kirkby, 1978; Raven, 1980). Martini and Thellier (1980) reported similar results with white clover. They used the ^{10}B (n, α) ^{7}Li nuclear reaction to study boron transport in the plant after foliar application and concluded that more than 98% of the applied boron remained at the treated area of the leaves, presumably due to boron-ester bond formation between boric acid and the alcoholic groups of cell wall. Less than 2% of the applied boron was distributed to the other parts of the plant which was transferred from the oldest parts to

the newly formed leaves. Raven (1980) suggested that redistribution of boron in the phloem is very limited because

(1) boron concentration in phloem sap was limited by toxicity of the boron in the cytoplasmic transport channel, or

(2) the inability to maintain high boron concentrations in a transport channel which is surrounded by a boron-permeable membrane and is adjacent to xylem sap with low boron concentrations.

2.6 Soil factors affecting boron requirement and uptake by plants

2.6.1 Soil pH

Soil pH is one of the most important factors affecting the availability of boron to plants. Studies by Peterson and Newman (1976) and Gupta and MacLeod (1977) have shown that a negative relationship between soil pH and uptake of boron by plants occurs when soil pH levels are higher than 6.5. Since only boron that is in the soil solution is available to plants (Hatcher et al., 1959), these results indicate that boron distribution between the liquid and solid phase is strongly dependent on soil pH. The soil pH may also reflect the balance between $B(OH)_3$ and $B(OH)_4^-$, with the proportion of the latter increasing at high pH. If only $B(OH)_3$ was taken up by plants, the effect of pH on uptake of boron by plants does not necessarily require change in distribution between liquid and solid phase. However, this relationship is not consistent, and deviations from this effect occur, owing to factors such as crop species (Gupta, 1972, 1977).

Liming soils to pH more than 6.5 induced boron deficiency in susceptible crops (Batey, 1971). The severity of lime-induced boron deficiency, however, depends on a number of variables, including the moisture status of the soil (Berger, 1949), the nature of the crop (Bradford, 1966), and the period of time from lime application (Dermol and Trinder, 1947). Peterson and Newman (1976) studied the effect of pH on the availability of added boron at pH levels of 4.7, 5.3, 5.8, 6.3 and 7.4. Boron uptake by tall fescue

(*Festuca arundenacea* Schreb.) was relatively uniform for the first four pH levels but a drastic drop in uptake occurred at pH 7.4 indicating that all the effect is due to fixation rather than uptake of B(OH)3 and $B(OH)4^-$. Plant uptake of boron at the five pH levels showed no relationship with the amount of water soluble boron but the data were in agreement with those of Wear and Patterson (1962) as the plant boron concentration was higher at a lower pH level than at a higher level. Gupta (1968) also suggested that there was no relationship between hot water soluble boron and pH on 108 soil samples from eastern Canada (pH 4.5-6.8). The decreasing of availability of boron at pH levels in excess of approximately 6.5 is probably related to the decreasing of boron concentration in soil solution as a consequence of adsorption onto clay and hydroxy-aluminum surfaces (Keren and Bingham, 1985). At pH < 7 boron is present in the soil as $B(OH)_3$, which is not adsorbed very extensively by the colloidal fraction. As the soil pH rises, the concentration of $B(OH)_4^-$, and hence adsorption, increases.

2.6.2 Parent material

The bulk of boron in soil comes originally from soil minerals, thus the boron content of soil is primarily related to the boron content of the parent material from which the soil was derived. Soils from marine shales (Norrish, 1975) and sedimentary rocks contain much higher concentrations of boron than igneous rocks and granitic material (Bingham et al., 1970; Whitestone et al., 1942; Liu et al., 1983). Gupta (1979) suggested that tourmaline is a boron containing mineral that is present in soils formed from acidic rocks and metamorphic sediments. However, boron from this source is not readily available for plant growth. Soil derived from materials of volcanic origin also have a high level of boron (Morgan, 1980). Hence, high boron soil is common in areas along the major world fault lines. Plant availability of boron is also reduced in soils derived from volcanic ash (Sillanpaa and Vlek, 1985) and in soil rich in aluminum oxides (Bingham et al., 1970; see section 2.3.1).

2.6.3 Organic matter

Although most cultivated soils contain a small amount of organic matter (1-5%), it can significantly modify their chemical properties (Keren and Bingham, 1985). Okazaki and Chao (1968) reported that in acid soils, organic matter is one of the main sources of boron since relatively little boron adsorption in the soil occurs at low pH levels. Although boron in soil organic matter is not immediately available to plants, it is considered to be the main source of available boron when released through mineralization (Gupta et al., 1985). The influence of organic matter on the availability of boron in soil is amplified by decreases in the pH and the clay content of the soil. The strongest evidence that organic matter affects the availability of soil boron is derived from studies that demonstrate a positive correlation between organic matter and hot water soluble boron (Gupta, 1968). Addition of material such as compost rich in organic matter resulted in a large increase in concentration of boron in plant tissues and in phytotoxicity (Purves and Mackenzie, 1973). This is probably because of the high concentration of boron in the compost used in this experiment. However, in soil with low organic matter content (1.2%), the effect of the organic matter on availability of boron in the soil is negligible (Mezuman and Keren, 1981).

There are indications that boron is strongly adsorbed in limed peat as leaching of boron from peats has been found to be low (Prasad and Woods, 1971). The necessity to add boron to peat soils has been recognized (Prasad and Byrne, 1975). Results indicate that the reduced boron uptake at high pH is partly due to a chemical reaction between limed peat and added boron. Gupta (1979) reported that boron toxicity symptoms could not be observed from crops grown on peats at boron fertilizer rates that usually produce toxicity on mineral soils. Prasad and Byrne (1975) also found that there was no boron toxicity symptom in sweetcorn grown on a peat soil even when the hot water soluble boron concentration was as high as 10 mg kg^{-1}.

2.6.4 Soil Texture

Soil texture is an important factor affecting the availability of boron in certain soils (Wear and Patterson, 1962). There is evidence that the movement of boron in sandy soils is greater than that in soil of heavier texture (Kubota et al., 1949). Page and Cooper (1955) also reported that after the addition of 12.5 cm of water to acid sandy soil, about 85% of applied boron was leached from the soil. Movement of boron is less rapid in heavy-textured soils because of increased adsorption by the clay particles (Reisenauer and Hoeft, 1973). If other things are equal, light-textured soils contain less available boron than heavy-textured soils and boron deficiency is more common in them (Davies, 1980). Gupta (1968) reported on his studies of soil from eastern Canada that a greater quantity of hot water soluble boron was found in the fine-textured soils than in the coarse-textured soils while the highest percentage of total boron in the hot water soluble form occurred in the fine-textured soils. The observed relationship between boron and soil texture could be attributed to the fact that much of the boron present in the soil occurs as an anion, particularly in the alkaline range, and is adsorbed to clay particles. The lower amounts of boron in sandy soils are likely to be related to higher leaching of boron, which would also explain the lower percentage of the total boron that occurred in the hot water soluble form (Gupta, 1968).

In general, more applied boron is required in fine-textured soil than that in coarse-textured soil to produce similar boron concentrations in plants. Singh et al. (1976) reported that in gram (*Cicer arietinum*), boron concentrations of 3.5 ppm in solution in sandy loam and 4.5 ppm in clay loam resulted in tissue boron concentrations of 232 ppm and 221 ppm, respectively. Similarly, Eaton (1935) reported boron injury to be comparatively greater at lower applied boron concentrations in coarse textured than fine textured soil.

2.6.5 Soil moisture

Moisture appears to affect the availability of boron more than that of other elements. Boron deficiency is observed in dry seasons or in late summer when moisture is

low (Hobbs and Bertramson, 1949; Baker and Mortensen, 1966). Drought stress or moisture stress in the surface soil induces boron deficiency in many crops including alfalfa (*Medicago sativa* L.) (Barber, 1957), apple (*Malus domestica* Borks.) (Faust and Shear, 1968) and cotton (*Gossypium hirsutum* L.) (Miley and Woodall, 1967). Bouma (1967) reported that root growth is limited by boron deficiency which would intensify drought stress. According to Batey (1971), turnip (*Brassica rapa*) in Wales normally became boron deficient in soil with < 0.3 mg kg^{-1} of extractable boron but deficiency was observed in a dry summer in fields with extractable boron levels of 0.5-0.6 mg kg^{-1}. Gupta et al. (1976) also found that moisture had a significant effect on plant boron uptake when boron was applied to the soil. The boron concentration of barley, with added boron, ranged from 162 to 312 mg kg^{-1} under normal conditions, but only from 87 to 135 mg kg^{-1} when the area near the boron fertilizer band was kept dry.

The analysis of soils for predicting boron deficiency (Berger and Truog, 1945) indicates that available soil boron is often concentrated in the surface zone although Cartwright et al. (1986) observed that the concentration of boron in the low rainfall regions of southern Australia reached a maximum in the subsoil. Thus, for the former situation, drying of surface layers should restrict water and boron uptake from this zone, and consequently, restrict the boron supply to plant meristems (Moraghan and Mascagni, 1991). The explanation of how moisture stress induces boron deficiency in plants would appear to lie not in fixation processes, but in the inability of the plant to extract boron from soil due to the lack of moisture in the root zone (Davies, 1980). Some investigators suggested that the cause of drought-induced boron deficiency was that lack of moisture restricted mineralization and availability to plants of organically bound boron in soil (Berger, 1962; Evans and Sparks, 1983; Flannery, 1985). Studies by Kluge (1971) indicated that boron deficiency in plants during drought may be only partially associated with the level of hot water soluble boron in soil. The reduction in volume of soil solution, of mass flow and diffusion rate and the limited transpiration flow in the plants during drought periods may be causative factors of boron deficiency in spite of an adequate supply of available boron in the soil.

Boron toxicity in plants is chiefly affected by the concentration of boron in soil water (Keren and Bingham, 1985). Tips and margins of older leaves are first affected because boron distribution in plants is related to transpiration patterns (Marschner, 1986). Thus, environmental factors that influence the transpiration rate will influence the boron toxicity in plants (Lovatt, 1985; Nable et al., 1990b).

2.7 Functions of boron in plants

2.7.1 Cell division and enlargement

The effects of a lack of boron on root growth have been reported by many investigators. Neales (1959, 1960) reported that root elongation continued for a period of 48 to 80 hours when corn, bean and pea were grown in the absence of boron while elongation of flax roots was sustained for only 48 hours in a boron-deficient medium.

The earliest symptom of boron deficiency that can be observed in squash plants grown in boron-deficient solution culture is the cessation of root elongation (Cohen, 1972). Many investigators have questioned which of the two processes boron is necessary for, cell elongation or cell division. Kouchi and Kumazawa (1975) found that the primary effect of boron deficiency on tomato root tips was the inhibition of cell division and cell enlargement in the root apices. Several investigators have suggested that boron is required for cell division (Hass and Klotz, 1931; Whittington, 1957). Cohen and Lepper (1977) concluded that the cessation of root elongation brought about by boron deficiency was caused by a failure of cell division in meristematic cells, and not by cellular elongation. This suggests that boron acts as a regulator of cell division. The growth response of diatom cells to boron indicated that cell division stopped early after removing boron from the culture, and the cells increased in size due to swelling rather than to a blockage in the process of cell separation (Smyth and Dugger, 1981).

Alexander (1941), Neales (1960) and Sommer and Sorokin (1928) suggested that the primary influence of boron is not on cell division since abortive lateral primordia are

formed in boron-deficient roots. Normal mitosis occurred as lateral meristems began development but cell division ceased soon thereafter. Skok (1958) also reported that boron-deficient roots showed an increase in lateral root initiation with a decrease in root elongation. Boron deficiency and phytohormone interactions have been studied by Birnbaum et al. (1974) in unfertilized cotton (*Gossypium hirsutum* L.) ovules grown *in vitro*. They found that the *in vitro* cultures of cotton ovules in the absence of boron showed reduced fiber cell growth (i.e. elongation). However, cell division was rapid and resulted in a mass of undifferentiated callus tissue. Therefore, they concluded that boron is not required for cell division but, in contrast, its absence promotes the division-inducing (callus forming) capacity of gibberellic acid (GA_3). They further suggested that boron is required for fiber elongation in response to IAA. Skok (1957) observed that cell maturation rather than division appeared to be more affected by boron deficiency in sunflower seedlings. Results of Robertson and Loughman (1974a) indicated that it is unlikely that responses associated with boron deficiency are caused by interference with cell division, but they may be related to the role of boron in the metabolism, transport, or action of auxin-type hormones in broad beans (*Vicia faba* L.).

2.7.2 Cell differentiation and maturation

Skok (1958) proposed that boron functions primarily in differentiation and maturation of plant cells rather than in cell division. Dugger (1983) suggested that in bean and Clematis, root growth of cuttings showed a response to boron, but the effect was on root initiation rather than on growth and differentiation of the initiated roots. Sommer and Sorokin (1928) studied roots of pea and found that in the absence of boron, differentiation of lateral root primordia and of isolated xylem elements occurred prematurely. Similar results were found in excised tomato roots by Albert and Wilson (1961). Neales (1960) and Albert and Wilson (1961) also observed premature differentiation on lignified tissues in root of *Vicia faba* and excised tomato roots, respectively. In the studies of seedlings of *Vicia faba*, Robertson and Longhman (1974b) reported that boron deficiency did not reduce

the ability of cells to divide, nor did it affect cell differentiation capacity. They further observed that deficiency caused a change in the normal polarity of elongation and division resulting in apparent hyperplasia of the stele.

2.7.3 Phenolic compounds and lignin biosynthesis

Boron deficiency was reported to lead to an accumulation of phenolic compounds (Reed, 1947). This resulted in a decreased level of lignin which is a product of phenolic polymerization in boron-sufficient tissue. The excessive amounts of phenolic compounds are the cause of necrosis and ultimate death from this deficiency (Watanabe et al., 1964). Spurr (1952) found that fluoresence in boron-deficient tissue of celery occurred because of the localised accumulation of caffeic and chlorogenic acids. It was later suggested that the necrosis caused by boron deficiency arises from an increase in caffeic acid (Dugger, 1983). However, in boron-deficient oil palm, there were no leucoanthocyanins, which were normally present where other phenolic compounds accumulated (Rajaratnam and Lowry, 1974). Boron deficiency also caused an increase in flavonol, flavonones, and flavonol-3-glucosides in tomato leaves (Shkol'nik and Abysheva, 1975). Flavonol and flavonones are in the flavonoids group which is the most important single group of phenolics (Harborne, 1989).

Sunflower (*Helianthus annuus*) grown under conditions of boron deficiency accumulated phenolic compounds which resulted in a reduction of IAA oxidase activity (Shkol'nik et al., 1964). However, it was reported recently that early effects of boron deficiency are not attributed to changes in endogenous IAA levels (Hirsch et al., 1982; Hirsch and Torrey, 1980).

In general, with the exception of some marine diatoms, lignified plants require boron (Pilbeam and Kirkby, 1983). Boron may be essential for the biosynthesis of lignin from coumaryl, coniferyl and sinapyl alcohols (McClure, 1979). Lewis (1980a) proposed that the primary role of boron was in the biosynthesis of lignin and differentiation of xylem. In boron-deficient sunflower, the ability of leaves to synthesise lignin apparently

decreased with the increasing severity of symptoms (Perkins, 1957) and there was less lignification in boron-deficient root tissue than in normal tissue (Dutta and McIlrath, 1964). Boron regulated the hydroxylase and oxidase activities of phenolases which are involved in the biosynthesis of caffeic and hydroxyfurulic acids.

The variation in boron requirement among plant species probably occurs because of interspecific variation in lignin composition. The lignin of monocots and bryophytes, two groups of plants with a low requirement of boron, consist mainly of coumaryl alcohol. On the other hand, the higher boron requirement of dicots may be related to the additional conversion of *p*-coumaric acid, the immediate precursor of coumaryl alcohol, to coniferyl and sinapyl alcohols (Pilbeam and Kirkby, 1983).

2.7.4 Cell wall biosynthesis

Several investigators have tried to define the role of boron in plant cell wall biosynthesis. Boron deficiency in celery plants altered cell walls, and apparently affected the rate and process of carbohydrate condensation into wall materials (Spurr, 1957). Whittington (1959) suggested that the abnormality of cell wall formation in boron-deficient field bean radicles prevented the cell wall from becoming organized for mitosis. In boron deficient field bean radicles, ^{14}C-glucose was incorporated into pectic substances at a higher level than that in boron-sufficient radicles. This role of boron in plant growth is described as a bonding agent between cell wall and polysaccharides (Slack and Whittington, 1964). Wilson (1961) also observed the effects of boron deficiency on cell walls of tobacco. For parenchyma grown in tissue culture there was a doubling of the amount of cell wall fraction with no change of the cellulose : pectic substance ratio compared to tissue grown under control conditions. There was an increased level of hemicellulose and pectic substance in the root tissue of boron-deficient oil palm seedlings as compared to boron-sufficient seedling roots (Rajaratnam and Lowry, 1974).

The effect of boron deficiency at the ultrastructural level appeared as morphological changes in the cell wall and the cellular Golgi apparatus (Kouchi and Kumazawa, 1976). They suggested that the abnormalities observed were caused by

(1) alterations to the mechanisms of cell wall synthesis or breakdown,

(2) the abnormality of the Golgi apparatus and

(3) the secretion of cell wall components by Golgi vesicles.

Cell wall thickening in root apical meristems, which occurred less than 3 to 6 hours after interruption of boron supply, was the result of an increase in hemicellulose and pectin, and an irregular deposition of vasicular aggregations of new cell wall material intermixed with membrane material (Hirsch and Torrey, 1980).

2.8 Level of boron in plants

The boron concentration of plants grown under natural conditions varies widely for plant species and kinds of soil. Shacklette et al. (1978) reported that trees and shrubs, which had a boronconcentration of 50-500 mg kg^{-1}, generally contain two to ten times as much boron as do vegetables. The lowest boron amounts, however, have always been found in seeds and grains, cereal grains in particular (Kabata-Pendias and Pendias, 1984).

In general, boron concentration in the plant reflects, to a considerable degree, boron requirement of the plant (Jones, 1991). For example, monocotyledons contain less boron than do dicotyledons, which agrees with their requirement for this element (Berger, 1949). Members of the Papilionaceae and Cruciferae families have relatively high boron requirements and, therefore, generally contain fairly high (> 25 mg kg^{-1}) boron concentrations in their leaves (Jones, 1991). A distinction must be made between the requirement for and the tolerance to boron. Plants with a high requirement do not necessarily have a high tolerance. Lucerne and cabbage have a high requirement, but they are classified only as semi-tolerant plants (Bradford, 1966). Grasses have low requirement but some species, e.g., cocksfoot, can withstand relatively massive amounts of boron-containing herbicides without being killed (Oram, 1961). Pea and barley have a low

requirement, but are considered as semi-tolerant (Davies, 1980). Most classifications of species reported above are based on a single genotype. However, in view of the large degree of intraspecific variation (see Section 2.11.2), this is not valid. It is not acceptable to generalise to the whole species on the basis of limited genetic material.

The critical levels of boron toxicity and deficiency in crops vary considerably according to species, stage of development at sampling, plant part sampled and the method used for extracting boron from plants. However, in many plants, boron deficiency in the field occurs when the concentration in fully mature leaves is < 15 mg kg^{-1} and the boron sufficiency range is between 20 and 100 mg kg^{-1} (Gupta, 1979; Adriano, 1986), whereas boron toxicity occurs when plant tissue concentration exceeds 200 mg kg^{-1} (Gupta, 1979). However, Gupta (1971) reported the critical value for toxicity as 16 and 20 mg kg^{-1} in boot stage tissue for wheat and barley, respectively. However, there are considerable problems with the establishment of critical values and use of leaf or shoot analysis to diagnose boron toxicity in barley and wheat. These problems are a consequence of the pattern of distribution of boron in leaves, the effects of environmental conditions on boron accumulation by plants, and the leaching of boron from leaves (Nable et al., 1990b; Nable and Moody, 1992).

The sufficiency range varies from one part of the plant to another. Lockman (1972) reported that the sufficient range for boron in sorghum [*Sorghum bicolor* (L.) Moench] was 1-6 mg kg^{-1} at dough stage in the third leaf below the head of the 82 to 97-days-old plants, whereas it was 1-13 mg kg^{-1} in the whole plant of 23 to 39-days-old plants. Robertson et al. (1976) and Gupta (1979) reported the boron sufficiency range and sampling criteria for a number of crops.

2.9 Distribution of boron toxicity and deficiency

Boron toxicity in plants may be common in semiarid regions with alkaline soils (Cartwright et al., 1984). Toxicity can occur under three main conditions:

(1) in soils developed from parent materials that contained high levels of boron (Eaton, 1944),

(2) in soils irrigated with high boron water leading to boron accumulation and concentration in the soils (Wilcox, 1960),

(3) in soils using overfertilization with minerals high in boron (Mackay et al., 1962).

Boron toxicity has been reported in a number of countries. In India, well water containing a high concentration of boron has been reported to be used for irrigation in arid and semi-arid regions of Uttar Pradesh, Haryana, Rajasthan, Punjab, Agra (Chauhan and Powar, 1978; Chauhan and Asthana, 1981) and Patti (Amritsar) (Singh and Kanwar, 1963). Symptoms of boron toxicity were observed in a wetland rice field at an IRRI farm in The Philippines and which was irrigated by high-boron deep well water (Ponnamperuma et al., 1979; Cayton, 1985). The yield reduction in rice was estimated at 10-20% for tolerant varieties in blocks irrigated by high-boron wells during dry seasons when rainfall was nil (Cayton, 1985). Boron toxicity has also been observed in crops grown on soils with high boron availability in newly developed fields of the San Joaquin Valley in California (Kubota, 1980). Plants with high concentrations of boron have been observed in other regions of the Western USA (Welch et al., 1991). Boron toxicity in barley and wheat has been identified in Turkey, Syria, Tunisia and the dryland areas of Egypt and suspected in Libya and Algeria (ICARDA Annual Report, 1993). There was approximately a 26-45% yield difference in a comparison between boron tolerant (5044-5800 kg ha^{-1}) and sensitive (3348 kg ha^{-1}) lines of barley in a high boron field at Kazan Research Farm in Turkey (ICARDA Annual Report, 1993).

High concentrations of boron have been recorded in many soil and plant samples obtained from widespread regions of the cereal growing districts of southern Australia (Cartwright et al., 1984; 1986). The areas with the potentially toxic levels of boron include upper Eyre Pennisula, upper Yorke Pennisula, parts of Murray Mallee in South Australia (Cartwright, 1986), parts of the cereal belt of Western Australia (Khan et al., 1985) and also western Victoria (Ralph, 1992). Soils of South Australia in which boron toxicity has been found are almost invariably sodic and rich in calcium carbonate (Cartwright et al.,

1986). This calcium tends to adsorb the boron at the top of the B horizon preventing its leaching out of the soil and thus presenting a layer of high boron soil to advancing plant roots (Rathjen et al., 1987). Identifying the distribution of high levels of soil boron in southern Australia was demonstrated by soil surveys (Cartwright et al., 1986) together with chemical analysis of barley grain harvested from South Australia and western Victoria. Maps depicting regions of low and high concentrations of boron in barley grain were constructed by CSIRO Division of Soil and published by Ralph (1992).

Generally, leaching is the major problem which causes boron deficiency in the soil of humid regions (Gupta et al., 1985). Miljkovic et al. (1966) reported that plant-available boron in the humid soil is located in the top 15 cm in the organic matter fraction. Thus, boron deficiency is frequently observed in plants grown on regosols, sandy podzols, alluvial soils and low humic gleys (Gupta et al., 1985). Boron deficiency has been reported in many countries of the world. In Australia reports of boron deficiency are confined to the high rainfall, acid soil regions of the eastern States, for example in clover in some parts of New South Wales (CSIRO Research Report, 1985-86), *Pinus radiata* in Victoria (Hopmans and Flinn, 1984) and Brassicas in Tasmania (Lamp, 1964). Boron deficiency in peanut has been reported to be common in northern and north-eastern Thailand (Bell et al., 1990). In China, the geographical distribution of boron deficiency in crops coincided with the distribution of boron deficient soil (Zheng et al., 1982). Deficiency of boron has been reported in many areas of Canada (Mackay et al., 1962), and in the United States boron deficiency was reported in 43 states (Sparr, 1970). Other countries in which boron deficiency has been reported include New Zealand (Sherrell, 1983), Sweden (Erikson 1979), Nigeria (Singh and Balasubramanian, 1983) and England (Wallace, 1951). Boron deficiency has also been found in some arid regions of India (Garg et al., 1979) and Pakistan (Khan et al., 1979).

2.10 Response of plants to boron

2.10.1 Deficiency symptoms

Since boron is relatively immobile following translocation in the transpiration stream and a concentrated distribution in the distal portion of older leaves (Oertli, 1960), boron deficiency can always be detected first in the youngest leaves and the growing points of shoots and roots (Bergman, 1984). In most plants, boron deficiency shows up as shortened internodes and arrested top growth. The terminal bud dies and lateral buds produce side shoots which result in a bushy or rosette appearance of plants (Gupta, 1979). Under severe stress, boron-deficient plants may develop chlorosis, drop their flower buds and fail to develop seeds (Keren and Bingham, 1985). In vegetables such as rutabaga and cauliflower, boron deficiency is indicated by dark-brown spots on areas in the storage tissue (Gupta, 1979). Boron deficiency causes sterility and consequently grain set failure in wheat without visual symptoms of deficiency on foliage (Rerkasem et al., 1991). Results of a study of the effect of boron on pollen germination when it was supplied in an agar medium for *in vitro* germination indicated that the percentage of germinated pollen and length of the pollen tube increased with the increasing medium boron (Cheng and Rerkasem, 1993). However, Bussler (1964) suggested that boron deficiency is manifested in individual species of plants by various visually perceptible characteristic micro- and macromorphological changes. Details of symptom expression for a large number of crop species are presented in a monograph on boron deficiency and toxicity by Eaton (1944) and in reviews by Berger (1949), Bradford (1966), and Gupta (1979).

2.10.2 Toxicity symptoms

Since boron distribution in plants is related to the transpiration pattern, the toxicity effects occur preferentially in the tips and edges of leaves, particularly in older leaves (Marschner, 1986), and spread from the lower to the top leaves (Bergman, 1984). The

pattern of chlorosis and necrosis follows the leaf venation (Oertli and Kohl, 1961; Gupta, 1979). Although different plant species vary greatly in their tolerance to an excess of boron, most plants are similar in their boron-toxicity symptoms. The toxicity symptoms consist of marginal and tip chlorosis, which is quickly followed by necrosis (Shorrocks, 1974). Acute boron toxicity results in premature leaf drop and eventual death of the plant (Keren and Bingham, 1985). There is evidence that the gradients of boron concentrations in leaves and severity of symptoms of boron toxicity coincide, thus indicating a relatively direct effect of boron upon symptom developments (Oertli and Kohl, 1961).

Foliar symptoms of boron toxicity in barley have been described by Christensen (1934), Gupta (1971) and Cartwright et al. (1984). The symptom is characterized by chlorosis and necrosis extending from the tips of the oldest leaf, with brown spots forming initially at the margins and later over the distal half or more of the leaf. In wheat, the symptoms are similar to barley, but brown spots do not develop within the affected region (Paull et al., 1990). The symptoms of boron toxicity were similar for both peas (*Pisum sativum*) and medics (*Medicago spp.*) and consisted of chlorosis and necrosis initially developing along the margins of the leaves and progressing to the leaf centre (Bagheri et al., 1992; Paull et al., 1992b). Symptoms developed first and were most severe on the older leaves. Excellent descriptions of boron toxicity symptoms are given by Eaton (1944), Bradford (1966), and Gupta (1979).

In field conditions where plants are under water stress because of drought, the symptoms of boron toxicity are very similar to those of drought stress in wheat (Paull et al., 1990) and most other crops. However, the symptoms of toxicity and drought can be differentiated in field grown barley because the occurrence of brown spots will indicate boron toxicity. Therefore, barley can be used as an indicator of boron toxicity in the field.

2.10.3 Yield

A reduction in yield due to the uptake of either extremely low or high concentrations of boron has been reported in many crop species.

Under boron deficient conditions, the number of grains/pod and seeds/pod increases with the application of boron for field peas and lucerne, respectively (Salinas et al., 1981; Misra and Patil, 1987). Rerkasem (1991) reported yield reductions of 21% and 70% in green gram (*Vigna radiata*) and black gram (*Vigna mungo*), respectively, in boron deficient conditions in Thailand. Bell et al. (1990) reported that seed quality of peanut, black gram, green gram and soybean grown in Thailand was more sensitive to low boron in soils than was seed dry matter. Seeds with a symptom of boron deficiency known as "hollow heart" (Harris and Brolman, 1966) were classified as low quality seeds. Application of boron was reported to increase the number of grains per ear (Ganguly, 1979) and 1000 grain weight (Iqtidar et al., 1979). Rerkasem et al. (1993) also reported depression of grain set under boron deficiency conditions in warm wheat-growing areas of Thailand.

In high boron conditions, there were reductions in the size of heads and number of heads per plant of wheat, barley and oats (Gupta, 1971). In South Australia a yield reduction of approximately 17% in a barley crop was attributed to boron toxicity in a red-brown earth. Boron concentrations in saturation extracts of the subsoil under plants that were severely affected ranged up to 17.9 g boron/cm^3 (Cartwright et al., 1984). In wheat, high concentrations of boron reduced tillering and delayed maturity (Paull et al., 1990). The yield effect of a wheat gene, *Bo1*, that confers tolerance to boron, was evaluated over a range of soil types in southern Australia by comparing boron tolerant and sensitive derivatives from a backcrossing program (Moody et al., 1993). The advantage of the tolerant lines ranged up to 11% with an average yield advantage of 3.3% in all trials conducted over a range of soil types. In contrast to boron deficiency of cereals, where the principal yield effect is upon fertility, boron toxicity affects both straw and grain yield (Gupta, 1971; Khandelwal and Lal, 1991). Mehrotra et al. (1980) reported that grain number was reduced by the reduction of spikelets of some wheat genotypes under boron

toxic conditions. Grain production appears to be more sensitive than straw growth to boron toxicity and reductions in grain yield, without reductions of straw yield, occurred in response to increasing levels of applied boron for wheat (Chauhan and Powar, 1978), lentil (Chauhan and Asthana, 1981) and peas (Chauhan and Powar, 1978; Salinas et al., 1981).

2.11 Genetic control and mechanism of response to boron

2.11.1 Interspecific genetic variation in response to boron

For many years it has been recognized that plant species and cultivars within a species may differ in response to nutrient levels in the soil. The magnitude of the differences between varieties in response to soil nutrient levels has prompted research into breeding varieties specially adapted to soils low in available nutrients (Brown and Jones, 1977).

Interspecific genetic variation was recognized in early investigations. Differences in response to high boron concentrations were reported for fruit trees (Haas, 1929), commercially grown plants (Eaton, 1935; Eaton, 1944), vegetables and cereals (Purvis and Hanna, 1938), and ornamentals (Francois and Clark, 1979). In these experiments, each crop species was represented by a single variety. The species were then classified as sensitive, semi-tolerant and tolerant to a high concentration of boron. There was conflict between researchers about the tolerance of some species and this probably occurred because these classifications were based on only a single variety. In general, the range of variation within a species would be expected to be the same as that across species.

The data of Oertli and Kohl (1961) indicated that the differences in boron toxicity symptoms observed between species were attributable to differences in the rate of uptake and local accumulation of boron, rather than the tolerance of tissues to boron. It has been claimed that, in general, the species which are more tolerant to high concentrations of boron also require more boron for normal growth. For example, tobacco which was the most tolerant of five species in an experiment also had the highest boron requirement (Gandhi

and Mehta, 1959). However, Davies (1980) demonstrated that plants with a high boron requirement do not necessarily have high tolerance. This is supported by Bradford (1966) who classified lucerne and cabbage, which have a high boron requirement, as only semi-tolerant. Since the classification of these species was based on a single variety, all of these experiments should be reassessed.

In studies of the response to high boron concentration in a diverse group of species, Eaton (1944) and Francois and Clark (1979) suggested that there was almost no association between the boron concentration in leaves and the boron tolerance rating. This, however, is not true in the case of comparisons among related species. For instance, Jerusalem artichoke (*Helianthus tuberosus*) is considerably more sensitive to boron, and has higher concentrations of boron in leaves, than sunflower (*Helianthus annuus*.) (Eaton and Blair, 1935).

Wheat and barley were classified as the same level of tolerance (semi-tolerant) to high concentrations of boron (Eaton, 1935). However, Bingham et al. (1985) observed that the tolerance of wheat to high boron concentrations is less than that of barley. This conflict of classification was attributed to either different varieties chosen to represent the two species, or the large variation of wheat yield at the low boron treatments (Bingham et al., 1985). However, the large degree of genetic variation in response to boron of wheat and barley (Nable, 1988; Paull et al., 1988b) indicates that the classification of species for response to boron on the basis of a single genotype is not valid.

2.11.2 Intraspecific genetic variation in response to boron

Knowledge of within species differences in response to mineral stress conditions is implicit in the selection of tolerant varieties by plant breeders. Considerable research has been undertaken in many crop species to evaluate intraspecific variation in response to boron stress, both toxicity and deficiency, and a number of tolerant germplasms have been used as sources of tolerance in breeding programs.

Field trials of wheat in a boron deficient soil were conducted by Ganguly (1979). Although no symptoms of boron deficiency were observed on foliage, there was genetic variation with respect to yield. Grain yield and number of grains per ear of the variety Janak increased with the application of boron but these effects were not found in the variety Sonalika. Sonalika outyielded Janak, both with and without boron. The reduction in grain yield under conditions of boron deficiency was probably the result of seedlessness (Ganguly, 1979). The varieties Janak, VP262 and Sonalika were classified as susceptible, moderately susceptible and less susceptible to boron deficiency, respectively, on the basis of their response to boron application (Chatterjee et al., 1980). Rerkasem et al. (1993) reported a wide genotypic variation in reproductive responses to boron among eight wheat genotypes. In sand culture grain set index ranged from 9.5% in SW41 to 94.5% in Fang 60 in low boron (0.2 μM) and ≥ 90% in all genotypes for high boron treatments (10 μM). In this experiment, the variety Sonalika with grain set index about 70% could be classified as less susceptible compared with the sensitive variety SW41 and this is consistent with the work of Ganguly (1979).

Genetic variation in response to high levels of boron has been demonstrated at the Waite Agricultural Research Institute for a number of crop species of southern Australia. Seven varieties of wheat and two of barley, selected from 150 varieties on the basis of differences in response to high soil boron in a field trial (Cartwright et al., 1987), were compared in a pot experiment at a range of soil boron concentrations (Paull et al., 1988b). The most tolerant varieties, Halberd and ((Wq*KP)*WmH)/6/12, not only showed the least symptoms but also had the lowest tissue boron concentrations in each of the boron treatments. These data were consistent with the results from field experiments. In barley, although there was no significant difference in grain yield, WI-2584 was more tolerant than Stirling on the basis of dry matter production (Paull et al., 1988b). Nable (1988) also reported on genetic variation in response to high levels of boron in barley. Five barley varieties, selected from screening trials in soil culture to represent a range of responses to an excess boron supply from sensitive to tolerant, were tested for responses to a range of boron concentrations in solution cultures. In each level of boron supply, the tolerant

varieties Sahara 3763 and Sahara 3769 accumulated considerably less boron than did the sensitive variety Schooner, whereas the moderately tolerant varieties Sahara 3768 and Galleon had intermediate concentrations.

Genetic variation in response to high concentrations of boron has been reported in field peas (*Pisum sativum* L.) (Bagheri et al., 1992; Paull, et al., 1992b) and annual medics (*Medicago* spp.) (Paull et al., 1992b). Bagheri et al. (1992) reported that there was significant variation in response to high concentrations of boron for dry-weight yield, boron concentration in shoots and visual symptoms among the tested varieties of peas. These three characters were used as parameters for the classification of varieties in response to boron. The most tolerant Australian varieties Alma, Early Dun, Dundale and Maitland were found to be the lowest for boron concentration in shoots (Bagheri et al., 1992; Paull, et al., 1992b).

Genetic variation in response to boron has been observed in rice. Ponnamperuma et al. (1979) reported evidence of boron toxicity in a rice field irrigated for 15 years with deep-well water with a boron content of 3.0-5.3 ppm. They found that IR40 was more tolerant than IR8 to high levels of boron. An experiment using solution culture treated with increasing boron levels (0.5-20 mg l^{-1}) was conducted in a glasshouse (Cayton, 1985). The results showed that at the level resulting in a 10% yield reduction, IR42 contained more boron in plant tissues than those of IR36 and IR46. This indicated that IR42 tolerated more boron in plant tissues than IR36 and IR46. Cayton (1985) also reported variation in yield reduction among different varieties in a rice field with high boron soil (17 mgB kg^{-1}). Yields of tolerant and sensitive varieties were reduced 0-35% and 45-76%, respectively, compared with those in normal soil (8.5 mgB kg^{-1}). The reduction in grain yield may have been due to a decrease in grain filling (Cayton, 1985) since there was evidence that normal supply of boron enhances dephosphorilization and synthesis of starch and cellulose (Bergmann, 1983), whereas excess boron inhibited the formation of starch from sugar (Scott, 1960).

Stephenson and Gallagher (1987) reported a difference in boron response between two commercial macadamia nut (*Macadamia integrifolia*, Maiden and Betche) varieties,

Keauhou (246) and Kakea (508). Although there were no differences in tree appearance, kernel recovery, kernel weight and percentage first grade kernels were enhanced by boron sprays to Keauhou (246) trees, while the nut-in-shell yield also increased in Kokea (508).

Three varieties of strawberry were grown in sand culture and supplied with four levels of boron and three levels of phosphorous. The experiment showed that the varieties Redcoat and K68-108 required more boron than the variety Midway for maximum vegetative growth. At high boron treatments, Redcoat had the lowest leaf boron concentration while Midway, which appeared to be the most sensitive to high boron concentrations, had the highest boron concentration in leaves (Blatt, 1976).

Studies have been conducted for several crops at the Waite Institute to identify regions from which a high proportion of tolerant genotypes originated and thus allow better targeting of germplasm collections in the search for boron tolerance. Moody et al. (1988) conducted a survey of lines from the Australian wheat collection for their response to high levels of soil boron. The experiment was established in a glass house using large boxes of soil with a high level of available boron (80 mg kg^{-1}). 1576 wheat varieties showed large variation in their response to boron. In comparison with the check variety Halberd, classified as moderately tolerant (Paull, 1990), the tested genotypes were classified as highly sensitive (12%), sensitive (35%), moderately sensitive (33%), moderately tolerant (14%) and tolerant (6%). Varieties originating from the Asia/Asia Minor region, Afghanistan, India and Japan were predominantly tolerant, those from South American countries Argentina, Brazil, Uruguay and the Northern Andes also included a considerable proportion of tolerant genotypes, while those from the regions in the higher northern latitudes (North America and Northern Europe) were mostly sensitive. Most of the Australian varieties were classified as moderately sensitive (Moody et al., 1989).

Subsequent screening of collections of peas, medics and barley have produced results consistent with Moody et al. (1988) regarding the origin of boron tolerant genotypes. Bagheri et al. (1994) reported that most of the tolerant lines of peas identified originated from Asia and South America whereas most of the lines from Europe were classified as sensitive. Tolerant accessions of medics were identified by Paull et al. (1992b)

and most of these originated from the central and western Mediterranean region. Jenkin (1993) reported that thirteen out of almost 350 barley accessions from the collection held at the Waite Agricultural Research Institute were tolerant to high levels of boron. Three of those tolerant genotypes originated from North Africa, three from Turkey, two from USA and one from each of Algeria, Korea, Argentina, China and Australia. It is interesting that the geographical distribution of tolerance to high levels of soil boron mentioned above is similar across plant species. It seems likely that Asia/Asia Minor and South America are the centres of origin for boron tolerance in wheat, peas and medics. However, more germplasm of the crops from the regions of Asia/Asia Minor and South America needs to be screened in order to conclude that these regions are the centres of origin for boron tolerance.

2.11.3 Mechanism of tolerance to boron

The mechanism of tolerance to boron toxicity has been investigated for barley, wheat, peas and medics, using either soil, solution or tissue culture. Barley and wheat were studied in a solution culture experiment by Nable (1988). In both species, the accumulation of boron in roots and shoots of tolerant genotypes was considerably less than susceptible genotypes at each level of applied boron. Boron tolerance was governed by the ability of plants to restrict movement of boron into their roots and, consequently, into shoots. The mechanism by which the boron was excluded was not determined and may be due to either membrane composition, cell wall composition, or physical barriers (Nable and Paull, 1991; Paull et al., 1992a). Other factors that may differ between varieties and substantially influence passive absorption of boron include

(1) surface area of roots,

(2) composition of the root cell membranes and effects on permeability to boron, and

(3) concentrations of boron adsorption sites in the free space, in particular the cis-diol content. No information is presently available on how these factors may vary between varieties of crop species (Nable, 1988).

Response to boron was independent of temperature of the root medium over the range 5-25°C suggesting the mechanism is not dependent upon enzyme activity (Nable et al., 1990a) and is also expressed by undifferentiated tissue (Huang and Graham, 1990) indicating tolerance is not dependent on whole plant structure. An interesting observation by Nable et al. (1990a) was that the mechanism affecting uptake of boron by barley genotypes contrasting in boron tolerance also affected uptake of silicon, supplied as $Si(OH)_4$. There was no competitive interaction in the uptake of boron and silicon, indicating that the mechanism apparently operates independently on boron and silicon. Restricted uptake of boron by tolerant genotypes was also reported for medics (Paull et al., 1992b) and peas (Bagheri et al., 1992; Paull et al., 1992b). Nable and Paull (1991) suggested that the ability of plants to restrict boron transport not only governed the degree of boron tolerance but may also be inversely related to the susceptibility to boron deficiency of the genotypes.

2.11.4 Inheritance of response to boron

Kelly and Gabelman (1960) evaluated susceptibility to boron deficiency in 67 strains and varieties of table beets (*Beta vulgaris* L.). Inheritance was concluded to be complex because of a wide array of tolerance to low boron. However, segregation among progenies from crosses between tolerant and susceptible parents was not studied to confirm this conclusion. In contrast, Tehrani et al. (1971) reported that susceptibility to boron deficiency of red beet was controlled by a simple dominant gene. The different conclusions between the authors were probably because of the difference in experimental designs. Since segregating populations derived from crosses between tolerant and susceptible lines was not studied, the conclusion of Kelly and Gableman (1960) may not be correct. Different varieties used in the experiments may also result in different genetic effects in response to boron.

Wall and Andrus (1962) described a mutant of tomato (*Lycopersicon esculentum*), T3238, which developed the stem and petiole brittleness characteristic of boron deficiency

in a nutrient medium in which the Rutgers variety grew without deficiency symptoms. It was concluded that T3238 has the ability to absorb boron from the soil; however, translocation of boron from root to shoot was not as rapid as in the Rutgers variety. Brittle stem susceptibility was controlled by a single recessive gene (*btl*). Pope and Munger (1953) demonstrated that a single recessive gene controlled susceptibility to boron deficiency in celery (*Apium graveolens*).

Blamey et al. (1984) reported that the inheritance of tolerance to boron deficiency in sunflower could be explained predominantly by additive or additive epistatic gene action. They further suggested that the susceptibility of a hybrid to boron deficiency could be predicted from the performance of its parents since those parents with a high boron status readily passed this character to their offspring.

Gorsline et al. (1964, 1968) studied the inheritance of different concentrations of 11 elements in the ear leaf of corn using diallel analysis of 12 inbred lines. They concluded that the boron concentration in the ear leaf was under the control of additive gene actions.

F_1 hybrids from a full set of diallel crosses, excluding reciprocals, among seven Mexican wheat varieties were studied in Thailand in response to boron deficiency using sand culture with a low level of boron at 0.2 μM (Jamjod et al., 1993). The results indicated that tolerance to a low level of boron in wheat was expressed as a quantitative character and mostly controlled by additive gene actions.

The experimental designs used in the experiments of Blamey et al. (1984), Gorsline et al. (1964, 1968) and Jamjod et al. (1993), described above, were probably not adequate to elucidate the mechanism of tolerance to boron. Therefore, major genes responsible for tolerance to boron in these crops could not be identified.

There is a very limited number of references in the area of genetic control of tolerance to high concentrations of boron. However, Paull et al. (1991b) reported that there were three major genes *Bo1*, *Bo2* and *Bo3* involved in the control of tolerance to high boron concentrations in five wheat genotypes. The boron tolerant genes showed additive effects which was expressed as transgressive segregation in the progeny from the cross between G61450 (tolerant) and Halberd (moderately tolerant) (Paull et al., 1991b). Chromosomal

location of genes controlling tolerance to high boron concentration was studied by Paull et al. (1988a). They found that the substitution line of chromosome 4A from Kenya Farmer (KF) into Chinese Spring (CS) was significantly more sensitive to boron than CS and the 20 other CS/KF substitution lines. The substitution line [CS(KF4A)] also expressed the mid-leaf necrosis symptom which was observed only in sensitive genotypes, including Kenya Farmer (Paull et al., 1991b). The segregation in response to boron of F_2 derived F_3 lines of CS x CS(KF4A) indicated a single gene located on the long arm of chromosome 4A. Paull (1990) used the method of monosomic analysis (Sears, 1953) to identify further the chromosomes carrying boron tolerance genes. He concluded that 7B and 7D were the most probable chromosomes responsible for boron tolerance in two wheat varieties G61450 and Federation, respectively. The difference in critical chromosomes between the two varieties could explain the transgressive segregation that occurred from the combination of G61450 and Halberd, a descendant of Federation (Paull, 1990).

Genetic control of tolerance of barley to high concentration of boron was studied in the F_1, F_2 and F_2 derived F_3 populations of the crosses between varieties Sahara 3771 (highly tolerant), CM72 (moderately tolerant) and Stirling (sensitive) (Jenkin, 1993). The results indicated that boron tolerance in barley is expressed as a partially dominant trait and controlled by at least two and three major genes for CM72 and Sahara 3771, respectively.

Sources of boron tolerance have been sought in species related to wheat, while interspecific amphiploids and addition lines have been evaluated to determine locations of genes conferring tolerance. Paull et al. (1991a) reported that the amphiploid of *T. aestivum* (var. Chinese Spring) x *Ag. elongatum* is more tolerant to boron than Chinese Spring. This indicated that the boron tolerance of *Ag. elongatum* can be expressed in wheat background (Paull et al., 1991a). Paull et al. (1992a) compared the boron concentration in shoots of two ditelosomic addition lines of chromosome 7E with Chinese Spring and the disomic 7E addition line in solution culture at high levels of boron. The results indicated that a gene(s) conferring tolerance to boron was located on 7Eβ. In contrast, Manyowa (1989) reported that none of the addition lines of CS/*Ag. elongatum* expressed more tolerance to boron than

Chinese Spring. This may be due to the fact that only some of the addition lines were tested by Manyowa (1989). The addition lines used were from the first set produced by Dvorak and Knott (1974) which were demonstrated later to be incomplete and several of the addition chromosomes were in fact translocations (Hart and Tuleen, 1983). Manyowa (1989) also found that the addition lines of Imperial rye (*S. cereale*) and *Ae. sharonensis* into Chinese Spring expressed significantly more tolerance than Chinese Spring and concluded that chromosomes 2R, 3R, 5R, 3S, 5S and 7S were responsible for boron tolerance. Paull (1985) reported no difference in response to boron between Chinese Spring and Betzes barley, therefore the Chinese Spring/Betzes barley addition lines (Islam et al., 1981) were not tested for boron response.

Since the inheritance of tolerance to high boron concentration is under the control of a series of major genes (Paull et al., 1991b), transfer of the boron tolerant *Bo1* allele from Halberd to Schomburgk, a moderately sensitive variety, has been achieved by backcrossing (Moody et al., 1993). An evaluation of the yield advantage of the boron tolerant allele was conducted by comparing between boron tolerant and sensitive BC_3 derived lines. The tolerant lines, one of which was recently released as BT-Schomburgk, had a yield advantage up to 11% with the average yield advantage being 3.3% in all trials conducted in a range of soil types across the South Australian cereal belt and at Walpeup in Victoria. However, there was no significant difference in yield between the two groups of lines in normal soil boron conditions (Moody et al., 1993).

2.12 Summary and research objectives

At the time when the research reported in this thesis started there was some information on genetic control of tolerance to boron in wheat which can be summarized as follows;

(1) Tolerance to high concentrations of boron in wheat is expressed as a partially dominant character and controlled by major genes which act in an additive manner and have been

named *Bo1*, *Bo2* and *Bo3* (Paull, 1990; Paull et al, 1991b). The *Bo1* allele has been transferred from Halberd to Schomburgk to produce BT-Schomburgk (Moody et al., 1993).

(2) Although there was a report of evidence for transgressive segregation among the progeny of G61450 x Halberd (Paull et al., 1991b), there was no report on the number of genes controlling tolerance to high concentrations of boron for this cross.

(3) Chromosomes 7B and 7D have been reported to be the most probable locations of genes for tolerance to boron in Federation and G61450, respectively. However, these results were described as equivocal because of the unreplicated experiment and the use of boron toxicity symptoms, which is a quantitative character, as a criterion to assess the response of individual plants (Paull, 1990).

(4) Exotic germplasm more tolerant than Halberd have been identified (Moody et al., 1988). However, there was no information on the allelic relationships between the tolerant exotic germplasm and the local tolerant varieties such as BT-Schomburgk. Information on these relationships will be very useful for the identification of the most suitable donors in a backcrossing program.

Thus, the research described in this thesis was undertaken to provide the following genetic information of boron tolerance;

(1) Determine the number of genes controlling boron tolerance in G61450 x Halberd.

(2) Identify chromosomes responsible for the genes controlling boron tolerance in G61450 and Halberd and some other tolerant exotic germplasms.

(3) Determine the allelic relationships between tolerant exotic germplasm and BT-Schomburgk.

Chapter 3

Materials and methods - general procedures

3.1 Screening technique

The method employed for screening plants for reaction to boron consisted of growing seedlings in filter papers which had been soaked with a solution of boric acid. The development and full details of this technique are described in Chapter 4.

3.2 Statistical analysis

A randomized complete block design (Chapter 4, Chapter 5, Chapter 6 and Chapter 7) and a split plot design (Chapter 4) were used as experimental designs. Analysis of variances were calculated using the MSTAT microcomputer program version 4.0 written at the Michigan State University.

For the estimation of the number of genes responsible for tolerance to boron, F_2 populations (Chapter 5 and Chapter 6), F_2 derived F_3 (Chapter 5) and F_4 families (Chapter 8), and parents were tested under high boron concentrations and means and variances of their root lengths were calculated.

The details of the experimental procedures, for example the number of seeds for each F_2 population and F_3 family and the number of F_3 families, are described in the individual chapters.

3.3 Genetic analysis

The number of genes controlling boron tolerance was estimated from the F_2 populations, F_2 derived F_3 and F_4 families (Chapter 5, Chapter 6 and Chapter 8).

F_2 populations

To estimate the number of genes controlling boron tolerance, the seeds of individual F_2 populations and the parents of the crosses were tested under high boron concentrations

in filter papers. The distribution of the seedling root length of the F_2 population was examined. If the distribution was bimodal (Figure 3.1a) or trimodal (Figure 3.1b), it was possible to classify the F_2 seedlings into two (sensitive and intermediate-tolerant) or three (sensitive, intermediate and tolerant) categories. Chi-square analysis was used for testing the goodness of fit of the observed segregation ratios to frequencies expected for monogenic (1 sensitive : 3 intermediate-tolerant or 1 sensitive : 2 intermediate : 1 tolerant) or digenic (1 sensitive : 15 intermediate-tolerant) segregations.

However, when the distribution was continuous, it was not possible to assign the individual F_2 seedlings to discrete categories (Figure 3.1c). Initially an attempts was made to differentiate among the individual F_2 plants on the basis of performance of the parents as measured by their means and standard deviations, but there were problems with using these parameters.

In the initial attempt, each F_2 seedling was assigned to one of the three categories, namely sensitive, intermediate and tolerant. Seedlings were classified as sensitive when the root length was equal to or shorter than the mean root length plus two standard deviations of the sensitive parent, whereas F_2 seedlings with a root length equal to or longer than the mean root length minus two standard deviations of the tolerant parent were classified as tolerant. The seedlings with a response between the sensitive and tolerant groups were classified as segregating. Chi-square analysis was used for testing the goodness of fit of the observed segregation ratios to frequencies expected for monogenic or digenic segregation. However, this differentiation between the sensitive, intermediate and tolerant genotypes on the basis of the mean and standard deviation of the parents is very sensitive to the balance between Type I and Type II errors.

Type I errors occur when the null hypothesis is rejected even though it is true (Sokal and Rohlf, 1981). For example, if the curves of the distribution of the root lengths of parents and a F_2 population tested under a high boron concentration are compared (Figure 3.2), and F_2 seedlings are classified as the parental genotype when roots are equal to or shorter than the mean root length plus two standard deviation of the parent, the frequency

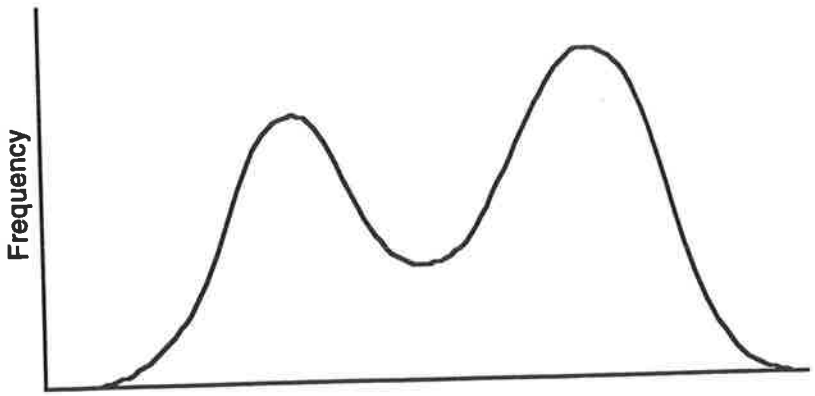
Figure 3.1 The distribution of root lengths of individual plants within F_2 populations.

(a) bimodal

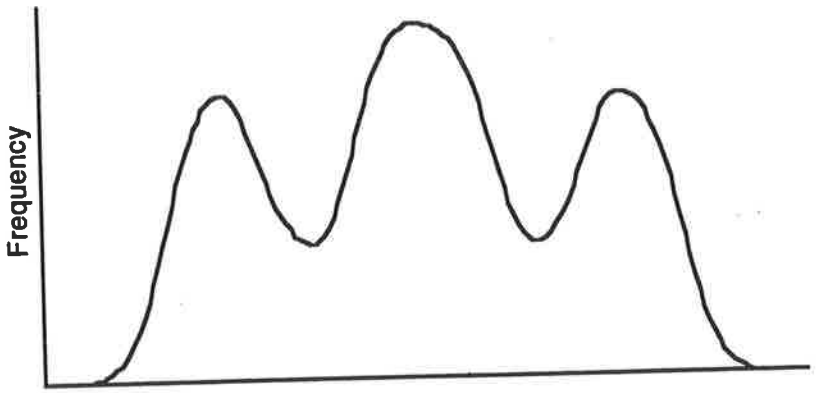
(b) trimodal

(c) continuous

(a)



(b)



(c)

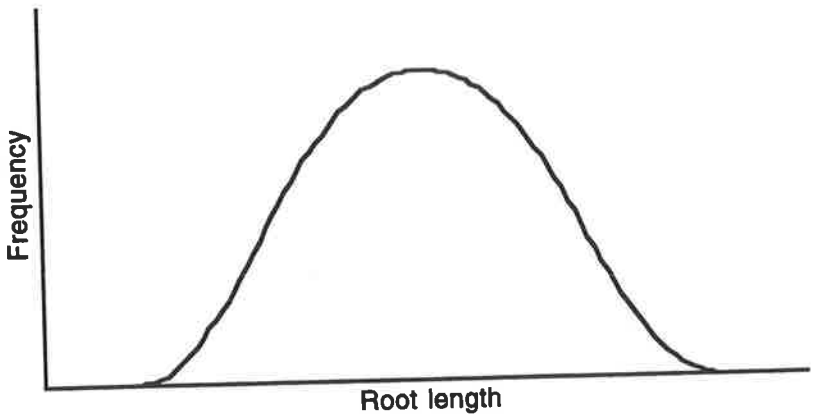
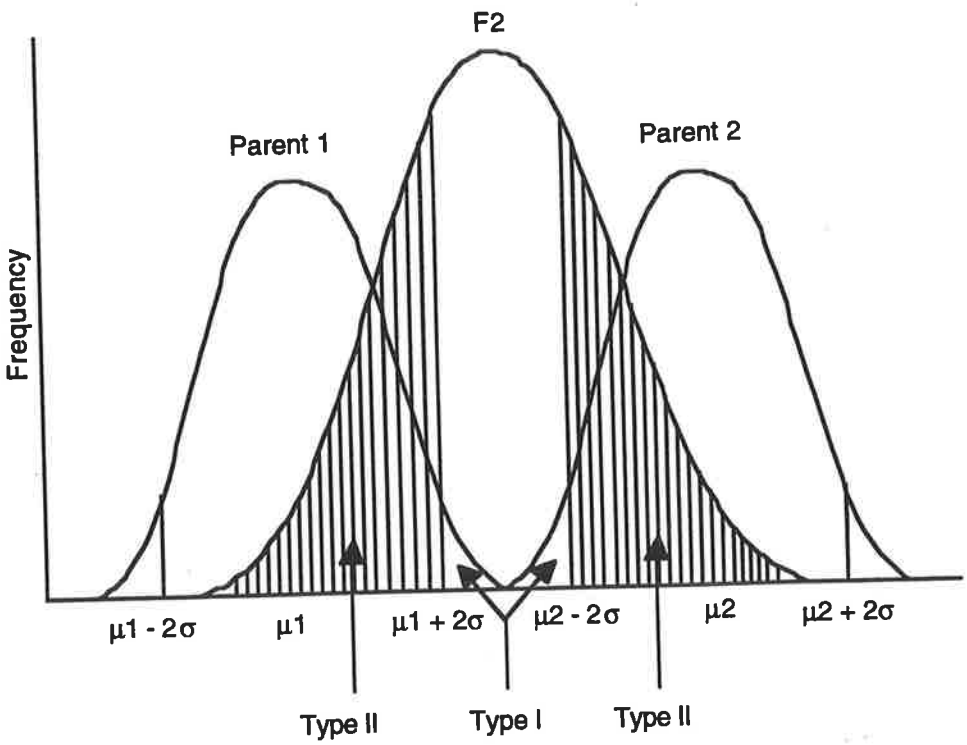


Figure 3.2 The comparison between frequency distributions of two parents and an F_2 population, illustrating Type I and Type II errors.
The shaded portions of the F_2 distribution represent plants classified as parental types on the basis of falling within the range (mean $\pm$ 2 st dev) for each parent. Type I errors occur when parental types fall beyond these regions and Type II errors occur when heterozygous plants fall within these regions.


F2
Parent 1
Parent 2
Frequency
μ1 - 2σ
μ1
μ1 + 2σ
μ2 - 2σ
μ2
μ2 + 2σ
Type II
Type I
Type II

at which a Type I error will occur is represented by the rejected regions (about 2.5 % of the population) (Figure 3.2).

In contrast to the error Type I, the error Type II occurs when the null hypothesis is accepted although in fact it is false (Sokal and Rohlf, 1981). For example, Type II errors occur when the distributions of one of the parents and the F_2 heterozygotes overlap (Figure 3.2). The Type II error will increase as the mean of the F_2 approaches the mean of the parent. Thus both of the errors will occur when the curves overlap, whereas only the Type I error will occur when the two curves do not overlap. Because of the under (error Type I) and over (error Type II) estimation of the parental genotypes in the F_2 population, this method of estimation of the number of genes controlling tolerance to boron could not be used when the distributions were not bimodal.

Alternatively, it is possible to estimate the number of genes by comparing the observed variance of a segregating generation with the expected variance calculated from the variance components of the parents and the F_1 population. Mather and Jinks (1977) partitioned the variance of the F_2 generation in terms of an additive-dominance model.

$$V_{F2} = 1/2\, d^2 + 1/4\, h^2 + E$$

$$V_{F3} = 3/4\, d^2 + 3/16\, h^2 + E$$

$$V_{F4} = 7/8\, d^2 + 7/64\, h^2 + E$$

Where V_{F2}, V_{F3} and V_{F4} are variances of F_2 and F_2 derived F_3 and F_4 populations, respectively, of the cross between two homozygous genotypes (AA and aa). In the model, the mid-point (m) is the midway between the means of the two homozygotes, d is the departure from the mid-point (m) of the means of each homozygous genotype, h is the departure from the mid-point of the heterozygous genotype (Aa) (Figure 3.3) and E is the environmental variance ($E = 1/4\, V_{P1} + 1/4\, V_{P2} + 1/2\, V_{F1}$; where V_{P1} and V_{P2} are the variances of the parents and V_{F1} is the variance of the F_1 hybrid between P_1 and P_2). Since the F_1 hybrids of the populations studied in this thesis were not tested, the variance of the F_1 was estimated from the average variance of the two parents ($V_{F1} = (V_{P1} + V_{P2})/2$).

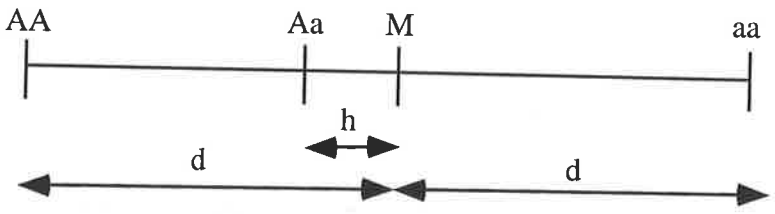
$V_{F2} = 1/2D + 1/4H + E$

$F_{F3} = 3/4D + 3/16H + E$

$V_{F4} = 7/8D + 7/64H + E$ (Mather and Jinks, 1977)

- V_{F2} and V_{F3} are the variances of F_2 and F_2 derived F_3 and F_4 populations, respectively
- D is the additive component, defined as d^2 for a single locus and $(d_a^2+d_b^2)$ for two loci
- d is the departure of AA from the midpoint of AA and aa for a single locus
- 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, for two loci
- H is the dominance component, defined as h^2 for a single locus and $(h_a^2+h_b^2)$ for two loci
- h is the departure of Aa from the mid-point of the homozygotes AA and aa
- h_a and h_b are the departures of the heterozygotes from the mid-points of the homozygotes for the two loci.
- E is the environmental variance

One gene



Two genes

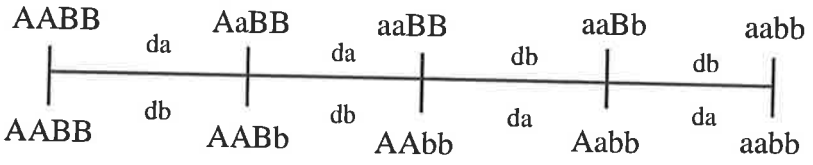
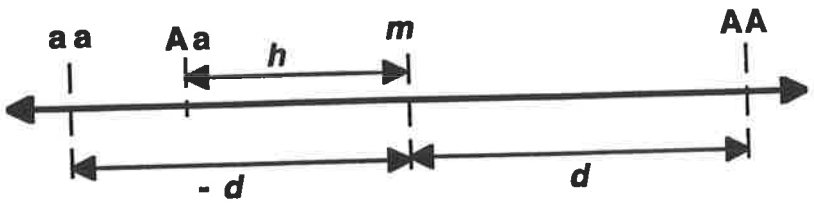


Figure 3.3 The *d* and *h* metrics of the allelic difference A-a. Deviations are measured from the mid-parent, *m*, midway between the two homozygotes AA and aa. Aa may lie on either side of *m* and the sign of *h* will vary accordingly (Mather and Jinks, 1977).



In the case of two genes with the assumptions of no linkage and no epistasis the equations for estimating the expected variances of populations are

$$V_{F2} = 1/2\ (d_a^2 + d_b^2) + 1/4\ (h_a^2 + h_b^2) + E$$

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

$$V_{F4} = 7/8\ (d_a^2 + d_b^2) + 7/64\ (h_a^2 + h_b^2) + E$$

where d_a and d_b are the departures from the mid-point (m) of the homozygous genotypes AABB and aabb, respectively, and h_a and h_b are the departures from the mid-point of the heterozygous genotypes AaBb, AaBB, AABb, Aabb and aaBb and the homozygous intermediate aaBB and AAaa.

Thus, the observed variances for segregating populations can be compared with the expected variances calculated for populations segregating at one or two genes from the above equations. The expected variance can be regarded as being significantly different from the observed variance when the expected variance is outside the range of the confidence interval ($P = 0.95$) of the observed variance.

The confidence interval (P=0.95) of the population variance was calculated as

$$(V_o \times df)/\chi^2 a \leq \textit{Confidence interval} \leq (V_o \times df)/\chi^2 b$$

where V_o = observed variance of a population, df = degrees of freedom of $n-1$, n = number of plants of an F_2 population or number of F_2 derived families of an F_3 population, $\chi^2 a$ = the lower level chi-square value at the probability of 0.95 and degrees of freedom of $n-1$,

χ^2_b = the upper level chi-square value at the probability of 0.95 and degrees of freedom of *n-1* (D. Pederson, pers. comm.).

F_2 derived population

An alternative method used to estimate the number of genes controlling tolerance to boron was the progeny testing of the F_3 or F_4 generations to determine the genotypes of F_2 plants. About 100 random F_2 derived families per population were tested with the parents in filter papers at a high boron concentration. The mean and variance of the seedling root length of each family were compared to those of the parents. A family was classified as either homozygous sensitive or homozygous tolerant when the mean and variance of the family were not significantly different from those of the sensitive or tolerant parent, respectively. The means of the families were significantly different from that of a parent when the means were not within the confidence interval of the mean of the parent. The confidence interval of the mean of a parent was calculated as

$$\textit{Confidence interval} = m \pm t\alpha_1 \times \sqrt{(V_p \times (1/n_1 + 1/n_2))}$$

where n_1 = number of plants within the family, n_2 = number of plants of the parent, m = mean of the parent, t = t-test value at the probability of α_1 and degrees of freedom of $(n_1 - 1) + (n_2 - 1)$; $\alpha_1 = 0.05/n_2$ (each plant is tested individually, thus n_2 tests are to be carried out and the probability of 0.05 is divided by n_2); V_p = variance of the parent (D. Pederson, pers. comm.).

When the variance of a family was the same as or close to those of the parents but the mean of the family was between the sensitive and tolerant parents, the family was classified as homozygous intermediate, whereas a family with a variance greater than those of the parents was classified as a segregating family. The variance of a family was significantly different from the two parents when the variance of the family was greater than the LSD of the parental variances. The LSD of parental and family variances were calculated as

LSD of the parental variances = $Vp \times F\alpha_1$

where Vp = variance of a parent, F = F-test at the probability of α_1 and degrees of freedom of $(n_1 - 1)$, $((n_2 - 1) + (n_3 - 1))$; n_1 = number of plants within a family, n_2 and n_3 = number of plants within each of the two parents; $\alpha_1 = 0.05/n_3$ (each plant is tested individually, thus n_3 tests are to be carried out and the probability of 0.05 is divided by n_3) (D. Pederson, pers. comm.).

Chi-square analysis was used for testing the goodness of fit of the observed segregation ratios of the F_3 families to the frequencies expected for monogenic (1 sensitive : 3 segregating-tolerant and 1 sensitive : 2 segregating : 1 tolerant) or digenic (1 sensitive : 15 intermediate-tolerant and 1 homozygous sensitive : 2 homozygous intermediate : 1 homozygous tolerant : 12 segregating) segregation.

3.4 Cytological methods

The chromosome complements of pollen mother cells (PMCs) were determined during monosomic analysis (Chapter 6) and backcross reciprocal monosomic analysis (Chapter 7). To determine the extent of chromosome pairing, PMCs were examined at metaphase I. Spikes at the early boot stage were collected and anthers from florets were squashed in aceto-orcein stain and examined microscopically to determine the stage of cell division. When an anther at metaphase I was identified, the remaining two anthers from the floret were fixed in 3 absolute ethanol : 1 glacial acetic acid for 24 hours at 4°C. The anthers were then hydrolyzed in 1N HCl at 60°C for 12 minutes and stained with Feulgen stain for 1-2 hours at room temperature. The stained anthers were squashed in 45% acetic acid for microscopic examination.

Chapter 4

Screening technique for boron tolerance

4.1 Introduction

In breeding crop-plants for tolerance to a mineral stress, procedures for assaying plant response to the pertinent stress factor and screening techniques are very important, The procedures should

(a) correctly measure the intensity of the appropriate stress,

(b) provide the maximum expression of genetic variation, avoiding the confounding effects of genotype by environment interaction,

(c) be accurate, rapid and inexpensive in order to permit a large number of segregants to be tested.

The procedure of screening for boron tolerance adopted by Moody et al. (1988) for evaluating a germplasm collection and by Paull (1990) for studying the genetics of boron tolerance consisted of growing plants in loamy top-soil to which a high level of boron had been applied. Plants were rated on the basis of vigor, indicated by the height of plants, stem diameter and extent of tillering and by leaf symptoms related to the uptake of boron by plants. These results were confounded to some extent by environmental variation, for instance evaporative demand, and other genetic effects (Paull, 1990), including the height of plants being effected by semi-dwarf genes in some varieties. This was of particular relevance when evaluating segregating populations derived from crosses between landraces or "old Australian" varieties, as the donor parents of boron tolerance, and semi-dwarf recurrent parents.

Paull et al., (1990) and Bagheri et al., (1992) scored leaf symptoms to distinguish between contrasting genotypes of wheat and peas (*Pisum sativum* L.), respectively. The major problem encountered was that the severity of symptoms increased with the age of the leaves, so only leaves of an equivalent age could be scored when comparing genotypes.

Boron tolerant genotypes of wheat and barley (Nable, 1988) and peas and medics (Paull et al., 1992b) maintain lower concentrations of boron in shoots and roots than more sensitive genotypes. In practice, measuring boron concentrations is expensive, slow and destructive. Therefore, this method cannot be used in early generations of a breeding program, such as in backcrossing, because it is not possible to harvest seeds from tolerant segregants. Tolerance is also expressed by excised root tips cultured on high boron media with tolerant genotypes either producing callus or developing longer root axes, depending upon the culture medium (Huang and Graham, 1990). Unfortunately, this method did not provide sufficiently accurate discrimination between all genotypes for accurate selection. None of these methods measure directly the fundamental expression of the effect of high boron on plants, which is probably a reduced rate of growth of the root meristem, contributing to reduced grain yields. In the agricultural situation, this reduced root growth decreases the amount of water available to the plants increasing water stress under low humidity conditions.

While these methods proved effective both in elucidating the inheritance of boron tolerance and in the breeding of boron tolerant varieties, they were comparatively slow and inaccurate. A new more accurate and more rapid method which could be quantified was a priority for the studies reported in this thesis.

A filter paper technique involving culturing seedlings in filter papers soaked with dilute solutions of a herbicide paraquat had been developed to distinguish the response of resistant and susceptible biotypes of the weed, barley grass (*Hordeum glaucum* Steud.) (Powles, 1986). Seeds of the resistant and susceptible biotypes were incubated in the dark at 19°C on filter paper presoaked with water containing appropriate concentrations of the herbicide. Plants of the resistant and susceptible biotypes differed in the rate of primary shoot elongation. The shoots of the seeds from resistant biotypes were able to elongate at higher concentrations of the herbicide than those from the susceptible biotypes and at comparatively high concentrations very little shoot elongation occurred for the susceptible biotypes (Powles, 1986).

The filter paper technique used for screening for tolerance to boron in the experiments described in this chapter was developed following an initial proposal and investigations by D. Smith, of the wheat breeding group, Roseworthy Campus, University of Adelaide. The experiments reported here were conducted to establish this rapid and non-destructive test as being a suitable method for assessing response to high concentrations of boron.

Two experiments were conducted to identify the optimum boron concentrations for the differentiation of response to boron between wheat varieties. Then, the seedling root lengths of a range of varieties, measured in the filter paper technique at different boron treatments, were compared with other accepted parameters of boron response, such as boron concentrations in shoots, toxicity symptoms and shoot dry weight for plants grown in soil, to establish the validity of the new test.

4.2 Materials and methods

The wheat varieties examined in these experiments, their pedigrees, responses to boron and Australian Winter Cereals Collection accession numbers are presented in Table 4.1.

4.2.1 Response of diverse varieties by the filter paper technique

Seven wheat varieties (Table 4.2) with diverse responses to boron were tested at four boron treatments, in the filter papers technique, to identify the optimum concentration of boron to distinguish between varieties and to determine the relationships between root length, number of roots and shoot length. [The use of the filter paper technique as a method for screening of tolerance to boron is being published in Plant and Soil.]

As it has been shown that the function and growth of roots is impaired where there is an inadequate supply of zinc (Webb and Loneragan, 1990), boron and calcium (Haynes and Robbins, 1948), the control (B0) and all other solutions included 0.5mM $Ca(NO_3)_2.4H_2O$, 0.0025mM $ZnSO_4.7H_2O$ and 0.015mM H_3BO_3.

Table 4.1 Varieties used in experiments, Australian Winter Cereals Collection (AUS) accession numbers, their pedigrees, origins and responses to boron toxicity.

Variety	AUS	Pedigree	Origin	B response[a]
India 126	4743	Unknown	India	T[b]
G 61450	6141	Mentana/Kenya//Quaderna	Greece	T
Benventuto Inca	1929	Mentana/Lin Calel M.A.	Argentina	T
Turkey	1473	Unknown	Turkey	T
AUS 4041	4041	Abyssinia10	Ethiopia	T
AUS 4903	4903	Iraq22	Iraq	T
Klein Granador	22082	BuckRelen/Bage/2/Klein Petiso12.300/Massav2No.5 -P.Gaboto/Janel	Argentina	T
Lin Calel	2881	Unknown	Argentina	T
Halberd	11612	(((Scimitar x KenyaC6042) x Bobin) x Insignia49)	Australia	MT
Bonza	59	Yaqui50/Kentana48	Columbia	MT
BT-Schomburgk	25600	((Halberd x Aroona) x Schomburgk #3)/3/27	Australia	MT
Schomburgk	23325	(((W3589 x Oxley) x Warigal #2) x Aroona #2)/65/1	Australia	MS
Mokoan	22680	WW15/Olympic/2/ Kalyansona/Olympic	Australia	MS
Condor	16036	Penjamo62/4*Gabo56/2/ TZPP/Nainari60/4/2*Lerma Rojo/2/Norin10/Brevor (Seln.14)/3/3*Andes	Australia	MS

Table 4.1 (continued).

Cultivar	AUS	Pedigree	Origin	B response
(W1xMMC)	26183	(Warigal x ((Siete Cerros x Mengavi) x Crim))/1/10	Australia	S
Kenya Farmer	6121	Gaza/2*Bobin//Button/ Kenya73D2IlC	Kenya	VS

[a] Data of boron response were derived from: Moody et al. (1988) and Paull et al. (1991b).
[b] T = tolerant, MT = moderately tolerant, MS = moderately sensitive, S = sensitive, VS = very sensitive.

Fifteen seeds of each variety were surface sterilized with 0.5% sodium hypochlorite and pre-germinated for two days at 4°C and one day at 15°C (Plate 4.1a). The sterile seeds were placed embryo downwards at a spacing of 2 cm across the middle of a filter paper (Ekwip® 32*46 cm grade R6) (Plate 4.1b) soaked with the control solution (B0) or boric acid solution (50, 100 and 150 mgB l^{-1}, designated as B50, B100 and B150, respectively) and drained for 2-3 minutes. The filter papers were rolled up and covered with aluminium foil (Plate 4.1c), then stored upright at 15°C. After 12 days, the length of the shoot and longest root and the number of roots of each seedling were measured (Plate 4.1d).

The experiment was conducted as a randomized complete block design with two replicates.

4.2.2 Comparison between response of varieties in filter papers and high boron soil

Previous investigators (Nable, 1988; Paull et al., 1992b) have reported that the boron accumulation in plant shoots was the most reliable measurement of boron tolerance for a range of crops, including wheat. The second experiment was conducted to compare the response of 14 wheat varieties (Table 4.6) by the filter paper method with boron accumulation in shoots of plants grown in a high boron soil.

This experiment was arranged as a split plot design with two and five replicates for the filter paper and pot experiments, respectively. The procedures as described in the first experiment were used for the filter paper experiment with fifteen seeds of each variety per plot and three solutions B50, B100 and B150. The length of the longest root of each seedling was measured after 12 days.

The soil in which the plants were grown was from the surface horizon (0-10cm) of a red brown earth (Typic Haploxeralf) (Soil Survey Staff, 1975). Boron, as boric acid, was applied and uniformly mixed through the soil at a concentration of 10 or 20 mg kg^{-1} (designated as B10 and B20). The soil was placed in 200 mm diameter pots lined with plastic bags to prevent leaching. Three pregerminated seeds of each variety were sown per pot.

Plate 4.1 Illustrations of the filter paper technique.

(a) Seeds being pre-germinated.

(b) The pre-germinated seeds placed on the filter paper.

(a)

(b)

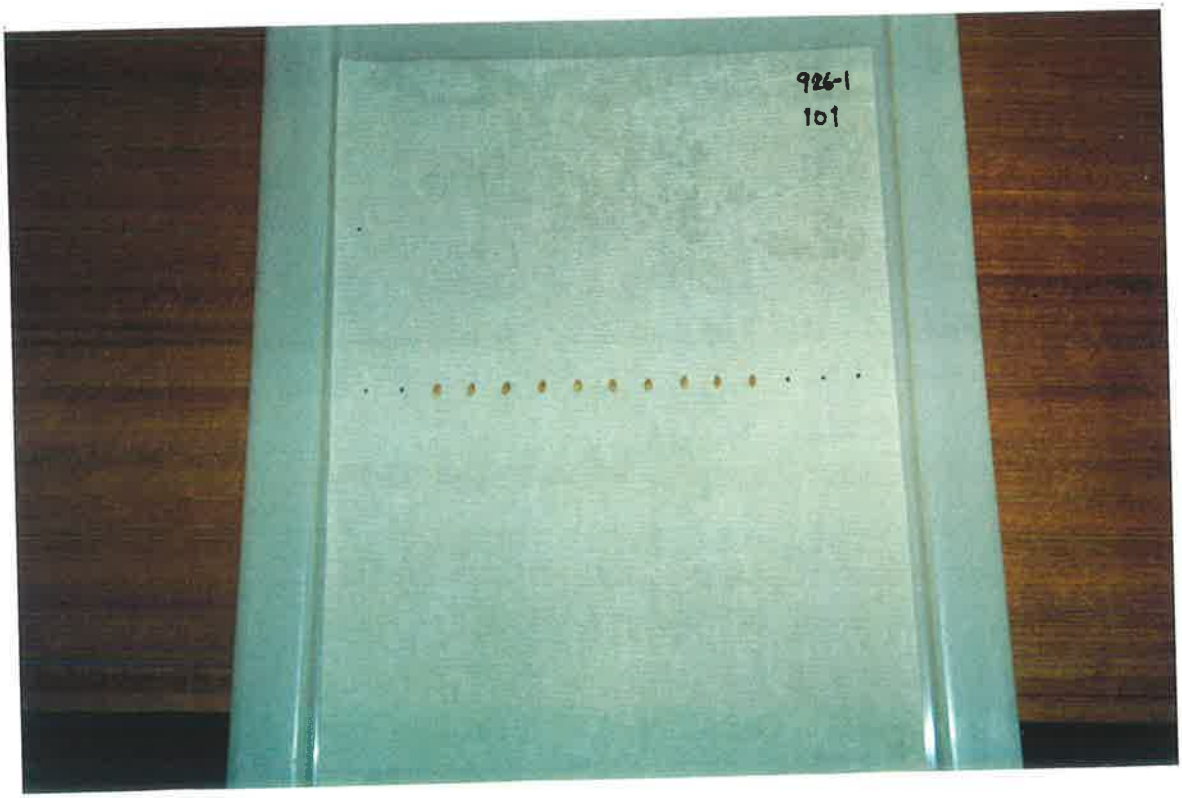


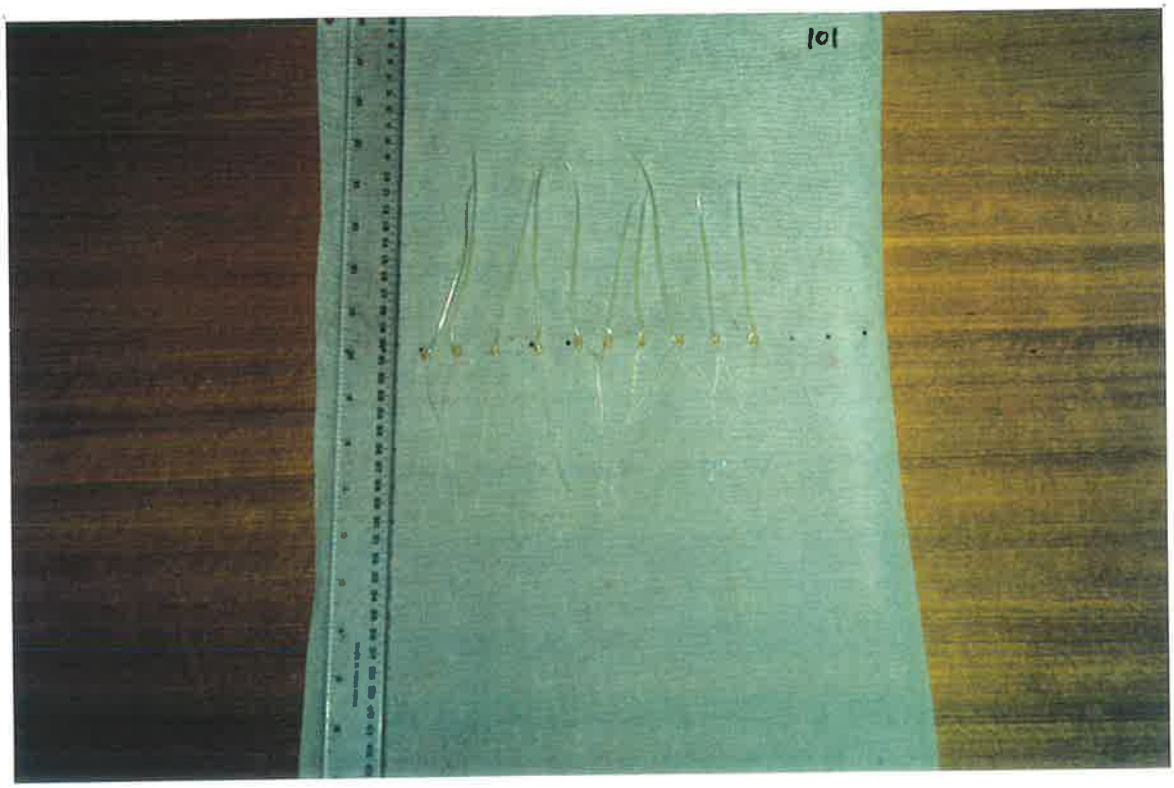
Plate 4.1 (continued).

(c) Filter papers rolled up and covered with aluminium foil.

(d) Seedling development after twelve days of treatment.

(c)

(d)



The youngest fully expanded blade of the main shoot was rated for expression of symptoms indicative of boron toxicity eight weeks after sowing using a scale adapted from Kluge and Podlesak (1985) (Table 4.7). The plants were harvested at ground level eight weeks after sowing, dried, weighed and the concentration of boron in the shoots was determined by ICP spectrometry following digestion in nitric acid (Zarcinas et al., 1987).

An arc sine transformation was used to normalize the boron toxicity symptom data before the results were tested by analysis of variance.

4.3 Results

4.3.1 Root length of diverse varieties by the filter paper technique

Root length

There were highly significant effects for both genotypes and boron treatments and a highly significant interaction ($P < 0.01$) between varieties and boron treatments for the growth of roots (Table 4.2). In high boron treatments, the root lengths differed significantly between genotypes and root lengths were longer for the genotypes rated as tolerant in earlier studies by Moody et al., (1988) and Paull et al., (1991b) (Table 4.2).

At B0, the root lengths of the very sensitive variety Kenya Farmer and the moderately sensitive Schomburgk were not significantly different from the moderately tolerant variety Halberd and the tolerant G61450, but significantly longer than the tolerant Bonza and Benventuto Inca (Table 4.2). At B50, there was no significant difference between Halberd, G61450 and Benventuto Inca, whereas in B100, Halberd had significantly shorter roots than G61450, Bonza and Benventuto Inca. At B150, Halberd, G61450 and Benventuto Inca were not significantly different from each other, however, the roots of these three varieties were shorter than those of Bonza and India 126. The roots of India 126 were significantly longer than all other varieties at all treatments, including the control. At B50, there was no significant difference between the root lengths of Halberd and Schomburgk, whereas, in B100 and B150, Halberd had significantly longer roots than Schomburgk. Kenya Farmer had shorter roots than Schomburgk at all three levels of added boron. These results indicate that genetic variation in response to boron is

Table 4.2 Mean length of the longest seminal root of 15 seedlings of seven wheat varieties and relative root length (%) when tested in the filter paper technique at four boron treatments.

Variety	B[a]	Root length (cm)					Relative root length (%)			
		B0	B50	B100	B150	Mean	B50/B0[b]	B100/B0	B150/B0	B150/B50
India 126	T	22.7	18.2	15.6	12.9	17.3	80	69	57	71
Bonza	T	15.6	15.1	13.0	9.7	13.3	97	83	62	64
G61450	T	19.9	14.4	11.5	7.5	13.3	72	58	38	52
Benventuto Inca	T	15.5	14.3	11.5	6.9	12.0	92	74	45	48
Halberd	MT	19.5	13.2	9.9	6.3	12.2	68	51	32	48
Schomburgk	MS	20.9	9.8	4.9	3.0	9.6	47	23	14	31
Kenya Farmer	VS	20.9	3.4	1.6	0.8	6.7	16	8	4	24
Mean		19.2	12.6	9.7	6.7	12.1				

LSD(0.05) Varieties 0.8, Treatments 0.7, Interaction 1.6

[a] boron responses are quoted from Table 4.1,

[b] relative root length, e. g. B50/B0 = (root length at B50/root length at B0) x 100.

expressed in the filter paper procedure at boron concentrations of B50 or greater. The root lengths of all varieties at B0 and B100 are shown in Plate 4.2.

There was no significant relationship between growth of roots in the absence of boron and in the boron treatments, with correlations between B0 and B50, B100 and B150 being r = -0.18, -0.23 and -0.09, respectively (Figure 4.1). There were, however, highly significant relationships ($P < 0.01$) between root length at different boron treatments with correlations between B50 and B100 and B150 and between B100 and B150 being r = 0.98, 0.94 and 0.97, respectively (Figure 4.1), which indicate that the responses of the varieties, as determined by seedling root lengths were consistent at the three high boron levels.

As there were differences in root lengths, between varieties, at the control treatment, relative root lengths ((root length at boron treatment/control) x 100) were calculated (Table 4.2). The relative root lengths of all of the tolerant varieties were greater than those of Halberd which in turn was greater than the moderately sensitive variety Schomburgk and the sensitive variety Kenya Farmer which had the lowest relative root length. At B150/B50, Benventuto Inca and Halberd had the same relative root length. The ranking of the tolerant varieties on the basis of relative root length was consistent between B50/B0 and B100/B0 but these differed from B150/B0 which in turn differed from B150/B50. In contrast to B150/B0, the relative root length of Benventuto Inca was greater than that of India 126 at B50/B0 and B100/B0. At B150/B0 Benventuto Inca was more tolerant than G61450, however, at B150/B50 they were similar (Table 4.2). G61450 had longer roots at B0 than Benventuto Inca but similar lengths at B50.

Shoot length

Varieties and boron treatments showed highly significant effects with respect to length of shoots and a highly significant interaction ($P < 0.01$) (Table 4.3). At B0, there was no significant difference in shoot lengths between the very sensitive variety Kenya Farmer, the moderately sensitive Schomburgk and the tolerant varieties G61450, Bonza and Benventuto Inca but at B100 and B150 the shoot lengths of Kenya Farmer were significantly shorter than those of the other varieties. There was no significance difference

Plate 4.2 Response to two levels of boron of seven wheat varieties when tested in the filter paper technique.

(a) B0

(b) B100

From left to right : India 126, Bonza, G61450, Benventuto Inca, Halberd, Schomburgk, Kenya Farmer.

(a)


Seedling root length of wheat varieties at B0
India126
Bonza
G61450
B. Inca
Halberd
Schomburgk
Kenya Farmer

(b)

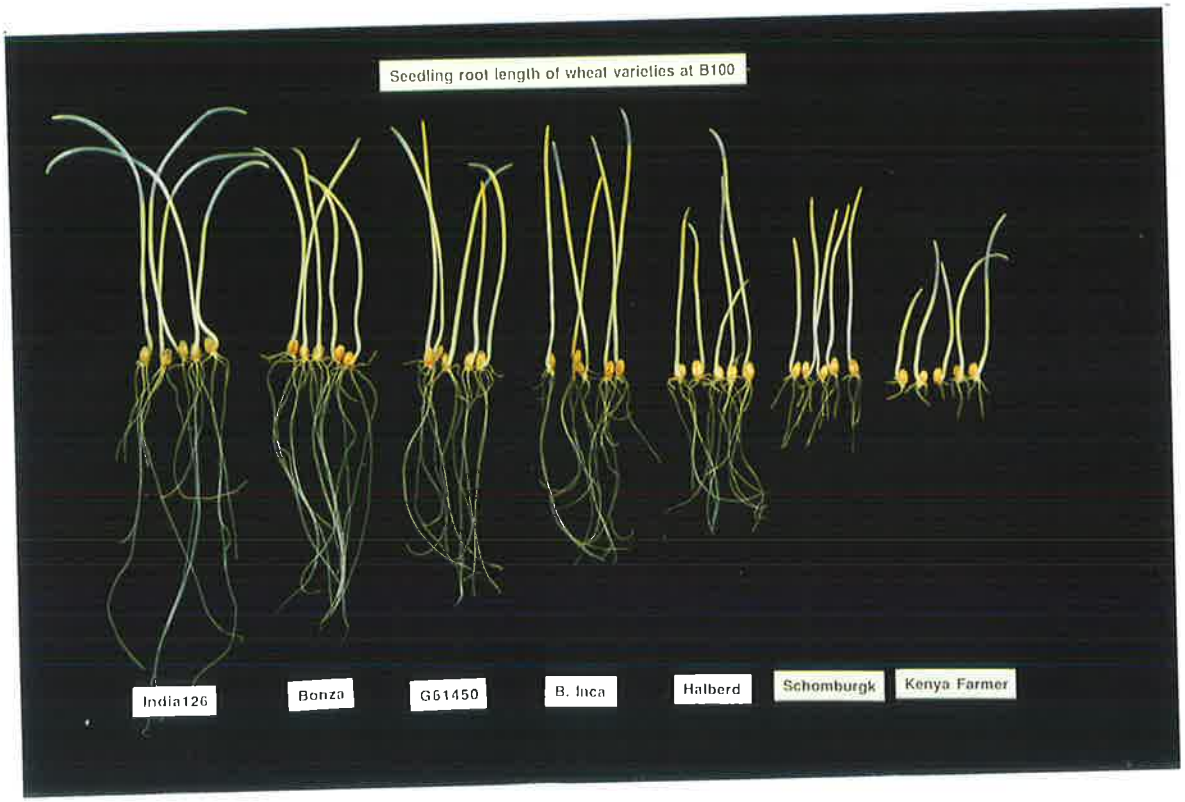

Seedling root length of wheat varieties at B100
India126
Bonza
G61450
B. Inca
Halberd
Schomburgk
Kenya Farmer

Figure 4.1 Relationships between seedling root length of seven wheat varieties tested in the filter paper technique at four boron treatments.

(a) B0 v B50

(b) B0 v B100

(c) B0 v B150

(d) B50 v B100

(e) B50 v B150

(f) B100 v B150

Note: ** significant at $P < 0.01$

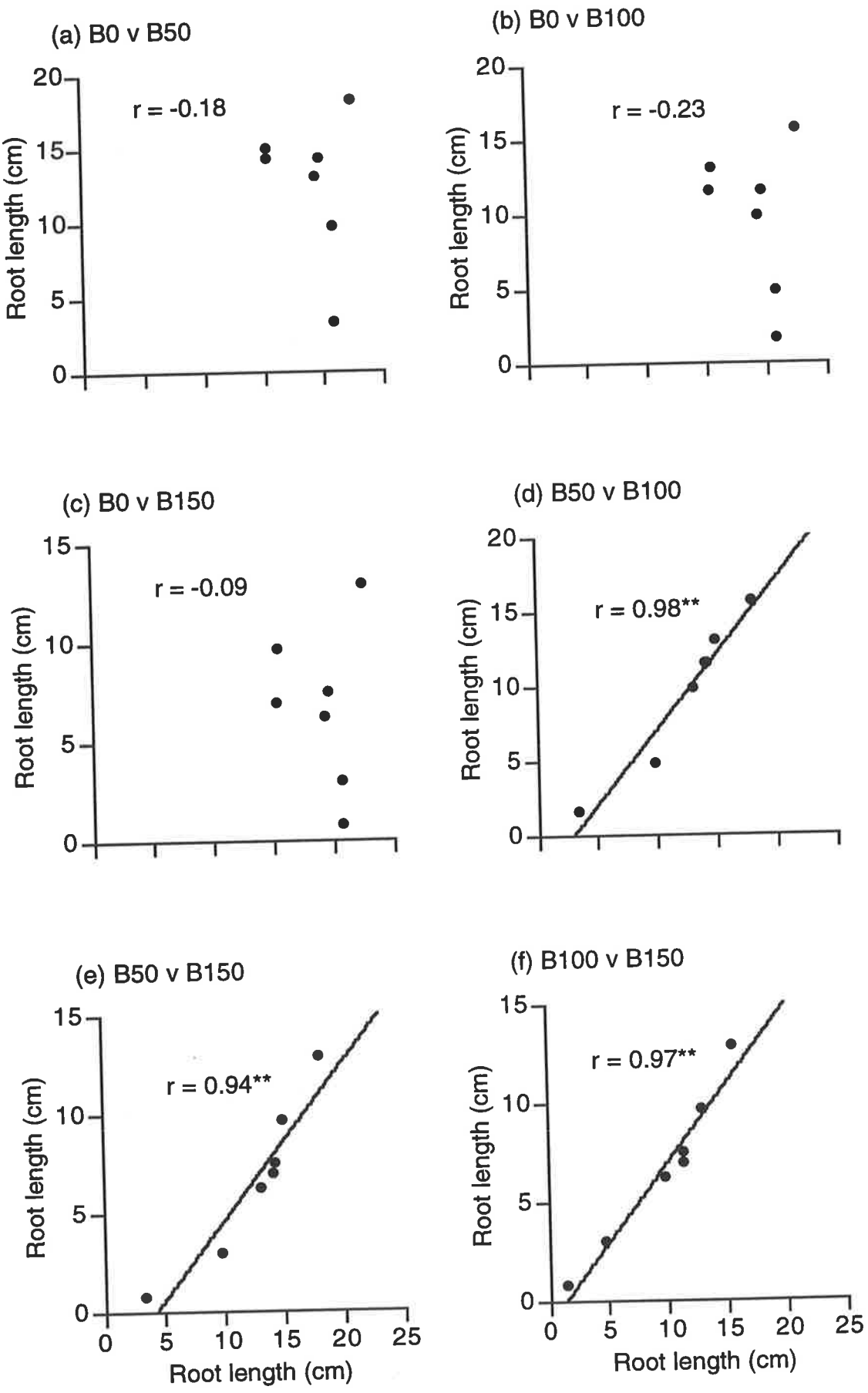

(a) B0 v B50
r = -0.18
Root length (cm)
(b) B0 v B100
r = -0.23
Root length (cm)
(c) B0 v B150
r = -0.09
Root length (cm)
(d) B50 v B100
r = 0.98**
Root length (cm)
(e) B50 v B150
r = 0.94**
Root length (cm)
Root length (cm)
(f) B100 v B150
r = 0.97**
Root length (cm)
Root length (cm)

Table 4.3 Mean of 15 seedlings for the length of shoots and relative shoot length (%) of seven wheat varieties when tested in the filter paper technique at four boron treatments.

Variety	B[a]	Shoot length (cm)					Relative shoot length (%)			
		B0	B50	B100	B150	Mean	B50/B0[b]	B100/B0	B150/B0	B150/B50
India 126	T	18.4	16.5	14.9	12.3	15.5	90	81	67	75
Bonza	T	9.6	9.9	10.9	8.2	9.6	103	114	85	83
G61450	T	11.0	9.6	8.2	5.6	8.6	87	75	51	58
Benventuto Inca	T	10.2	7.8	5.7	4.6	7.1	77	56	45	59
Halberd	MT	12.8	10.7	7.3	6.2	9.2	84	57	48	58
Schomburgk	MS	10.9	9.8	7.4	5.9	8.5	90	68	54	60
Kenya Farmer	VS	10.6	7.7	3.3	0.3	5.6	73	31	3	4
Mean		11.9	10.8	8.3	6.1	9.1				

LSD(0.05) Varieties 0.8, Treatments 0.7, Interaction 1.6

[a] boron responses are quoted from Table 4.1,

[b] relative root length, e. g. B50/B0 = (root length at B50/root length at B0) x 100.

between Schomburgk, Halberd and G61540 at the three levels of boron. Shoots of India 126 were significantly longer than those of all other varieties at all treatments.

There were differences between varieties in relative shoot lengths at B50/B0, B100/B0, B150/B0 and B150/B50 (Table 4.3). The relative shoot lengths of Bonza were the greatest, whereas those of Kenya Farmer were the least and this was consistent between B50/B0, B100/B0, B150/B0 and B150/B50. However, there was no consistency in the ranking of the other genotypes. There was also inconsistency between the relative shoot lengths and the previous assessments of level of tolerance to high boron. For example, the tolerant variety G61450 had almost the same relative shoot length as the moderately sensitive variety Schomburgk, which suggested that the relative shoot length should not be used as a selection criterion for boron tolerance.

Number of roots

There were highly significant effects for varieties and boron treatments with respect to the number of roots per plant and also a significant interaction ($0.01 < P < 0.05$) (Table 4.4). These effects could be attributed principally to the response of the very sensitive Kenya Farmer. There was no significant difference in number of roots between moderately sensitive, moderately tolerant and tolerant varieties at the three high levels of boron.

Correlation between root lengths, shoot lengths and number of roots

The lengths of the longest roots at B50, B100 and B150 were significantly correlated with shoot lengths at B100 and B150. However, there was only one statistically significant correlation between the length of the longest root and number of roots and none between the length of shoots and number of roots (Table 4.5).

Table 4.4 Mean number of roots per plant for 15 seedlings of seven wheat varieties when tested in the filter paper technique at four boron treatments.

Variety	B[a]	Number of roots				
		B0	B50	B100	B150	Mean
India126	T	4.0	4.0	4.0	4.0	4.0
Bonza	T	4.5	4.5	4.5	4.5	4.5
G61450	T	5.0	5.0	5.0	5.0	5.0
Benventuto Inca	T	4.0	3.5	4.0	3.5	3.8
Halberd	T	5.0	4.5	4.5	4.5	4.6
Schomburgk	MS	4.0	4.0	5.0	3.5	4.1
Kenya Farmer	VS	3.5	5.0	4.0	2.0	3.6
Mean		4.3	4.4	4.4	3.9	4.2

LSD(0.05) Varieties 0.5, Treatments 0.2, Interaction 1.0

[a] boron responses are quoted from Table 4.1

Table 4.5 Correlation coefficients between root length, shoot length and number of roots for seven wheat varieties tested in the filter paper technique at four boron treatments.

Statistic and treatments	Shoot length				Number of roots			
	B0	B50	B100	B150	B0	B50	B100	B150
Root length								
B0	0.66	0.55	0.23	0.15	-0.19	0.26	0.07	-0.20
B50	0.48	0.63	0.79*	0.87**	0.50	-0.43	0.04	0.79*
B100	0.48	0.61	0.79*	0.83*	0.47	-0.34	-0.10	0.74
B150	0.60	0.74	0.90**	0.90**	0.35	-0.30	-0.16	0.66
Shoot length								
B0					-0.05	-0.21	-0.33	-0.14
B50					0.08	-0.20	-0.14	0.30
B100					0.19	-0.21	-0.03	0.53
B150					0.27	-0.38	0.02	0.61

* significant at $P < 0.05$, ** significant at $P < 0.01$ for one tailed test.

4.3.2 Comparison between response of varieties in filter papers and high boron soil

Root length

There were highly significant effects for varieties and boron treatments and a significant interaction between boron treatments and varieties for growth of roots (Table 4.6). The results indicate that the response of varieties to boron varied between different boron treatments, a finding consistent with the first experiment.

Higher boron treatments were required to discriminate between tolerant and moderately tolerant varieties than between moderately tolerant and moderately sensitive varieties. For example, at B50, root lengths of Halberd and BT-Schomburgk were significantly longer than those of Mokoan, Condor and (W1xMMC), but not significantly different from the tolerant varieties G61450, Benventuto Inca, Lin Calel and AUS 4041, whereas at B100, the roots of Halberd were significantly shorter than those of all the tolerant varieties. At B150, there was significant variation in root length among the tolerant varieties. There were highly significant relationships ($P < 0.01$) between root lengths at different boron treatments with the correlations between B50 and B100 and B150 and between B100 and B150 being r = 0.92, 0.92 and 0.97 ($P < 0.01$), respectively (Figure 4.2) and again these were consistent with the results from the first experiment.

The relative root lengths at B100/B50 and B150/B50 of all of the tolerant varieties were greater than those of the moderately tolerant varieties (Table 4.6). At B150/B50, Halberd had a greater relative root length than BT-Schomburgk, Mokoan and Schomburgk, but was not much different from Condor and (W1xMMC), and there was little variation in relative root length among BT-Schomburgk, Mokoan and Schomburgk. Whereas at B100/B50, the relative root lengths of Halberd, BT-Schomburgk and (W1xMMC) were similar and greater than those of Mokoan, Condor and Schomburgk. There was variation in relative root lengths among the tolerant varieties. At B150/B50, AUS 4041, and Klein Granador had greater relative root length than India 126; these three varieties had the greatest relative root lengths overall. AUS 4903, Lin Calel, Benventuto Inca, Turkey 1473 and G61450 differed little in response to boron. At B100/B50, the relative root length of G1450 was similar to AUS 4041, Klein Granador and India 126.

Table 4.6 Mean length of the longest root of 15 seedlings and relative root length of 14 wheat varieties tested in the filter paper technique at three boron treatments.

Variety	B[a]	Root length (cm)				RRL[b] (%)	
		B50	B100	B150	Mean	B100/B50[c]	B150/B50[d]
India 126	T	13.9	12.5	8.6	11.7	90	62
Klein Granador	T	12.7	11.8	8.9	11.1	93	70
AUS 4903	T	13.5	11.3	7.7	10.8	84	57
AUS 4041	T	11.1	10.8	7.9	9.9	97	71
Lin Calel	T	11.6	9.9	6.2	9.2	85	53
Benventuto Inca	T	11.5	9.2	6.3	9.0	80	55
Turkey 1473	T	13.2	8.8	7.4	9.8	67	56
G 61450	T	9.6	8.7	5.3	7.9	91	55
Halberd	MT	10.6	6.7	4.5	7.3	63	42
BT-Schomburgk	MT	9.8	6.4	3.2	6.5	65	33
Mokoan	MS	8.2	4.1	2.5	4.9	50	30
Condor	MS	6.1	3.1	2.3	3.9	51	38
Schomburgk	MS	7.4	3.9	2.6	4.7	53	35
(W1xMMC)	S	6.5	4.1	2.5	4.4	63	38
Mean		10.4	7.9	5.4	7.9		

LSD(0.05) Varieties 0.9, Treatments 1.6, Interaction 1.5

[a] Boron response,

[b] RRL = relative root length,

[c] (root length at B100/root length at B50) x 100,

[d] (root length at B150/root length at B50) x 100.

Figure 4.2 Relationships between seedling root length of 14 wheat varieties tested in the filter paper technique at three boron treatments.

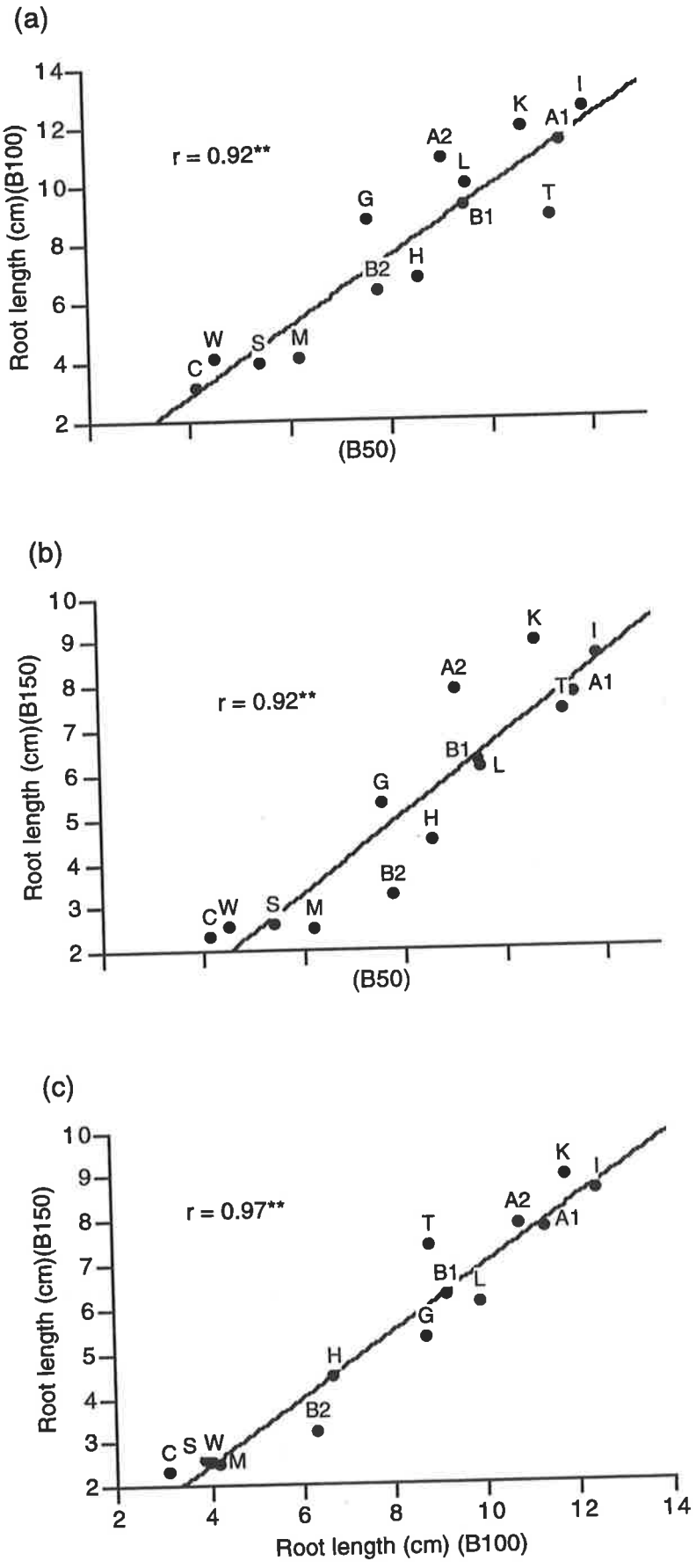
(a) B50 v B100

(b) B50 v B150

(c) B100 v B150

Note: the name of each variety is represented by a capital letter; A_1 = AUS 4903, A_2 = AUS 4041, B_1 = Benventuto Inca, B_2 = BT-Schomburgk, C = Condor, G = G61450, H = Halberd, I = India 126, K = Klein Granador, L = Lin Calel, M = Mokoan, S = Schomburgk, T = Turkey 1473, W = (W1 x MMC),

** significant at $P < 0.01$


(a)
Root length (cm)(B100)
r = 0.92**
(B50)
C
W
S
M
G
A2
L
K
A1
I
T
B1
B2
H
(b)
Root length (cm)(B150)
r = 0.92**
(B50)
(c)
Root length (cm)(B150)
r = 0.97**
Root length (cm) (B100)

The ranking of the varieties India 126, G61450, Benventuto Inca, Halberd and Schomburgk in this experiment (Table 4.6) was consistent with that of the first experiment (Table 4.2).

Boron concentrations in shoots

There were highly significant effects of varieties and boron treatments, while the interaction between boron treatments and varieties was highly significant ($P < 0.01$) for concentrations of boron in shoots of plants grown in the high boron soil (Table 4.7). Concentrations were lower for the more tolerant than the sensitive genotypes.

At B10, there was no significant difference between the moderately sensitive, moderately tolerant and tolerant varieties. However among the tolerant varieties, the concentration of boron in shoots of AUS 4041 was the lowest while Klein Granador was the highest (Table 4.7). The concentration of boron in the shoots of the sensitive line (W1xMMC) was significantly higher than the moderately sensitive Condor, Schomburgk and Mokoan (Table 4.7). At B20, the concentrations of boron in shoots of Halberd and BT-Schomburgk were significantly higher than those of the tolerant varieties G61450, AUS 4041, Benventuto Inca, Turkey 1473 and India 126. These results, which were consistent with those for root length in filter papers (Table 4.6), indicate that the higher boron treatment maximized the variation among the more tolerant genotypes. There was no significant variation in concentrations of boron in shoots among the tolerant varieties, with the exception of Lin Calel and Klein Granador which contained significantly higher concentrations of boron in shoots (comparable to Halberd) at the B20 treatment. This was not consistent with the data from the root length experiment (Table 4.6) and the report of Moody et al. (1988) which indicated that these two varieties were more tolerant than Halberd.

Shoot dry weight

There was significant variation in shoot dry weight among varieties at each level of boron and shoot dry weight was greater for the more tolerant genotypes (Table 4.7). At

Table 4.7 Boron concentrations in shoots, shoot dry weight and boron toxicity symptoms for 14 varieties compared under high boron conditions in soil.

Variety	B[a]	B in shoot (mg kg^{-1})		Shoot dry weight (gm)		B symptom [b]	
		B10	B20	B10	B20	B10	B20
India 126	T	118.0	312.8	2.1	1.7	1.2 (6.2)	1.3 (6.5)
K. Granador	T	203.5	553.2	1.8	1.3	1.6 (7.2)	1.6 (7.2)
AUS 4903	T	152.6	368.6	1.7	1.0	1.5 (7.0)	1.5 (7.0)
AUS 4041	T	102.2	245.9	2.3	1.7	1.2 (6.2)	1.3 (6.5)
Lin Calel	T	131.1	491.9	2.7	1.9	1.1 (6.0)	1.9 (7.9)
Benventuto Inca	T	164.0	269.3	2.3	1.3	1.4 (6.8)	1.5 (7.0)
Turkey 1473	T	113.6	293.0	2.1	1.1	1.0 (5.7)	1.8 (7.7)
G61450	T	145.3	285.0	2.6	1.7	1.2 (6.2)	1.6 (7.2)
Halberd	MT	162.7	488.9	2.1	1.4	1.1 (6.0)	1.5 (7.0)
BT-Schomburgk	MT	121.9	413.8	1.1	0.7	1.2 (6.2)	1.9 (7.8)
Mokoan	MS	182.9	680.8	1.6	0.8	1.6 (7.2)	2.1 (8.3)
Schomburgk	MS	160.5	577.2	1.5	0.7	1.4 (6.8)	1.9 (7.9)
Condor	MS	179.0	600.0	1.2	0.6	1.6 (7.2)	2.4 (8.9)
(W1xMMC)	S	299.4	796.2	1.3	0.4	1.8 (7.6)	3.0(10.0)
LSD ($P < 0.05$)		97.9		0.4		0.3 (0.8)	

[a] Boron responses are quoted from Table 3.1.

[b] Symptoms were scored from 1 (no symptom) to 5 (severe symptoms) and the significance value refers to arc sine transformed data, presented in brackets, for the B10 and B20 treatments.

Visual rating	Description of damage for youngest expanded leaf
1	no visual symptoms
2	tip necrosis (1cm)
3	1/4 leaf blade severe chlorosis with > 1 cm tip necrosis
4	1/2 leaf blade necrosis
5	leaf dead

Adapted from Kluge and Podlesak (1985)

B10, the shoot dry weight of Halberd was significantly greater than that of Schomburgk, Condor, Mokoan and (W1xMMC). However, there was little difference between the moderately tolerant and tolerant varieties at both the B10 and B20 treatments. Shoot dry weights of BT-Schomburgk were significantly lower than those of Halberd at the two boron treatments. The unexpected low shoot dry weight of BT-Schomburgk might have occurred because of poor germination and uneven growth of seeds of this variety.

Symptoms of boron toxicity

Symptoms of boron toxicity of wheat have been described as chlorotic and necrotic lesions developing from the tips of the leaves, along the margins, towards the mid-rib and base (Paull et al., 1988b). There were highly significant effects for varieties and boron treatments and the interaction between boron treatments and varieties was also highly significant ($P < 0.01$) (Table 4.7). At both levels of boron, symptoms of toxicity for Halberd were significantly less than those for Schomburgk, Condor, Mokoan and (W1xMMC), but similar to almost all of the tolerant varieties. However at B20, the most severe symptoms occurred in (W1xMMC) and these were significantly different from the other varieties. The least symptoms were observed on AUS 4041 and India 126.

Correlations between root length, symptoms of toxicity and shoot dry weight

There were highly significant correlations between the parameters in the pot experiment at B20, namely boron concentration in shoots, toxicity symptoms and shoot dry weight, and the length of roots at all levels of boron in the filter paper experiment (Figures 4.3, 4.4, 4.5). At B20 of the pot experiment, there were also highly significant correlations between the three parameters boron concentration in shoots, symptoms of boron toxicity and shoot dry weight (Figure 4.6).

There were also significant correlations between shoot dry weight in the pot experiment at B10 and the length of roots at all levels of boron in the filter paper experiment. At B10, there was a statistically significant relationship between boron concentration in shoots and the length of roots at B50 and B100 (Figures 4.3a, 4.4a) and

Figure 4.3 Relationships between seedling root length at B50 in the filter paper technique and boron concentration in shoots, symptoms of boron toxicity and shoot dry weight at B10 and B20 in soil for 14 wheat varieties.

(a) seedling root length (B50) v concentration of boron in shoots (B10).

(b) seedling root length (B50) v concentration of boron in shoots (B20).

(c) seedling root length (B50) v symptoms of boron toxicity (B10).

(d) seedling root length (B50) v symptoms of boron toxicity (B20).

(e) seedling root length (B50) v shoot dry weight (B10).

(f) seedling root length (B50) v shoot dry weight (B20).

Note: the name of each variety is represented by a capital letter; A_1 = AUS 4903, A_2 = AUS 4041, B_1 = Benventuto Inca, B_2 = BT-Schomburgk, C = Condor, G = G61450, H = Halberd, I = India 126, K = Klein Granador, L = Lin Calel, M = Mokoan, S = Schomburgk, T = Turkey 1473, W = (W1 x MMC),

*, ** significant at $P < 0.05$ and 0.01, respectively, of one tailed test.

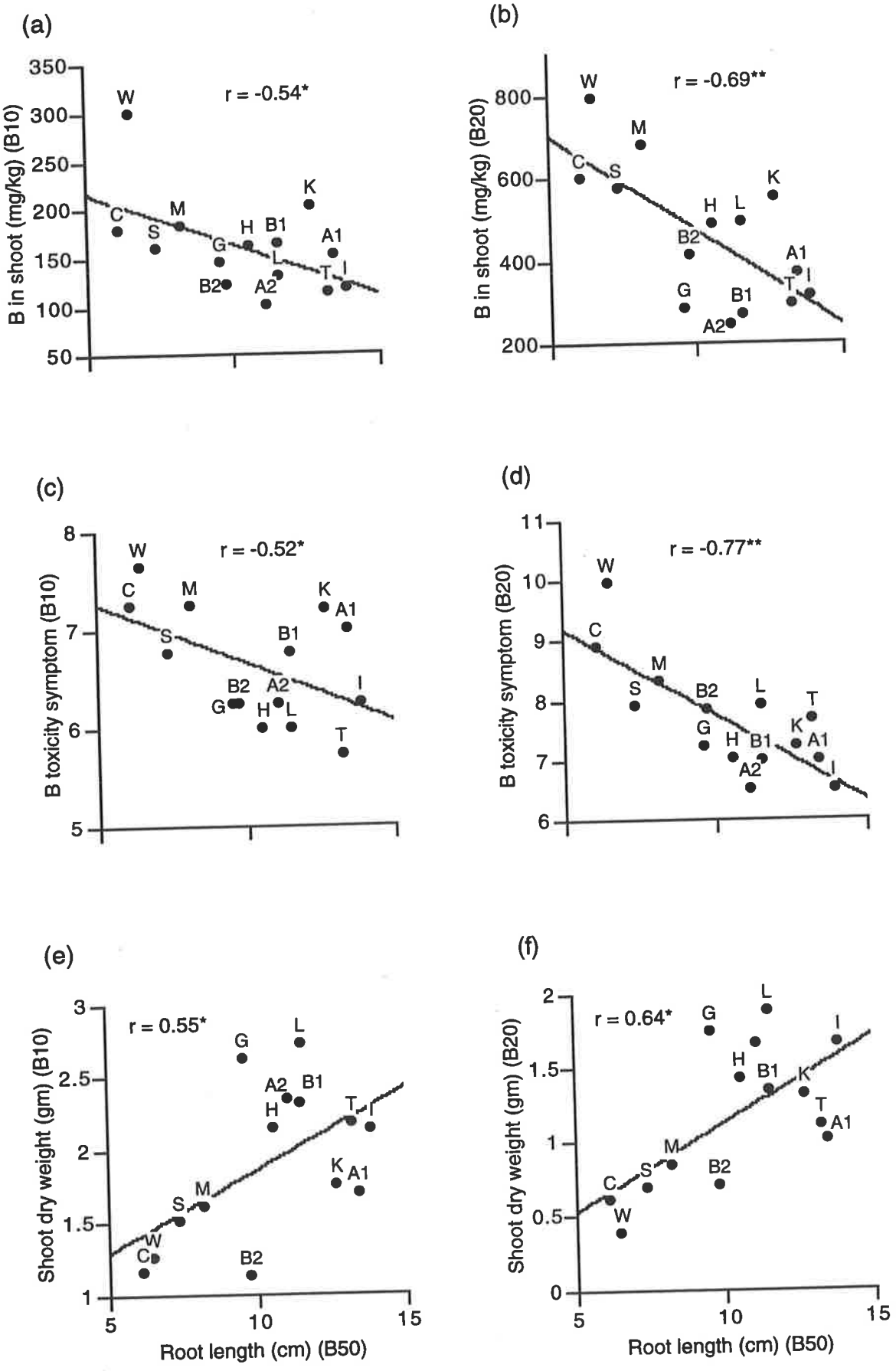

(a)
B in shoot (mg/kg) (B10)
r = -0.54*
(b)
B in shoot (mg/kg) (B20)
r = -0.69**
(c)
B toxicity symptom (B10)
r = -0.52*
(d)
B toxicity symptom (B20)
r = -0.77**
(e)
Shoot dry weight (gm) (B10)
r = 0.55*
Root length (cm) (B50)
(f)
Shoot dry weight (gm) (B20)
r = 0.64*
Root length (cm) (B50)

Figure 4.4 Relationships between seedling root length at B100 in the filter paper technique and boron concentration in shoots, symptoms of boron toxicity and shoot dry weight at B10 and B20 in soil for 14 wheat varieties.

(a) seedling root length (B100) v concentration of boron in shoots (B10).

(b) seedling root length (B100) v concentration of boron in shoots (B20).

(c) seedling root length (B100) v symptoms of boron toxicity (B10).

(d) seedling root length (B100) v symptoms of boron toxicity (B20).

(e) seedling root length (B100) v shoot dry weight (B10).

(f) seedling root length (B100) v shoot dry weight (B20).

Note: the name of each variety is represented by a capital letter; A_1 = AUS 4903, A_2 = AUS 4041, B_1 = Benventuto Inca, B_2 = BT-Schomburgk, C = Condor, G = G61450, H = Halberd, I = India 126, K = Klein Granador, L = Lin Calel, M = Mokoan, S = Schomburgk, T = Turkey 1473, W = (W1 x MMC),

*, ** significant at $P < 0.05$ and 0.01, respectively, of one tailed test.

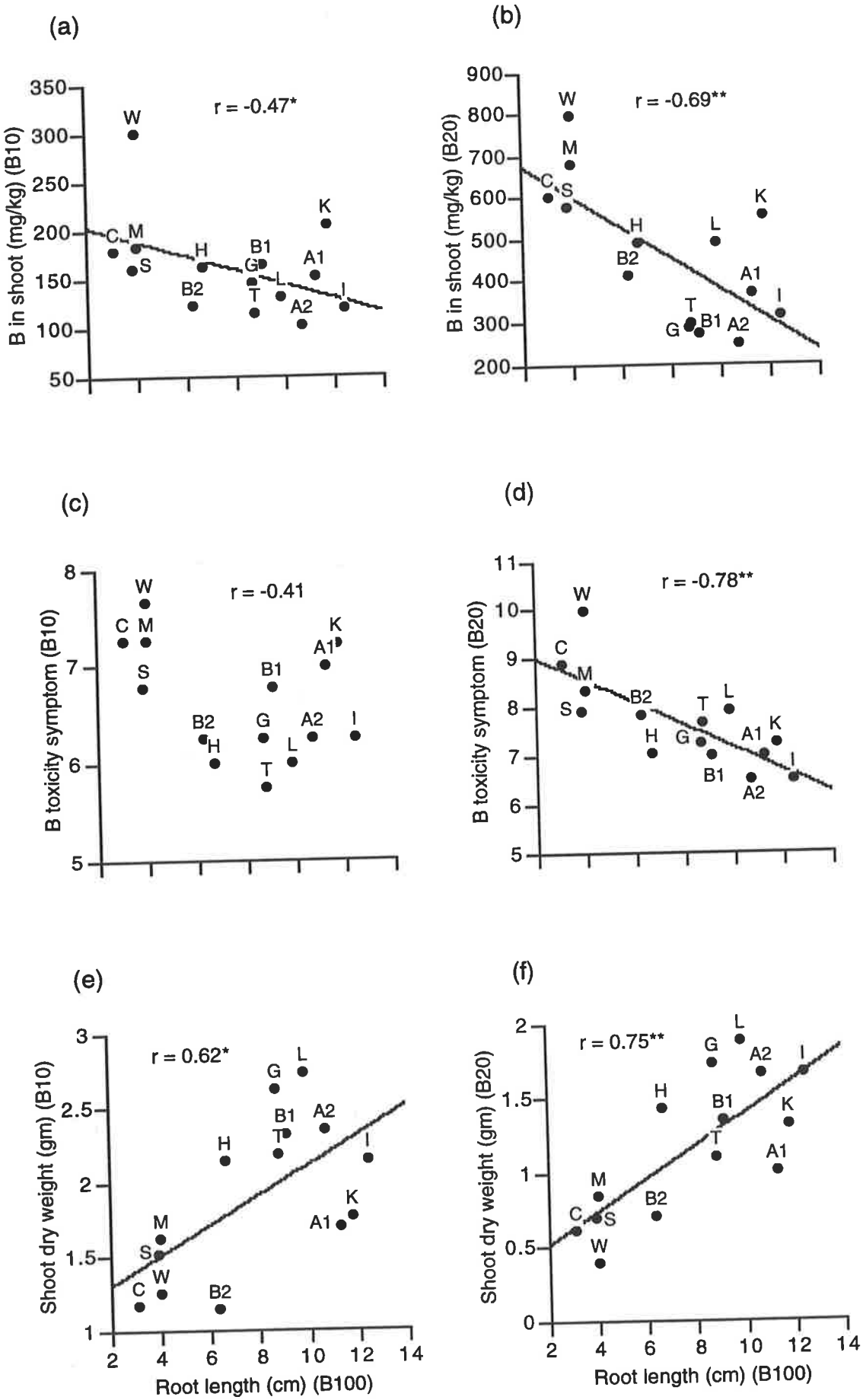

(a)
r = -0.47*
B in shoot (mg/kg) (B10)
(b)
r = -0.69**
B in shoot (mg/kg) (B20)
(c)
r = -0.41
B toxicity symptom (B10)
(d)
r = -0.78**
B toxicity symptom (B20)
(e)
r = 0.62*
Shoot dry weight (gm) (B10)
Root length (cm) (B100)
(f)
r = 0.75**
Shoot dry weight (gm) (B20)
Root length (cm) (B100)

Figure 4.5 Relationships between seedling root length at B150 in the filter paper technique and boron concentration in shoots, symptoms of boron toxicity and shoot dry weight at B10 and B20 in soil for 14 wheat varieties.

(a) seedling root length (B150) v concentration of boron in shoots (B10).

(b) seedling root length (B150) v concentration of boron in shoots (B20).

(c) seedling root length (B150) v symptoms of boron toxicity (B10).

(d) seedling root length (B150) v symptoms of boron toxicity (B20).

(e) seedling root length (B150) v shoot dry weight (B10).

(f) seedling root length (B150) v shoot dry weight (B20).

Note: the name of each variety is represented by a capital letter; A_1 = AUS 4903, A_2 = AUS 4041, B_1 = Benventuto Inca, B_2 = BT-Schomburgk, C = Condor, G = G61450, H = Halberd, I = India 126, K = Klein Granador, L = Lin Calel, M = Mokoan, S = Schomburgk, T = Turkey 1473, W = (W1 x MMC),

*, ** significant at $P < 0.05$ and 0.01, respectively, of one tailed test.

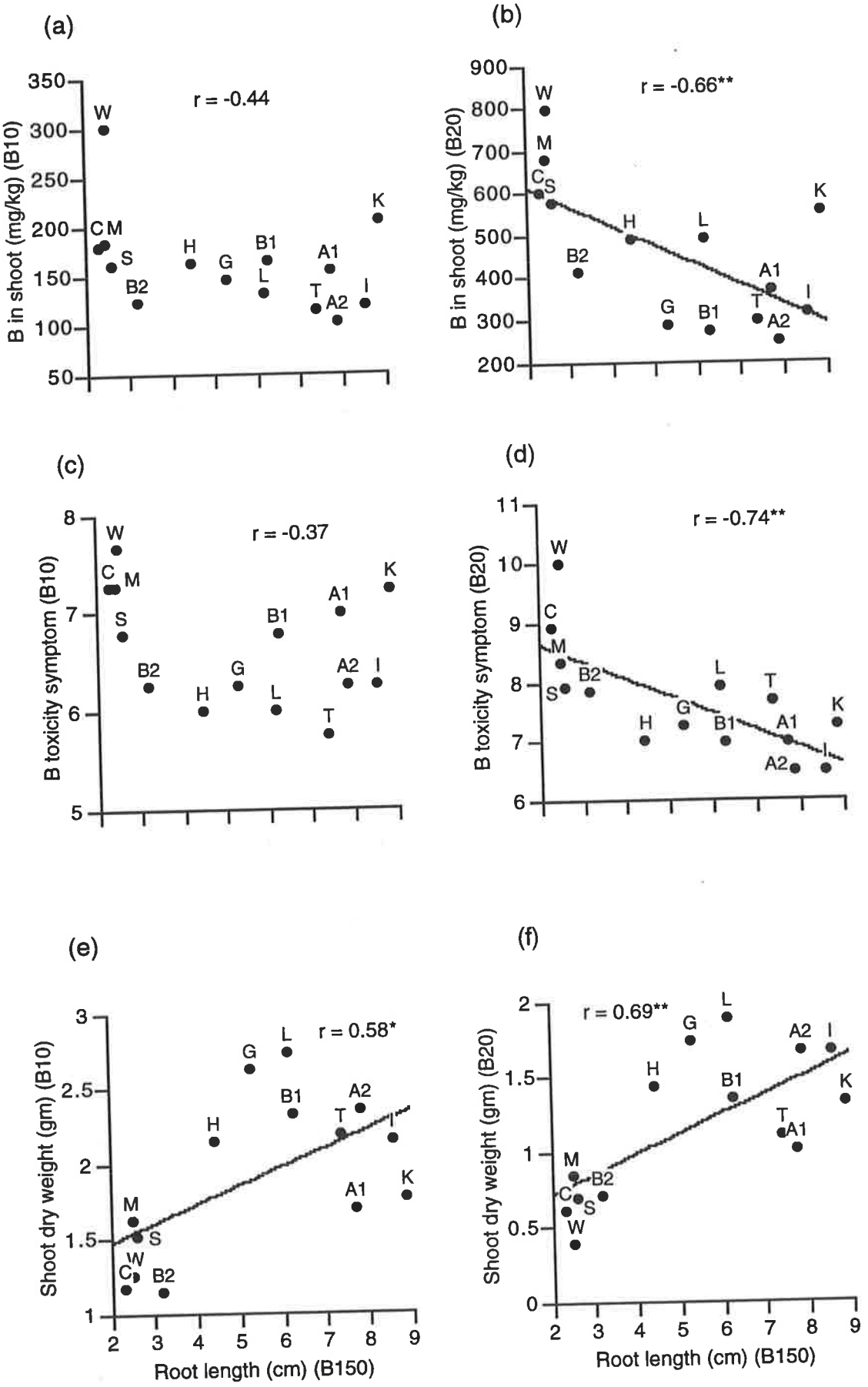

(a)
r = -0.44
B in shoot (mg/kg) (B10)
(b)
r = -0.66**
B in shoot (mg/kg) (B20)
(c)
r = -0.37
B toxicity symptom (B10)
(d)
r = -0.74**
B toxicity symptom (B20)
(e)
r = 0.58*
Shoot dry weight (gm) (B10)
Root length (cm) (B150)
(f)
r = 0.69**
Shoot dry weight (gm) (B20)
Root length (cm) (B150)

Figure 4.6 Relationships between boron concentration in shoots, symptoms of boron toxicity and shoot dry weight for 14 wheat varieties grown at two levels of soil boron.

(a) concentration of boron in shoots (B10) v shoot dry weight (B10).

(b) concentration of boron in shoots (B10) v symptoms of boron toxicity (B10).

(c) symptoms of boron toxicity (B10) v shoot dry weight (B10).

(d) concentration of boron in shoots (B20) v symptoms of boron toxicity (B20).

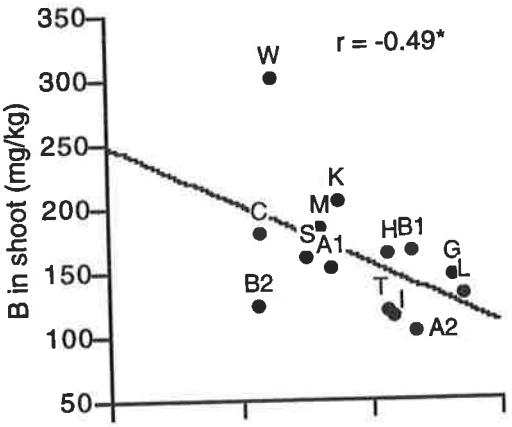
(e) concentration of boron in shoots (B20) v shoot dry weight (B20).

(f) shoot dry weight (B20) v symptoms of boron toxicity (B20).

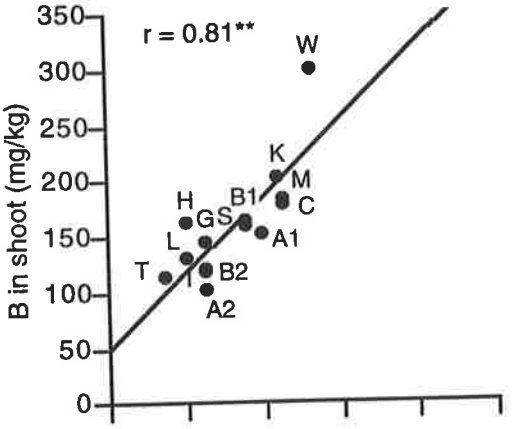
Note: the name of each variety is represented by a capital letter; A_1 = AUS 4903, A_2 = AUS 4041, B_1 = Benventuto Inca, B_2 = BT-Schomburgk, C = Condor, G = G61450, H = Halberd, I = India 126, K = Klein Granador, L = Lin Calel, M = Mokoan, S = Schomburgk, T = Turkey 1473, W = (W1 x MMC),

*, ** significant at $P < 0.05$ and 0.01, respectively, of one tailed test.

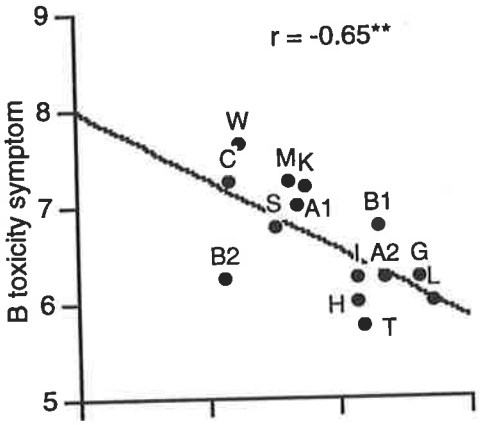
(a) B10



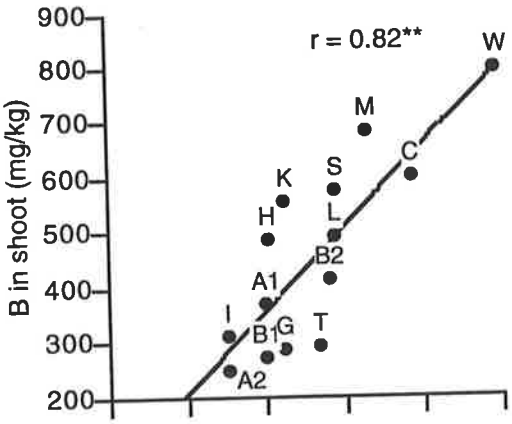
(b) B10



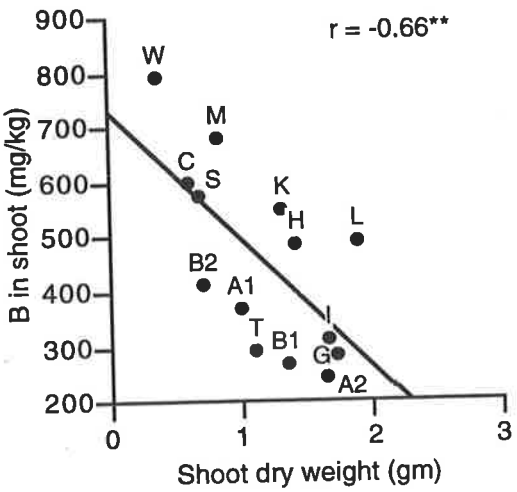
(c) B10



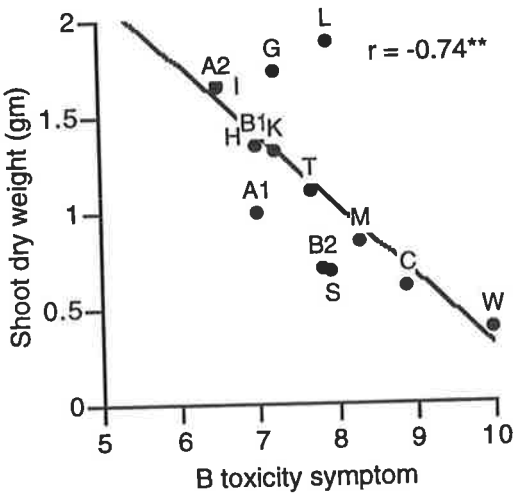
(d) B20



(e) B20



(f) B20



between toxicity symptoms and the length of roots at B50 (Figure 4.3c). However, there was no significant correlation between boron concentration in shoots at B10 and root lengths at B150 (Figure 4.5a) and between toxicity symptoms and root lengths at B100 (Figure 4.4c) and B150 (Figure 4.5c). The probable reason for this was the small variation in shoot boron concentration and toxicity symptoms among the varieties at B10.

At B10 of the pot experiment, the correlation between toxicity symptoms and boron concentration in shoots (Figure 4.6b) and shoot dry weight (Figure 4.6c) were highly significant, and a significant correlation was also observed between shoot dry weight and boron concentration in shoots (Figure 4.6a).

4.4 Discussion

There was a broad agreement in respect to boron response of varieties between the results of the first filter paper experiment reported here and those of the previous research by Moody et al. (1988) and Paull et al. (1991b). The ranking of the tested varieties on the basis of tolerance to boron (Table 4.2) was consistent with the results of Moody et al. (1988) and Paull et al. (1991b).

As the correlations between the root length at B0 and the three boron treatments were non-significant (Figure 4.1), the differences in root length at the high boron treatments could not be attributed to inherent variation in root growth among the varieties. The highly significant relationships between root lengths at the three boron treatments (Figure 4.1) indicate the consistency of the seedling root length in response to high boron conditions. The highly significant interaction between varieties and boron treatments in the first experiment (Table 4.2) indicates that genetic variation for boron tolerance can be tested by the filter paper technique.

Appropriate levels of boron must be applied when comparing a number of genotypes of different tolerance. If the concentration is too low, there will be no discrimination due to other factors overriding the boron response, conversely the plants will die if the concentration is too high. Paull (1990) showed that for five wheat genotypes tested for response to boron in soil, lower concentrations of boron are required for

maximum discrimination between sensitive genotypes than when comparing between the more tolerant genotypes. The most appropriate treatments for identifying differences in root length, between moderately sensitive and sensitive genotypes, would appear to be B50 because at higher concentrations (B100 and B150) the length of the roots for both types of genotypes were similar (Table 4.6). For example, at B50, the moderately sensitive variety Mokoan was more tolerant to boron than the sensitive variety (W1xMMC), whereas at B100 and B150, these two varieties were similar in response (Table 4.6). However, both B50 and B100 are appropriate for comparing between moderately sensitive and moderately tolerant genotypes, but at B150, there was no significant difference between the two types of genotype. For example, at B50 and B100, there were significant differences in root length between the moderately tolerant variety BT-Schomburgk and the moderately sensitive varieties Mokoan, Condor and Schomburgk, while at B150, the root lengths of these varieties were similar. Significant variation in root length among the more tolerant genotypes was observed at both B100 and B150. For example, at B100 and B150, Klein Granador was more tolerant than Lin Calel and Benventuto Inca (Table 4.6)

The seedling root lengths at the three levels of boron in filter papers were highly significantly correlated with the three characters determined for plants grown in soil containing high levels of boron, namely, the concentrations of boron in the shoots, plant dry weight and symptoms of toxicity. This indicates that root length could be used as a selection criterion in a genetic study or breeding program for boron tolerance. There was a consistent ranking of the genotypes used in the filter paper experiments (Table 4.7) with those in previous reports (Moody et al., 1988). However, the seedling root length of the sensitive variety (W1xMMC) was not different from those of the moderately sensitive varieties Condor and Schomburgk at any of the treatments (B50, B100 and B150) (Table 4.6). In contrast, the concentration of boron in shoots of (W1xMMC) was significantly higher and the symptoms more severe than those of Condor and Schomburgk, indicating (W1xMMC) was more sensitive to boron than the latter two varieties.

The use of the filter paper technique as a screening method for tolerance to boron may be more appropriate than the use of concentration of boron in shoots and toxicity

symptoms of boron on leaves. Direct selection for the ability to produce long roots in the presence of boron might result in greater extraction of water and thus an increase in grain yield of the new varieties. As it is non-destructive, the filter paper technique can be used for selecting individuals in the segregating generations of a breeding program, whereas the determination of boron concentration can only also be applied to advanced generations and varieties grown under high boron conditions in the field.

Lower concentrations of boron in shoots and grain have consistently been found to be associated with boron tolerance for plants grown in soil or solution culture (Nable, 1988; Paull et al., 1988b; Rathjen et al., 1987), so the second experiment was conducted without a nil boron treatment for both soil and filter paper experiments. Although it might be argued that the interpretation of the dry matter data might be confounded to some extent by variation in dry matter production in the absence of boron, the ratio of yield at B20, relative to B10, can be calculated as an alternative. A low ratio would indicate a large decrease in yield and a sensitive response. The yields of varieties at B20, relative to B10 show that those varieties with lower ratios were indeed more sensitive than those with high dry weights (Table 4.7). For example, the relative yield (B20/B10) of the sensitive (W1xMMC) was 0.31 and the three moderately sensitive lines were approximately 0.50. All the other lines were greater than 0.50, with India 126 producing a relative yield of 0.81. In the filter paper experiment, B0 was omitted to maximise the number of genotype x treatment combinations that could be undertaken at one time, and because there was no relationship between root length in the absence and presence of boron in the first experiment. There was no evidence from these experiments that the omission of the B0 treatments was not justified.

The tolerant varieties can be divided into two groups namely highly tolerant and tolerant on the basis of the mean root length averaged over all treatments (B50, B100 and B150) (Table 4.6). The first group consisted of three varieties India 126, Klein Granador and AUS 4903, while AUS 4041, Lin Calel, Benventuto Inca, Turkey 1473 and G61450 belonged to the second group. The mean root lengths of all tolerant lines in all these treatments, except G61450 at B50, were longer than Halberd and BT-Schomburgk (Table

4.6). The means of the root length of moderately tolerant Halberd and BT-Schomburgk were significantly longer than those of the moderately sensitive Mokoan, Condor and Schomburgk, but the filter paper test was not able to discriminate between the moderately sensitive and the single variety in the sensitive group, (W1xMMC). Although the reason for this is unknown, it is interesting to note that Huang and Graham (1990) were not able to discriminate between moderately sensitive and sensitive genotypes when testing elongation of excised root tips on agar medium enriched with boron. However, the first filter paper experiment clearly discriminated between the very sensitive Kenya Farmer and Schomburgk (Table 4.2).

To differentiate between all 14 varieties included in the second experiment, a combination of the mean length of root averaged from all treatments (Table 4.6) and the concentration of boron in shoots at B20 (Table 4.7) was used. On this basis, India 126 was classified as the most tolerant and (W1xMMC) was the most sensitive although Kenya Farmer, which was only included in the first filter paper experiment would have been, almost certainly, more sensitive than (W1xMMC). There was some discrepancy in the ranking of lines between the filter paper and the pot experiments and this occurred in particular for Klein Granador and Lin Calel. There was no significant difference in the mean root length over all treatments between Klein Granador and AUS 4903, but the concentration of boron in AUS 4903 was lower than that of Klein Granador (Table 4.7). The high boron concentration observed in shoots of these two lines might indicate either a greater level of internal tolerance of boron in their shoots, compared to other lines, or it may be a consequence of genetically impure stocks or contamination of samples during analysis. Nevertheless, there was, in general, good agreement between the two experimental systems and the overall ranking of lines into the categories very tolerant, tolerant, moderately tolerant, moderately sensitive and sensitive.

Although there were significant relationships between root and shoot lengths at three levels of boron (B50, B100 and B150), shoot lengths and number of roots of different varieties under high boron conditions, these were not consistent with the levels of tolerance

of the varieties when grown in high boron soil, suggesting the two latter parameters are not appropriate for screening for boron tolerance.

The advantages of the filter paper technique

There are many advantages of using the filter paper technique as a method for screening for boron tolerance. These include the fact that it is rapid, non-destructive and produces an objective metric value that can be statistically analysed. The method is also reproducible as it is conducted in a controlled environment and not subject to seasonal variations in daylength, temperature regimes and precipitation, which influence glasshouse and field experiments. For instance, boron concentrations in plant tissues grown in a glasshouse are affected by day length and temperature (Ylaranta et al., 1979). Nable et al., (1990b) described considerable problems with using leaf or shoot analyses to diagnose boron toxicity in barley due to the patterns of boron distribution in vegetative tissue and the effects of differential transpiration rates and rain on accumulation of boron accumulation. Nable and Moody (1992) reported that precipitation resulted in a decrease in the boron concentration and content of whole shoots and young leaves of wheat harvested from a field trial conducted in a high boron soil. They concluded that foliar analyses are unreliable for diagnosing boron toxicity due to the change in boron concentration by rainfall. However, this does not preclude tissue analysis from testing for genetic variation in response to high concentrations of boron because even though the boron in shoots may be decreased by rain there is no evidence to suggest that the relative difference between varieties is altered.

The filter paper assay may be conducted in 15 days, including the initial period of seed imbibition which improves the uniformity of results. As the test is non-destructive, selected tolerant plants may be transplanted and used for seed multiplication or as parents in a crossing program.

Chapter 5

Determining the number of genes conferring tolerance to a high concentration of boron in G61450 and Halberd

5.1 Introduction

Boron tolerance of wheat is controlled by a series of partially dominant additive genes (Paull et al., 1991b). The *Bo1* allele, which confers tolerance to boron, was transferred from a moderately tolerant variety Halberd (full genotype *Bo1Bo1Bo2Bo2Bo3Bo3*) to a moderately sensitive variety Schomburgk (*bo1bo1Bo2Bo2Bo3Bo3*) to produce BT-Schomburgk. BT-Schomburgk has demonstrated a significant yield advantage over Schomburgk when grown under high boron conditions (Moody et al., 1993). It is possible that varieties more tolerant than BT-Schomburgk, and higher yielding in boron toxic conditions, could be bred by transferring other genes conferring boron tolerance from tolerant to moderately tolerant varieties using a backcrossing program.

Exotic germplasm more tolerant to boron than Halberd were identified at the Waite Institute (Moody et al., 1988). These lines offer the potential for increasing the level of boron tolerance in Australian varieties. Most of the tolerant exotic lines originated from Asia, Asia Minor and South America, whereas there was a low proportion of tolerant lines from regions in the more northerly latitudes (North America and North Europe) (Moody et al., 1988). The geographic diversity in origin between the exotic tolerant lines and Australian varieties indicates the possibility of different genes controlling tolerance to boron. Simple genetic control was observed within Australian materials, but transgressive segregation occurred between Halberd and a tolerant exotic line, G61450, that originated from Greece (Paull et al., 1991b). This suggested that there were at least two different genes controlling boron tolerance between the two genotypes. An understanding of the genetic control of tolerance to boron within these lines would increase the efficiency of

transferring the level of tolerance exhibited by G61450 to Halberd and other Australian varieties.

The objective of these experiments was to determine the genetic relationship, with respect to tolerance to boron, between Halberd and G61450. In this regard, the F_2 and F_3 progeny from crosses between G61450, Halberd and two inbred lines derived from transgressive segregants identified among the progeny of (G61450 x Halberd), were tested for segregation in response to boron. These four lines, and the Condor selection P44, the variety from which the monosomic series used in Chapter 6 was derived, were crossed to Schomburgk.

On the basis of the preliminary results of Paull (1990), it was hypothesized that the genetic relationships among the six populations are :

(a) one gene is responsible for the difference in boron tolerance between the tolerant line selected from (Halberd x G61450) and G61450,

(b) one gene is responsible for the difference between the tolerant line and Halberd. This gene is different to that in (a),

(c) the allele confirming greater sensitivity at the first locus (a) is responsible for the difference between the sensitive line selected from (Halberd x G61450) and G61450,

(d) the allele confirming greater sensitivity at the second locus (b) is responsible for the difference between the sensitive line and Halberd.

(e) two genes are responsible for the difference between the tolerant line and Schomburgk,

(f) no major gene difference exists between the sensitive line and Schomburgk,

(g) no major gene difference exists between Condor and Schomburgk.

5.2 Materials and methods

Plants in these experiments were tested for response to boron by measuring the root length of seedlings grown in filter paper saturated with a solution of boric acid, following the method described in Chapter 4.

Genetic materials

The pedigree and the response to boron for Halberd, G61450, Schomburgk and Condor are presented in Table 4.1 of Chapter 4.

Paull et al (1991b) selected four F_3 derived F_4 lines from the combination between G61450 and Halberd as boron tolerant and sensitive families. Concentrations of boron in shoots of the two tolerant lines, 418-3 (here designated 418T) and 426-2 (here designated 426T), were significantly less than G61450, whereas those of the two sensitive lines 414-2 (414S) and 442-1 (442S), were significantly greater than Halberd (Table 5.1). The selected families were used as genetic materials in the following experiments.

Boron response of parental lines

Five homozygous tolerant (418T-1, 418T-2, 418T-3, 426T-1, and 426T-2) and four homozygous sensitive (414S-1, 414S-2, 442S-1 and 442S-2) F_5 lines derived from single F_4 plants, were tested for boron tolerance and compared to G61450, Halberd and a moderately sensitive variety Schomburgk to select the most tolerant and sensitive lines as parents for genetic studies. Lines were tested in a solution of 100 mgB l^{-1}. The experimental design used was a randomized complete block design with two replications.

Boron response of segregating populations

F_2 populations

The F_2 generation of the cross between two selected lines, 418T-1 and 442S-1, and the crosses between both lines and the varieties G61450, Halberd and Schomburgk, and the F_2 of the cross between Schomburgk and a moderately sensitive Condor selection, were tested for segregation in response to boron.

The F_2 generation of each cross was also multiplied to the F_3 using approximately 100 random F_2 seeds per cross. The F_2 plants were harvested individually and progeny tested for tolerance to boron.

A total of approximately 132 F_2 seeds from each of the eight combinations were placed in filter papers with 11 F_2 seeds plus two seeds of each of their parents per paper.

Table 5.1 Concentration of boron (mg kg^{-1}) in whole shoots of F_4 plants, derived from tolerant and sensitive F_2 selections of (G61450 x Halberd), when grown in soil at B50 treatment. The concentrations of boron of lines within individual families were compared with Halberd (sensitive families) or G61450 (tolerant families), by an unpaired t-test. Derived from Paull et al. (1991b).

F_4 family	Mean B Conc (mg kg^{-1})[a]	t-test
Sensitive		
414-2	233	2.17*
407-1	209	1.40
425-1	208	0.97
442-1	248	2.25*
443-1	174	1.15
Tolerant		
400-1	128	0.03
418-3	107	2.39*
426-2	105	2.83**
410-1	119	1.35
436-3	122	0.54
G61450	128	
Halberd	191	

[a] boron concentration (mg kg^{-1}),

*, ** different from the parents at the 0.05 and 0.01 significance levels, respectively.

The distributions of the seedling root lengths of the F_2 populations were examined. When the distribution was bimodal or trimodal, the F_2 seedlings were classified into two or three categories using the cut-off points of the bimodal or trimodal classes to differentiate among the categories, as described in Chapter 3. Chi-square analysis was then used for testing the goodness of fit of the observed segregation ratios to the frequencies expected for monogenic or digenic segregation. If it was not possible to classify the F_2 seedling into categories due to a continuous distribution, the comparison between the observed and expected variances of each F_2 population was used for the estimation of the number of genes controlling tolerance to boron (Chapter 3). The expected variances were calculated from the following equations (described in Chapter 3) on the assumptions of no epistacy, no linkage and no dominance.

(a) 1 gene;

$$V_{F2} = 1/2D + E$$

(b) 2 genes;

$$V_{F2} = 1/2D + E$$

where d is the departure from the mid-point (m) of each homozygous genotype and E is the environmental variance ($E = 1/4\ V_{P1} + 1/4\ V_{P2} + 1/2\ V_{F1}$; V_{P1} and V_{P2} are the variances of the parents and V_{F1} is the variance of the F_1 hybrid between P_1 and P_2). Since the F_1 hybrids of the populations were not tested in this experiment, the variance of the F_1 was estimated from the average variance of the two parents ($V_{F1} = (V_{P1} + V_{P2})/2$) (see Chapter 3).

The expected variances were regarded as being significantly different from the observed variance when the expected variances were outside the boundaries set by the confidence interval of the observed variance, as described in Chapter 3.

F_2 derived F_3 populations

Progeny testing, using approximately 100 random F_2 derived F_3 families per cross, was conducted for all of the crosses. The objective in testing the F_3 generation was to distinguish between homozygous and heterozygous progenies which could not be

identified by testing the F_2 seeds per se. Twelve seeds of each F_2 derived family and twelve seeds of each parent of each cross were placed in separate filter papers. The methods (see Chapter 3) used for estimating the number of genes responsible for boron tolerance were :

(a) the classification of F_3 families into two categories (tolerant-segregating and homozygous sensitive) according to the cut-off point of the bimodal or trimodal distribution and the mean seedling root length of individual families,

(b) the classification of F_3 families into three categories (homozygous tolerant, segregating and homozygous sensitive) using the comparison between mean and variance of each F_3 family and that of the parents. A mean of the family outside the confidence interval of the mean of the parents (Chapter 3) or a variance greater than the LSD of the parental variances (Chapter 3) indicated that the family was heterogeneous.

(c) the comparison between the variance observed for individual populations and expected variances for alternative genetic models. The expected variances were calculated from the following equations, as described in Chapter 3.

For 1 gene; $V_{F3} = 3/4\ d^2 + E$

For 2 genes, $d_a = d_b = d/2$; $V_{F3} = 3/4\ d^2 + E$

The boron concentration used for all crosses was 100 mg l^{-1} with the exception of crosses between moderately sensitive genotypes (442S-1 x Schomburgk) and (Schomburgk x Condor) where the boron concentration was 50 mg l^{-1} (Section 4.2).

5.3 Results

Boron response of parental lines

The initial experiment showed significant differences between the tested lines. All of the five tolerant lines were significantly more tolerant to boron than the more tolerant parent, G61450, and three out of the four sensitive lines were significantly more sensitive than the more sensitive parent, Halberd, but not significantly different from Schomburgk. 418T-1 was the most tolerant line and the most sensitive lines were 442S-1, 414S-1 and

414S-2 (Figure 5.1 and Plate 5.1). 418T-1 and 442S-1 were selected as parents for the genetic studies.

Boron response of segregating populations

F_2 populations

(a) Populations that did not segregate.

There was no significant difference between 442S-1 and Schomburgk or between Condor and Schomburgk at B50 and all F_2 plants of the crosses (442S-1 x Schomburgk) and (Condor x Schomburgk) fell within the range of the parental standards (Figures 5.2a and 5.2b).

(b) Populations expected to be segregating at a single gene.

Since the distribution of the F_2 of (442S-1 x G61450) was trimodal (Figure 5.3a), the F_2 seedlings were classified into two categories (intermediate-tolerant and sensitive) and three categories (tolerant, intermediate and sensitive). The F_2 seedlings were classified as sensitive when the root length was less than or equal to 4 cm, whereas seedlings with a root length of more than 4 cm were classified as intermediate-tolerant. The result of chi-square analysis indicated that the segregation ratio of this cross was consistent with the monogenic ratio of 3 intermediate-tolerant : 1 sensitive. The intermediate and tolerant seedlings were further classified using 10 cm (Figure 5.3a) as the value to differentiate between the categories. Chi-square analysis indicated that the observed segregation ratio of this cross did not fit the expected 1 tolerant : 2 intermediate : 1 sensitive (Table 5.2).

Both of the expected variances for one and two gene models were not in the range of the confidence interval of the observed variance (Table 5.3). However, the observed variance was closer to the expected variance for a one gene than a two gene model, indicating the possibility of one gene controlling tolerance to boron for this cross.

Continuous distributions were observed in the F_2 populations of (442S-1 x Halberd) (Figure 5.3b), (418T-1 x G61450) (Figure 5.4a) and (418T-1 x Halberd) (Figure 5.4b). Thus, it was not possible to classify the F_2 seedlings into separate categories

Figure 5.1 Mean root lengths of seedlings of tolerant and sensitive F_4 derived F_5 families of (G61450 x Halberd), together with parents and the moderately sensitive Schomburgk, tested in filter papers at B100.

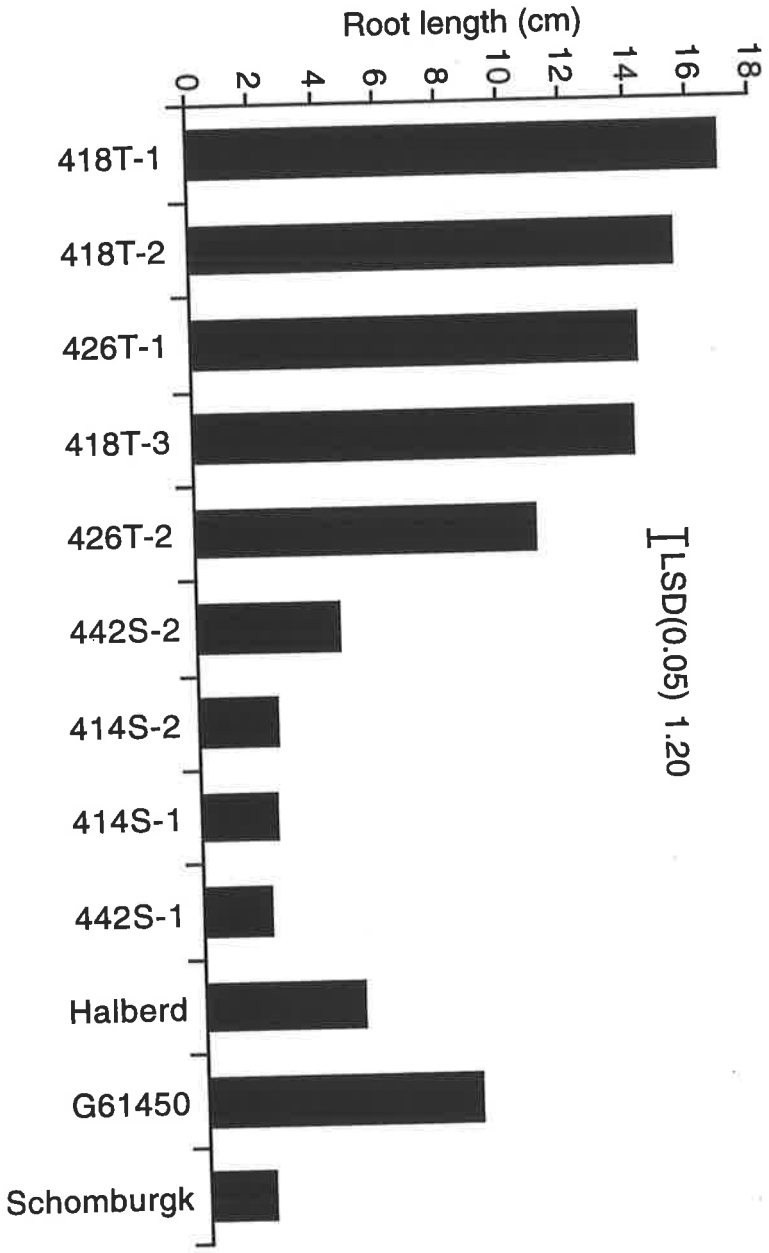
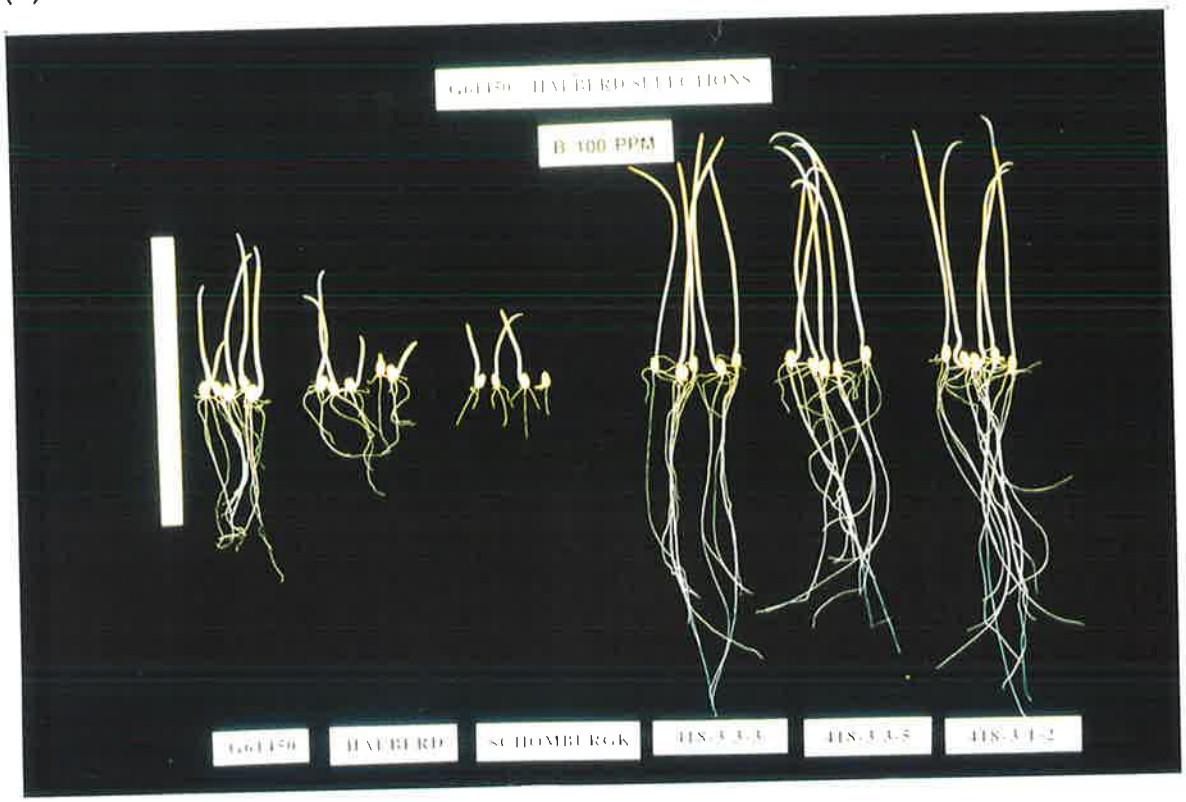

Root length (cm)
0
2
4
6
8
10
12
14
16
18
418T-1
418T-2
426T-1
418T-3
426T-2
442S-2
414S-2
414S-1
442S-1
Halberd
G61450
Schomburgk
LSD(0.05) 1.20

Plate 5.1 Response of F_5 lines of (G61450 x Halberd) in comparison with the two parents and Schomburgk when tested at B100.

(a) tolerant lines (the lines 418-3/1-2, 418-3/3-3 and 418-3/3-5 were the original selection numbers of 418T-1, 418T-2 and 418T-3, respectively)

(b) sensitive lines (the lines 442-1/2-1, 414-2/3-1 and 414-2/4-4 were the original selection numbers of 442S-1, 414S-1 and 414S-2, respectively)

(a)


B 100 PPM

(b)

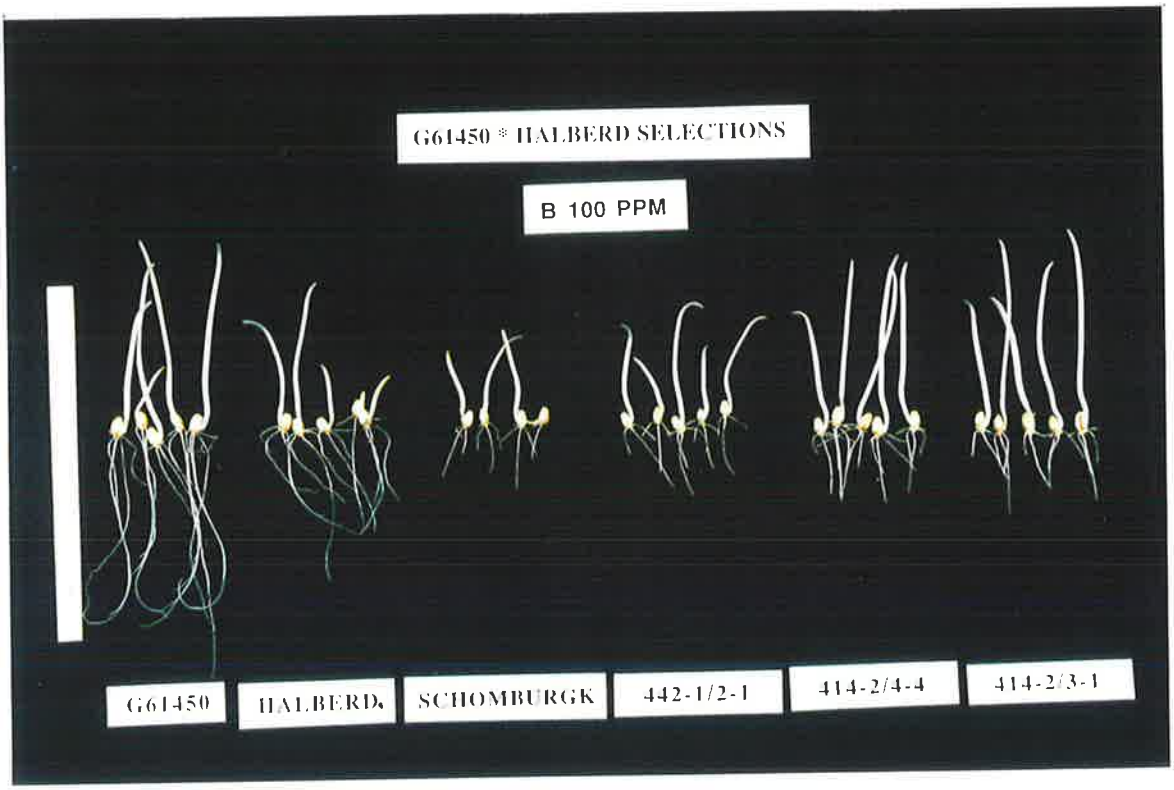

G61450 * HALBERD SELECTIONS
B 100 PPM
G61450
HALBERD
SCHOMBURGK
442-1/2-1
414-2/4-4
414-2/3-1

Figure 5.2 Root length of F_2 seedlings and parents, tested in filter papers at B50. (a) (442S-1 x Schomburgk), (b) (Condor x Schomburgk). Populations where no segregation was expected.

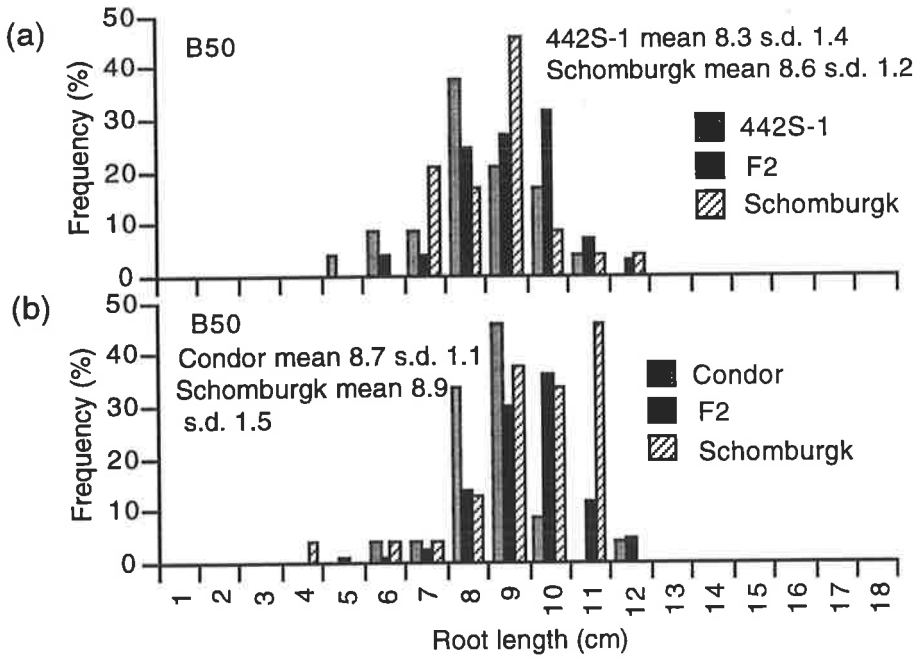


Figure 5.3 Root length of F_2 seedlings and parents, tested in filter papers at B100. (a) (442S-1 x G61450), (b) (442S-1 x Halberd). Populations where a single gene was expected to be segregating.

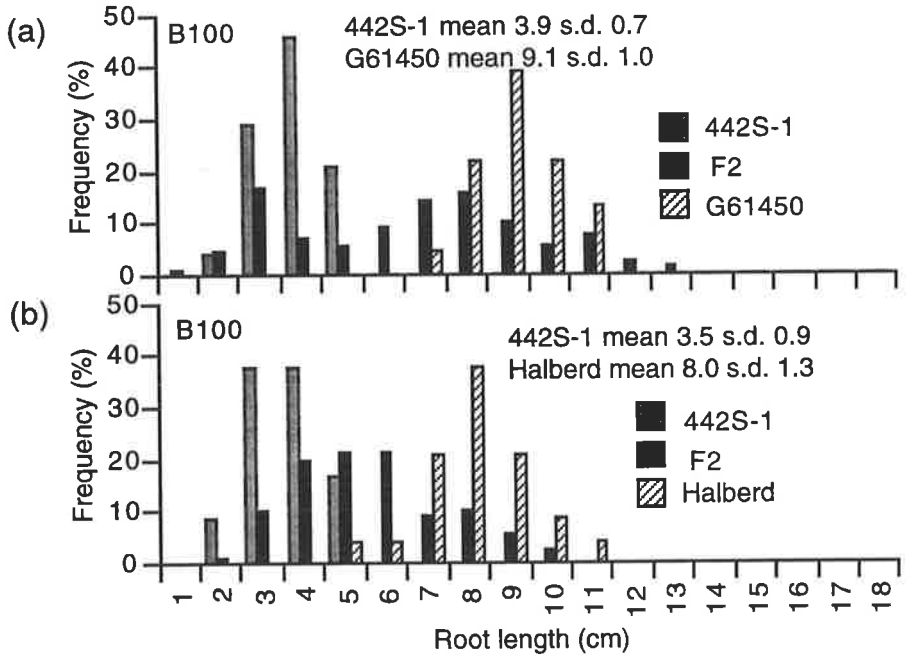


Figure 5.4 Root length of F_2 seedlings and parents, tested in filter papers at B100. (a) (418T-1 x G61450), (b) (418T-1 x Halberd). Populations where segregation was expected at a single locus.

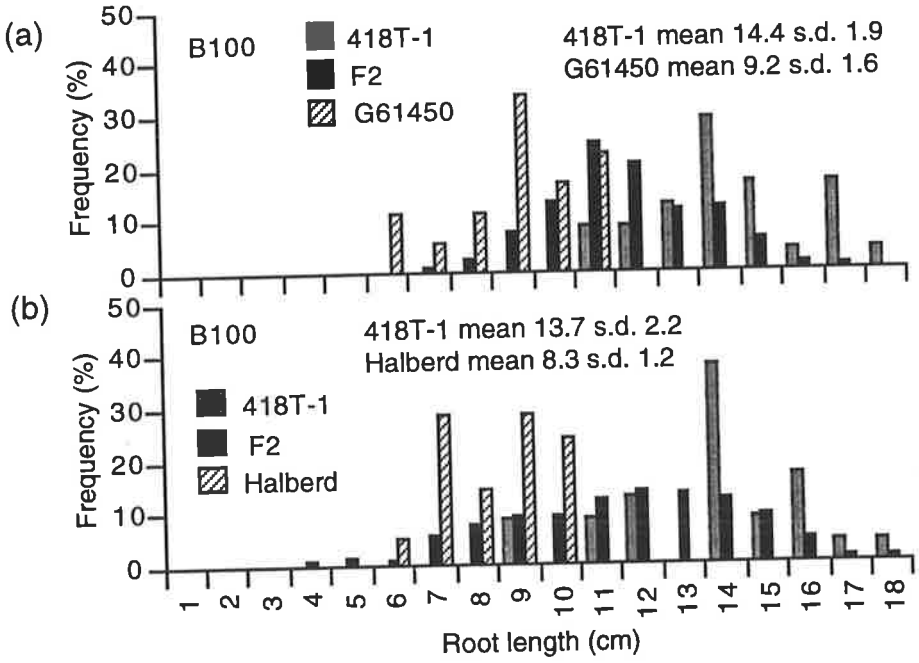


Figure 5.5 Root length of F_2 seedlings and parents, tested in filter papers at B100. (a) (418T-1 x Schomburgk), (b) (418T-1 x 442S-1). Populations where segregation was expected at two loci.

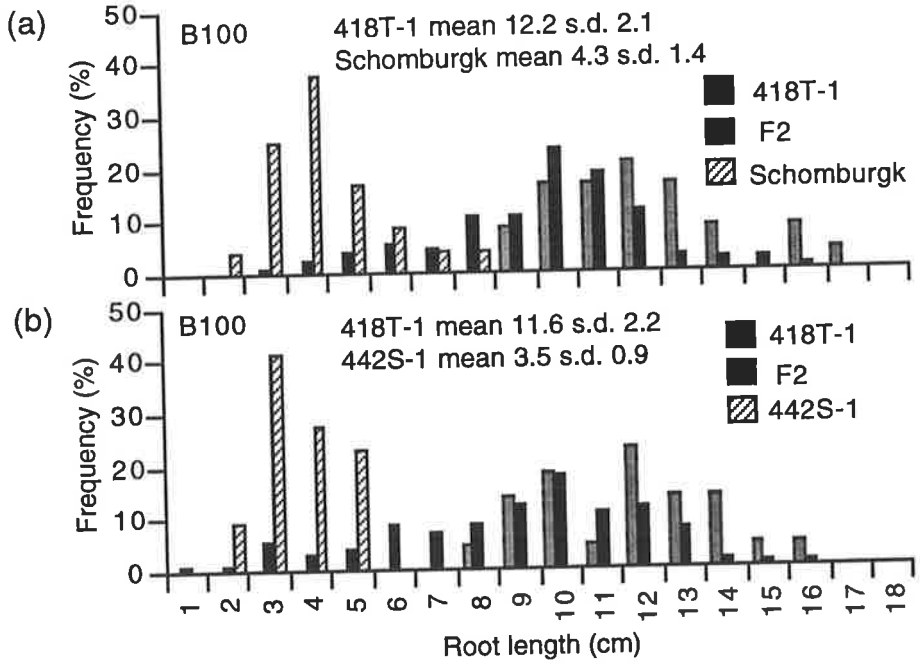


Table 5.2 Chi-square analysis of the observed and expected segregation ratios obtained for eight F_2 populations, tested in filter papers at B50 for (442S-1 x Schomburgk) and (Schomburgk x Condor) and B100 for the other populations.

Population (F_2)		Model	Frequency			
			Tol[a]+Int[b]	Sens[c]	χ^2_1	
(442S-1xSchomburgk)			No segregation observed			
(SchomburgkxCondor)			No segregation observed			
(442S-1xG61450)	Obs[d]		96	36		
	Exp[e]	3 : 1	99	33	0.36	
			Tol	Int	Sens	χ^2_2
(442S-1xG61450)	Obs		18	78	36	
	Exp	1 : 2 : 1	33	66	33	9.27
(442S-1xHalberd)			Continuous distribution			
(418T-1xG61450)			Continuous distribution			
(418T-1xHalberd)			Continuous distribution			
(418T-1xSchomburgk)			Continuous distribution			
(418T-1x442S-1)			Continuous distribution			

Probability (P) of chi-square at 1 and 2 degrees of freedom.

P	0.50	0.25	0.05	0.01
χ^2_1	0.45	1.32	3.84	6.63
χ^2_2	1.39	2.77	5.99	9.21

[a] Tolerant, [b] Intermediate, [c] Sensitive, [d] Observed value, [e] Expected value

Table 5.3 Comparisons between expected and observed variances of six F_2 populations when tested under high boron conditions.

Parents and F_2	d [a]	E [b]	Expected variance[c]		Observed variance	Confidence interval[d]
			1 gene	2 genes		
<u>One gene hypothesis</u>						
442S-1					0.6	
(442S-1 x G61450)	2.6	0.8	4.2	2.5	8.2	10.6-6.5
G61450					1.0	
442S-1					0.9	
(442S-1 x Halberd)	2.2	1.4	3.8	2.6	3.1	4.0-2.5
Halberd					1.8	
418T-1					3.6	
(418T-1 x G61450)	2.6	3.1	6.5	4.8	3.6	4.7-2.9
G61450					2.5	
418T-1					5.0	
(418T-1 x Halberd)	2.7	3.3	6.9	5.1	7.7	10.0-6.1
Halberd					1.5	
<u>Two genes hypothesis</u>						
418T-1					4.5	
(418T-1 x Schomburgk)	4.0	3.2	11.2	7.2	6.0	7.8-4.8
Schomburgk					1.9	
418T-1					4.8	
(418T-1 x 442S-1)	4.1	2.8	11.2	7.0	9.3	12.0-7.4
442S-1					0.8	

[a] d = the departure of one of a pair of corresponding homozygotes from their mid-point,
[b] E = environmental variance = 1/2 P_1 + 1/2 P_2 (Chapter 3),
[c] the expected variances were calculated on the assumption of no dominance, no linkage and no epistacy,
[d] confidence interval of observed variance at $P = 0.95$.

(Chapter 3). The comparison between the observed variance and the variances expected for one and two gene models indicated that the observed variance for (418T-1 x Halberd) was similar to the variance expected for segregation at a single gene (Table 5.3), and this agreed with the hypothesis for this cross. In contrast to the hypothesis, the observed variance of (418T-1 x G61450) was similar to the expected variance for two genes (Table 5.3). The observed variance of (442S-1 x Halberd) was not significantly different to either of the variances expected for one and two gene models (Table 5.3).

(c) Populations expected to be segregating at two genes.

Classification of the F_2 seedlings of (418T-1 x Schomburgk) and (418T-1 x 442S-1) into categories was not possible due to the distributions being continuous (Figures 5.5a and 5.5b), so comparisons between the observed and expected variances of these populations were used for estimation of the number of genes controlling tolerance to boron. The observed variance of (418T-1 x Schomburgk) was similar to that expected for two genes (Table 5.3). However, the observed variance of (418T-1 x 442S-1) was comparable with the expected variance for the one gene model (Table 5.3) and not in agreement with the hypothesis.

F_2 derived F_3 populations

(a) Population that did not segregate.

The testing of F_2 derived F_3 families of (442S-1 x Schomburgk) (Figure 5.6a) and (Condor x Schomburgk) (Figure 5.6b) were consistent with those of the F_2 populations (Figures 5.2a and 5.2b). All F_3 families of the two crosses fell within the range of the parental standards (Figures 5.6a and 5.6b). Also, there was no significant difference between 442S-1 and Schomburgk (Figures 5.2a and 5.6a) or between Condor and Schomburgk (Figures 5.2b and 5.6b) at B50. It was therefore concluded that the genetic control of response to boron is the same for Schomburgk, Condor and 442S-1.

Figure 5.6 Mean root length of F_3 families and parents (12-15 seedlings for each family and parent), tested in filter papers at B50. (a) (442S-1 x Schomburgk), (b) (Condor x Schomburgk). Populations where no segregation was expected.

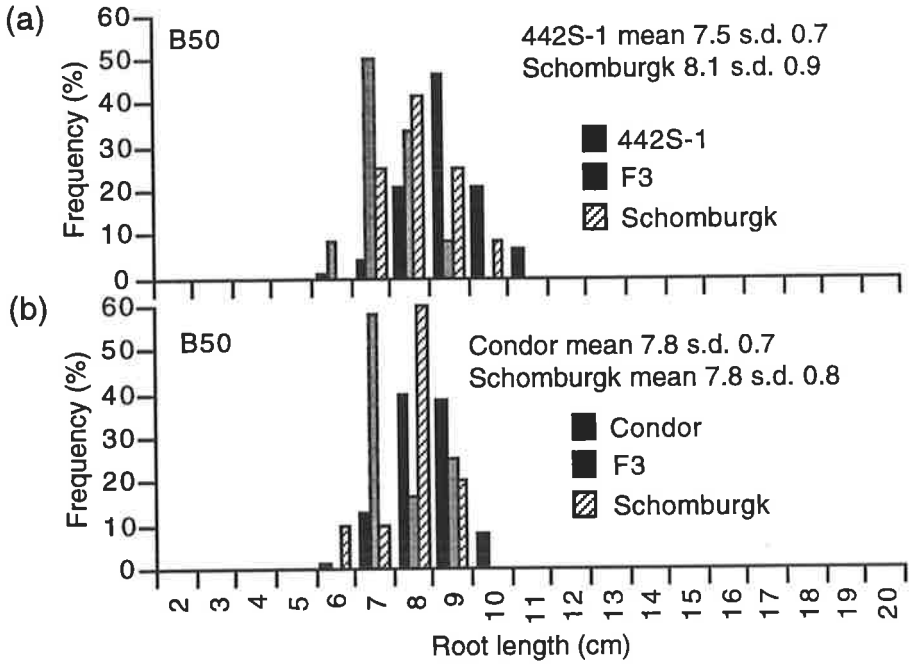


Figure 5.7 Mean root length of F_3 families and parents (12-15 seedlings for each family and parent), tested in filter papers at B100. (a) (442S-1 x G61450), (b) (442S-1 x Halberd). Populations where a single gene was expected to be segregating.

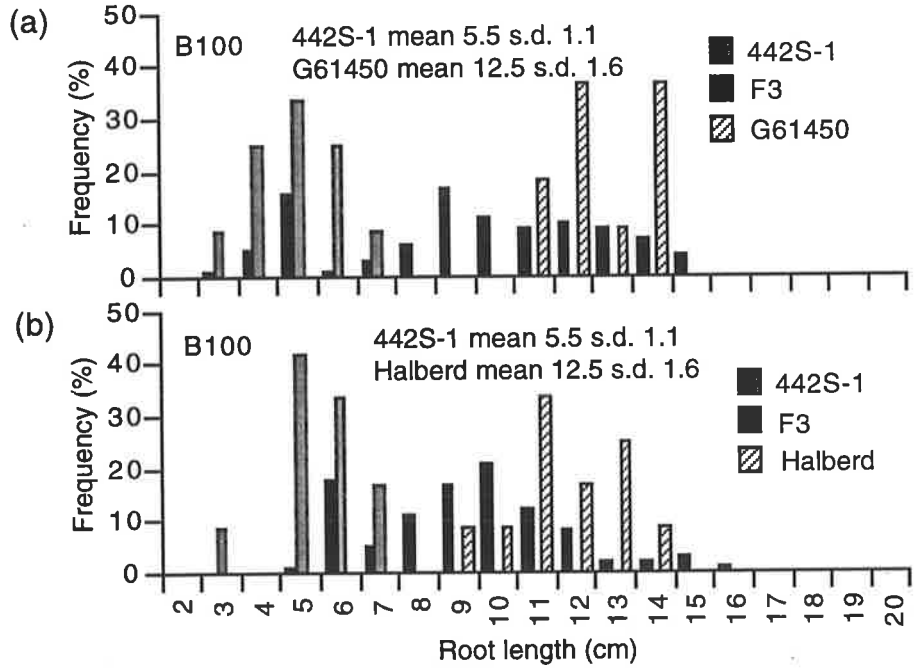


Figure 5.8 Mean root length of F_3 families and parents (12-15 seedlings for each family and parent), tested in filter papers at B100. (a) (418T-1 x G61450), (b) (418T-1 x Halberd). Populations where segregation was expected at a single locus.

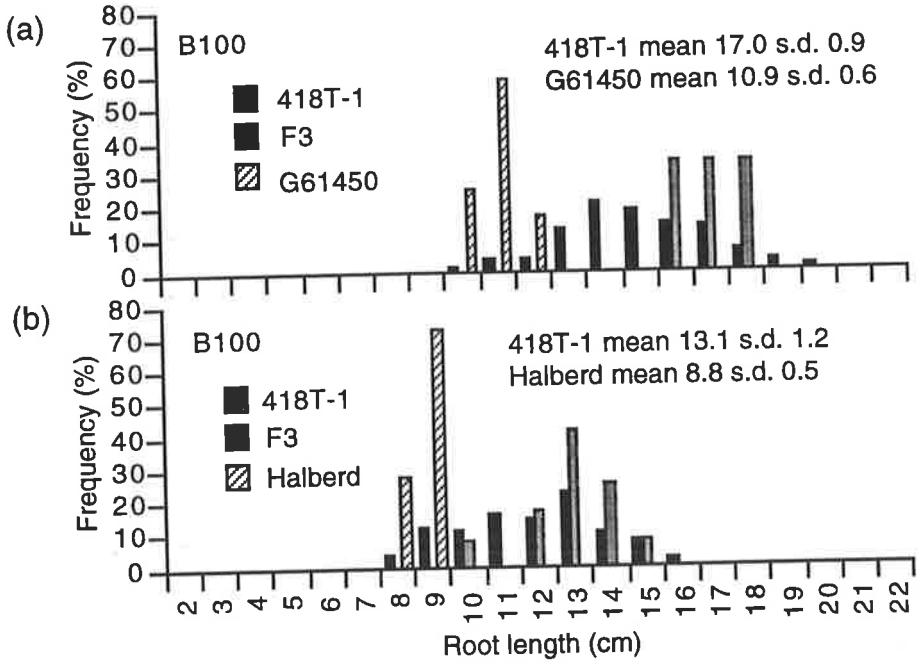
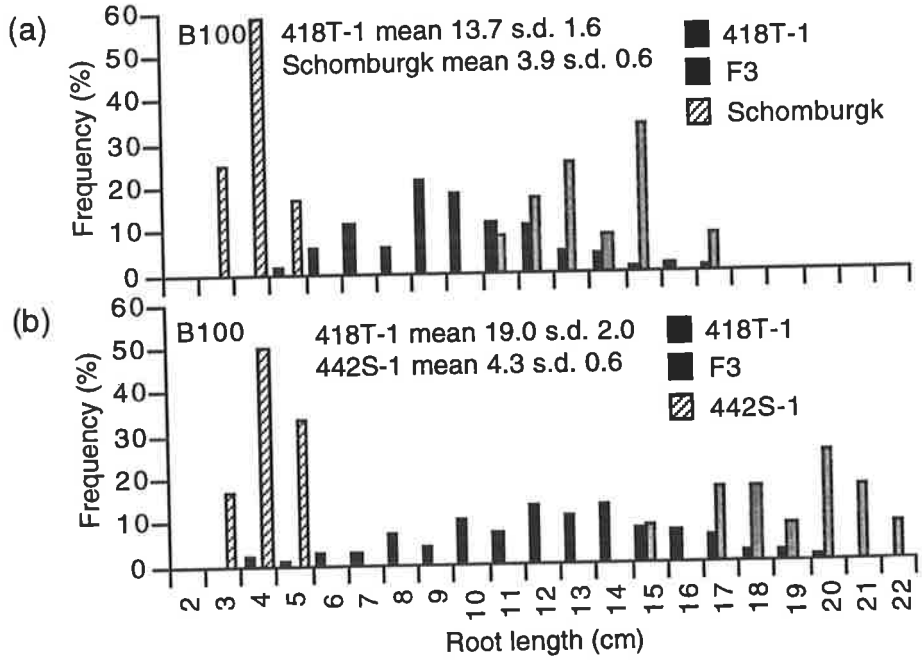


Figure 5.9 Mean root length of F_3 families and parents (12-15 seedlings for each family and parent), tested in filter papers at B100. (a) (418T-1 x Schomburgk), (b) (418T-1 x 442S-1). Populations where segregation was expected at two loci.



(b) Populations expected to be segregating at a single gene.

(442S-1 x G61450)

The F_3 families of (442S-1 x G61450) were classified as sensitive when the means of the seedling root lengths were less than or equal to 6.0 cm (Figure 5.7a) and the variance of the family was less than or similar to the LSD of the parental variances (4.6) (Figure 5.10a). Families with mean root lengths more than 6.0 cm were classified as intermediate-tolerant. The chi-square analysis was compatible with a 3 : 1 segregation ratio (Table 5.4).

The F_3 families of the cross (442S-1 x G61450) were also classified into three categories (homozygous tolerant, segregating and homozygous sensitive) by statistical methods (Chapter 3). A family with a variance less than or equal to the LSD of the parental variances (4.6) and a mean within the range of the confidence interval of mean of the sensitive or tolerant parent was classified as homozygous sensitive or tolerant, respectively, whereas a family with a variance greater than the LSD of the parental variances was classified as segregating. There were four families with means above the confidence interval of the tolerant parent (Figure 5.10a), however, because the variances of these families were not significantly different from the parent, these families were classified as homozygous tolerant. There were five families with means between the two parents (Figure 5.10a) but which had variances less than the LSD of the parental variances. Two of these families (Families 1 and 2) were individually inspected. As the root lengths of some of the plants within the two families fell between the two parents (Figure 5.11), the two were classified as segregating. It is probable that the other three families, with means which were between Family 1 and Family 2 (Figure 5.10a), were also segregating. Chi-square analysis indicated that the ratios of homozygous tolerant : segregating : homozygous sensitive of (442S-1 x G61450) was consistent with the monogenic segregation ratio of 1 : 2 : 1 (Table 5.4).

The expected variance for a one gene model of the F_2 derived F_3 population was within the confidence intervals of the observed variance for (442S-1 x G61450) (Table 5.5) indicating segregation at a single gene.

Figure 5.10 Means and variances of root length of 97 and 99 F_3 families of the crosses (442S-1 x G61450) and (442S-1 x Halberd), respectively, with parental lines, tested in filter papers at B100.

(a) (442S-1 x G61450),

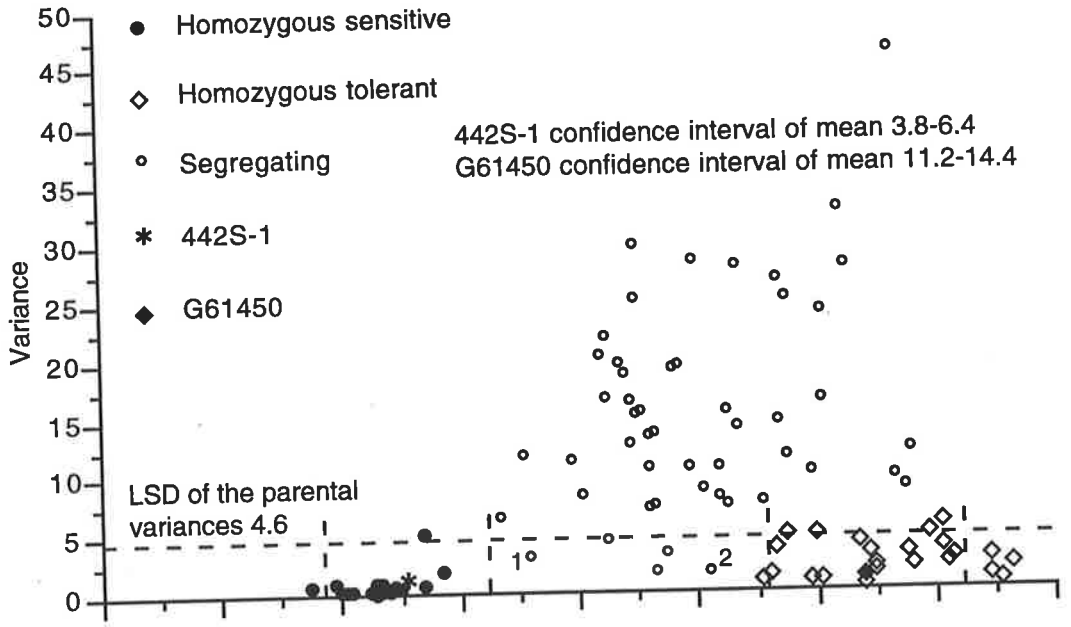
(b) (442S-1 x Halberd)

Note: The horizontal line is the LSD of the parental variances.

The vertical lines are the confidence intervals of means of the two parents.

The numbers on the diagram refer to the families which are depicted in Figures 5.11 and 5.12.

(a) (442S-1 x G61450)



(b) (442S-1 x Halberd)

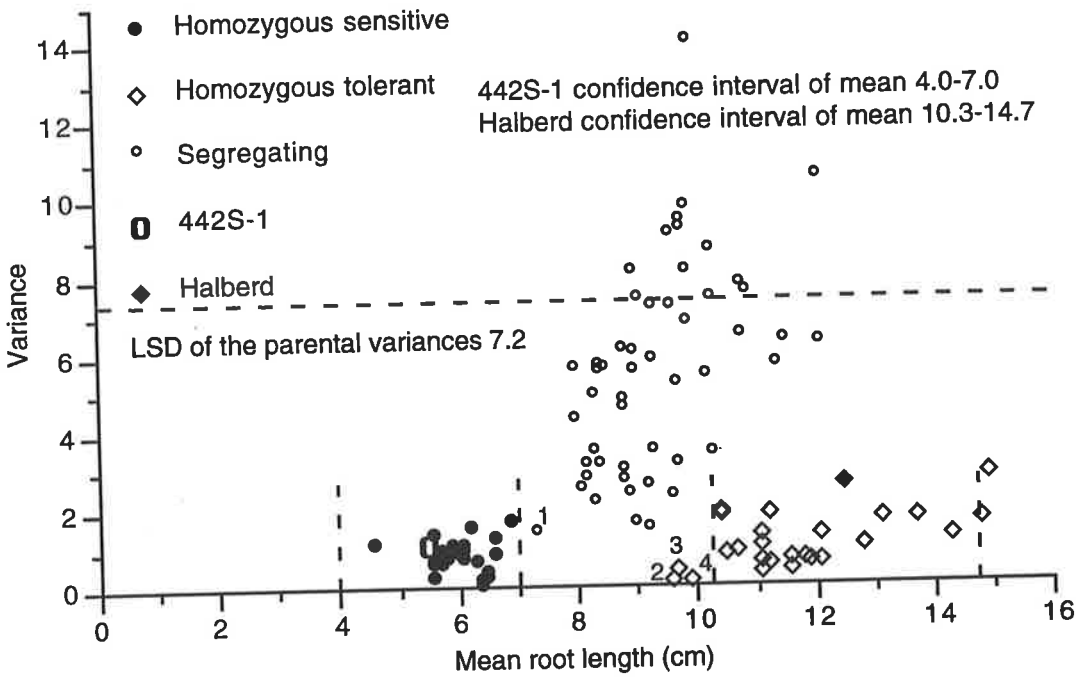


Table 5.4 Chi-square analysis of the observed and expected segregation ratios of F_2 derived families obtained for eight populations, tested in filter papers at B50 for (442S-1 x Schomburgk) and (Schomburgk x Condor) and B100 for the other populations.

Population (F_2 derived)		Model	Frequency		
			Tol[a]+Seg[b]	Sens[c]	χ^2_1
Classified on the basis of bimodal distributions					
(442S-1xSchomburgk)			No segregation observed		
(SchomburgkxCondor)			No segregation observed		
(442S-1xG61450)	Obs[d]		78	19	
	Exp[e]	3 : 1	72.75	24.25	1.52
(442S-1xHalberd)	Obs		76	23	
	Exp	3 : 1	74.25	24.75	0.16
Classified on the basis of statistical criteria					
(418T-1xG61450)	Obs		88	8	
	Exp	3 : 1	72	24	14.23
(418T-1xHalberd)	Obs		82	16	
	Exp	3 : 1	73.50	24.50	3.92
(418T-1xSchomburgk)	Obs		100	3	
	Exp	15 : 1	96.56	6.44	1.96
(418T-1x442S-1)	Obs		95	5	
	Exp	15 : 1	93.75	6.25	0.27
			Tol	Seg	Sens	χ^2_2
(442S-1xG61450)	Obs		23	52	22	
	Exp	1 : 2 : 1	24.25	48.5	24.25	0.52
(442S-1xHalberd)	Obs		25	51	23	
	Exp	1 : 2 : 1	24.75	49.50	24.75	0.17
(418T-1xG61450)	Obs		19	69	8	
	Exp	1 : 2 : 1	24	48	24	20.90
(418T-1xHalberd)	Obs		26	56	16	
	Exp	1 : 2 : 1	24.50	49	24.50	4.03

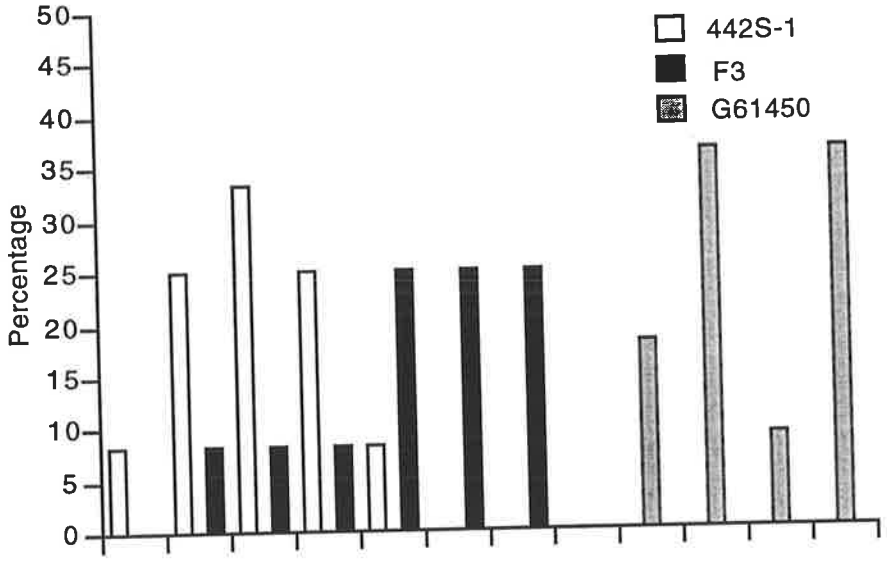
Probability (P) of chi-square at 1 and 2 degrees of freedom.

P	0.50	0.25	0.05	0.01
χ^2_1	0.45	1.32	3.84	6.63
χ^2_2	1.39	2.77	5.99	9.21

[a] Tolerant, [b] Segregating, [c] Sensitive, [d] Observed value, [e] Expected value

Figure 5.11 Response of individual plants within two F_3 families of (442S-1 x G61450) at B100 in comparison with individual plants of the two parents.

(a) Family 1



(b) Family 2

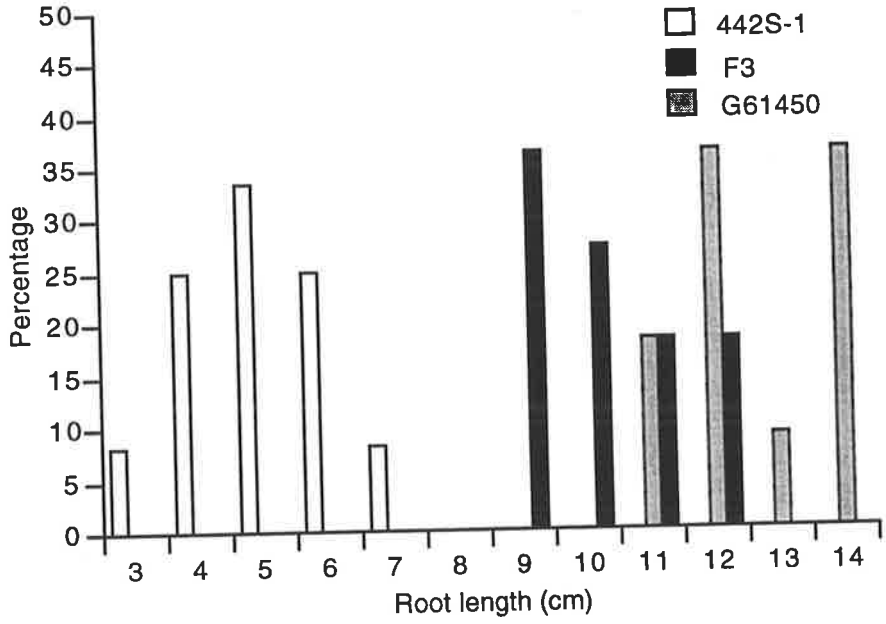


Table 5.5 Comparisons between expected and observed variances of six F_2 derived F_3 populations when tested under high boron conditions.

Parents and F_3	d [a]	E [b]	Expected variance[c]		Observed variance	Confidence interval[d]
			1 gene	2 genes		
One gene hypothesis						
442S-1					1.0	
(442S-1 x G61450)	3.9	1.2	12.6	6.9	10.8	14.6-8.3
G61450					1.4	
442S-1					1.2	
(442S-1 x Halberd)	3.5	2.0	11.2	6.6	5.9	8.0-4.5
Halberd					2.7	
418T-1					0.8	
(418T-1 x G61450)	3.1	0.6	7.8	4.2	3.9	5.3-3.0
G61450					0.4	
418T-1					1.4	
(418T-1 x Halberd)	2.2	0.9	4.5	2.7	4.1	5.6-3.2
Halberd					0.3	
Two genes hypothesis						
418T-1					2.6	
(418T-1 x Schomburgk)	4.9	1.5	19.5	10.5	6.0	8.1-4.6
Schomburgk					0.4	
418T-1					3.9	
(418T-1 x 442S-1)	7.4	2.2	43.3	22.7	11.6	15.6-8.9
442S-1					0.4	

[a] d = the departure of one of a pair of corresponding homozygotes from their mid-point,
[b] E = environmental variance = 1/2 P_1 + 1/2 P_2 (Chapter 3),
[c] the expected variances were calculated on the assumtion of no dominance, no linkage and no epistacy,
[d] confidence interval of observed variance at $P = 0.95$.

(442S-1 x Halberd)

The F_3 families of (442S-1 x Halberd) (Figure 5.7b) were classified as sensitive when root lengths were less than or equal to 7.0 cm. Chi-square analysis for the goodness of fit to a 3 : 1 (intermediate-tolerant : sensitive) ratio indicated segregation at a single gene for these two crosses (Table 5.4).

The families of (442S-1 x Halberd) (Figure 5.10b) were classified into three categories on the basis of their means and variances in comparison to those of the parents. As the LSD of the parental variances of (442S-1 x Halberd) (Figure 5.10b) was too high to use for differentiation, families with a mean within the confidence interval of the mean of the sensitive or tolerant parent and with a variance close to that of the parents was classified as homozygous sensitive or tolerant, respectively. There were four families which could not be classified because their means were intermediate to that of the two parents but with variances similar to or less than those of the two parents (Figure 5.10b), so the performance of the individual plants of these families was inspected. The root lengths of the plants within Family 1 overlapped the two parents while all of the plants within Families 2, 3 and 4 fell within the range of the tolerant parent (Figure 5.12). Therefore, Family 1 was classified as segregating whereas Families 2, 3 and 4 were classified as homozygous tolerant. Chi-square analysis indicated that the segregation of (442S-1 x Halberd) was consistent with the monogenic ratio of 1: 2 : 1 (Table 5.4).

The comparison between the observed and expected variances (Table 5.5) indicated segregation at two genes for (442S-1 x Halberd).

(418T-1 x G61450)

Continuous distribution was observed for F_2 derived F_3 populations of (418T-1 x G61450) (Figure 5.8a) so it was not possible to test the segregation directly. The F_3 families of (418T-1 x G61450) were classified into three categories on the basis of their means and variances in comparison to those of the parents. Since the LSD of the parental variances was very low in comparison to the variances of the families, it was not used to differentiate between the homozygous and heterozygous families (Figure 5.13a). There

Figure 5.12 Response of individual plants within four F_3 families of (442S-1 x Halberd) at B100 in comparison with individual plants of the two parents.

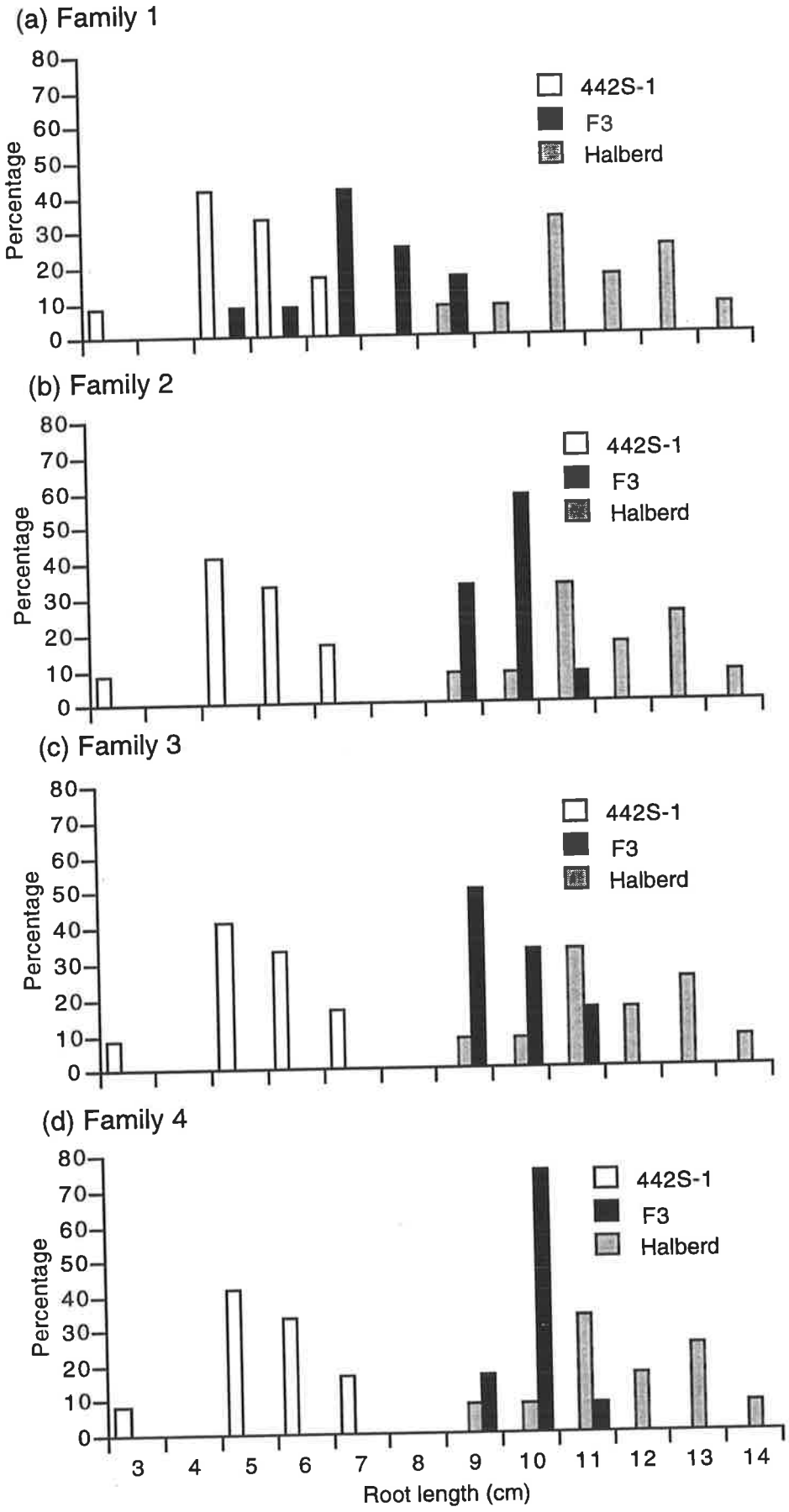

(a) Family 1
Percentage
442S-1
F3
Halberd
(b) Family 2
Percentage
442S-1
F3
Halberd
(c) Family 3
Percentage
442S-1
F3
Halberd
(d) Family 4
Percentage
442S-1
F3
Halberd
0
10
20
30
40
50
60
70
80
3
4
5
6
7
8
9
10
11
12
13
14
Root length (cm)

Figure 5.13 Means and variances of root length of 96 and 98 F_3 families of the crosses (418T-1 x G61450) and (418T-1 x Halberd), respectively, with parental lines, tested in filter papers at B100.

(a) (418T-1 x G61450)

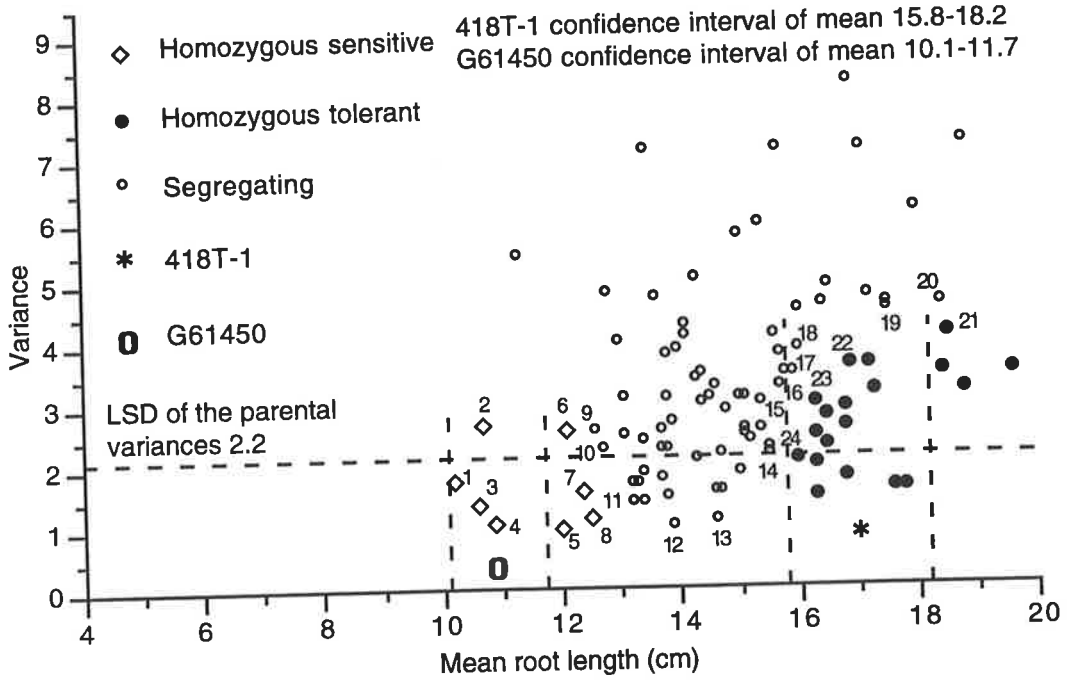
(b) (418T-1 x Halberd)

Note: The horizontal line is the LSD of the parental variances.

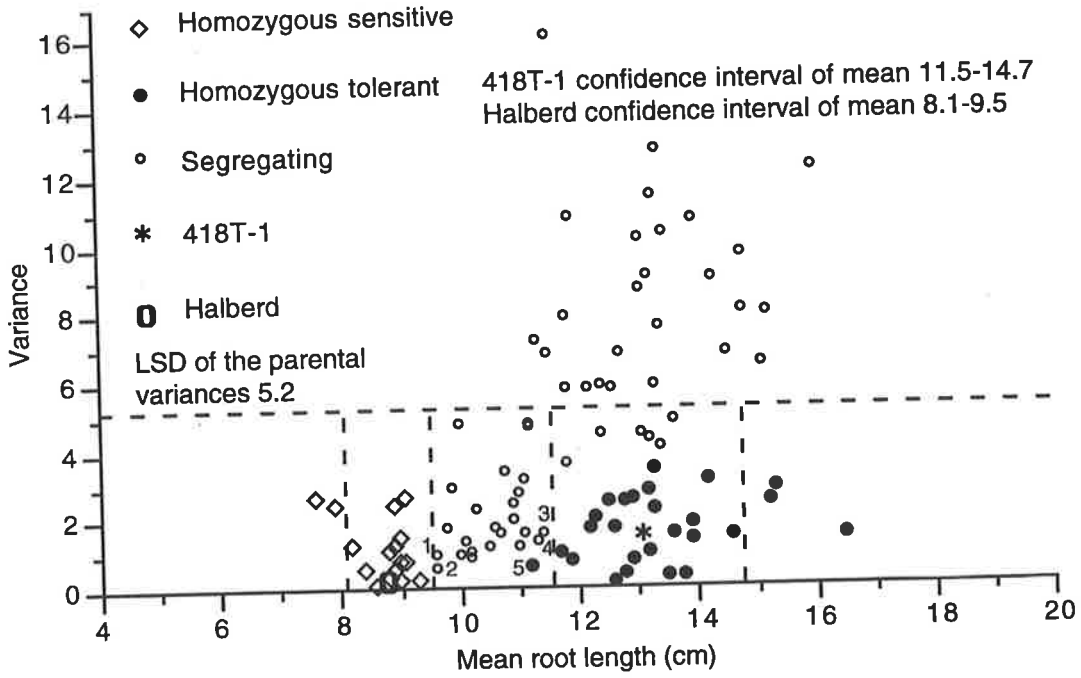
The vertical lines are the confidence intervals of means of the two parents.

The numbers on the diagram refer to the families which are depicted in Figures 5.14, 5.15, 5.16 and 5.17.

(a) (418T-1 x G61450)



(b) (418T-1 x Halberd)



were only four families with means within the confidence interval of the sensitive parent and eleven families within the range of the tolerant parent. The variances of the two parents were lower than those observed in other crosses, with the result that the variances of almost all of the families were higher than those of the two parents (Figure 5.13a). There were many families with their means outside the confidence interval of the two parents (Figure 5.13a). Thus, the performance of the individual plants of 24 families with their means within or outside the confidence interval of the parents were investigated. Families 1-8 and 21-24 were classified as homozygous sensitive and homozygous tolerant (Figures 5.14 and 5.16), respectively, because the variation in root length within these families was low and their means were close to those of the parents. Families 9-20 (Figures 5.15 and 5.16) were classified as segregating because of the high variation in root lengths within these families in comparison to the homozygous families. Chi-square analysis indicated segregation of (418T-1 x G61450) was not consistent with 1 homozygous tolerant : 2 segregating : 1 homozygous sensitive (Table 5.4). The deviation from the expected ratio was principally due to a low frequency of sensitive families and a high frequency of segregating families.

In contrast to the hypothesis of a one gene, the observed variance of (418T-1 x G61450) was similar to the expected variance for segregation at two genes (Table 5.5).

(418T-1 x Halberd)

Continuous distribution was also observed for F_2 derived F_3 populations of (418T-1 x Halberd) (Figure 5.8b). Therefore the F_3 families of (418T-1 x Halberd) were classified into three categories (homozygous tolerant, segregating and homozygous sensitive) using the comparison between the mean and variance of each F_3 family and those of the parents (Figure 5.13b). The LSD of the parental variances (5.2) of (418T-1 x Halberd) was considered too high to use to differentiate between the variances of the families and those of the parents (Figure 5.13b). Therefore, families were classified as homozygous sensitive or tolerant when the mean of the family was in the range of the confidence interval of the sensitive or tolerant parents, respectively, and the variance of the family was close to those

Figure 5.14 Response of individual plants within F_3 families of (418T-1 x G61450) (Families 1-8) at B100 in comparison with individual plants of the two parents.

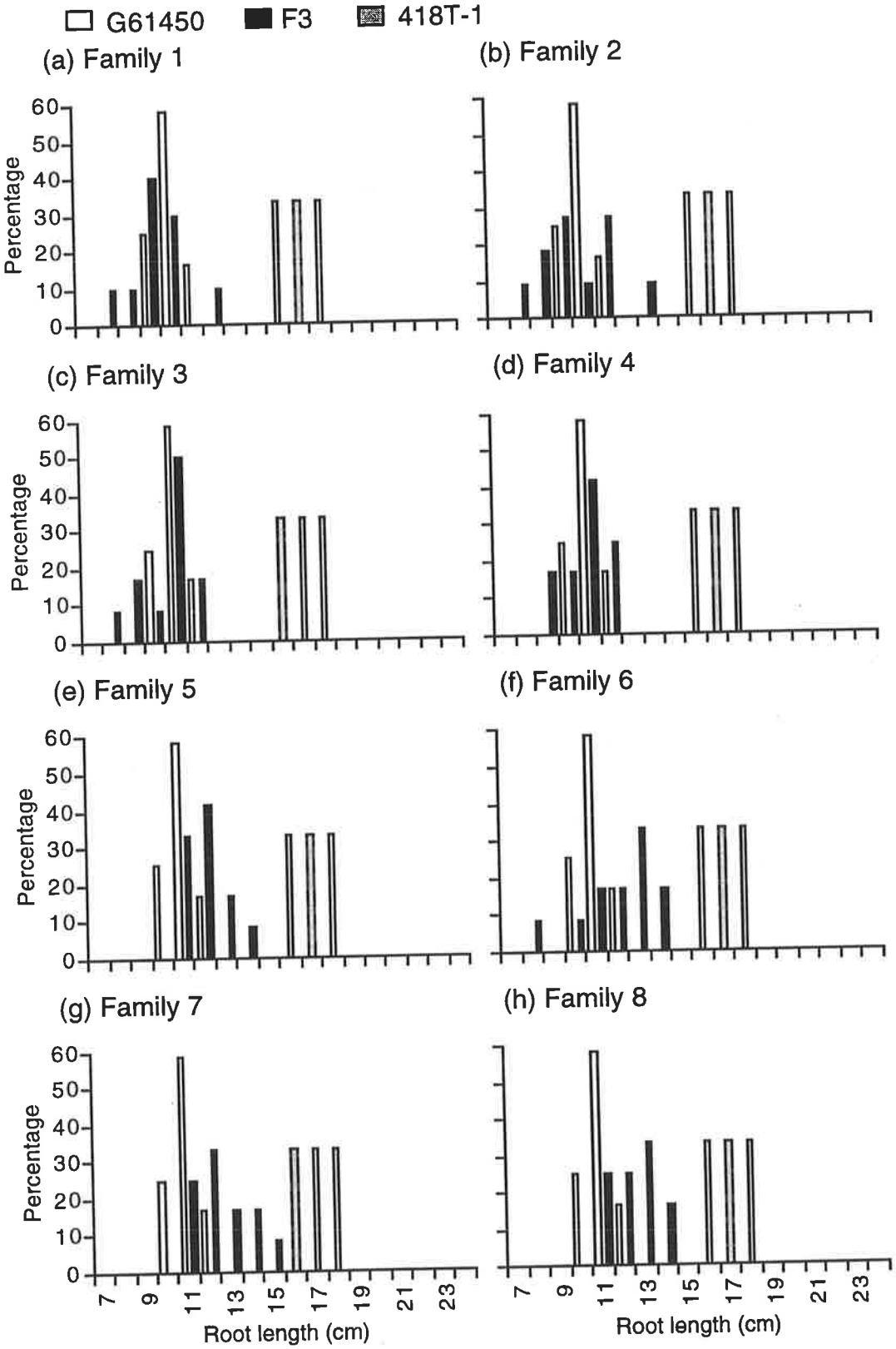

G61450
F3
418T-1
(a) Family 1
(b) Family 2
(c) Family 3
(d) Family 4
(e) Family 5
(f) Family 6
(g) Family 7
(h) Family 8
Percentage
0
10
20
30
40
50
60
7
9
11
13
15
17
19
21
23
Root length (cm)

Figure 5.15 Response of individual plants within F_3 families of (418T-1 x G61450) (Families 9-16) at B100 in comparison with individual plants of the two parents.

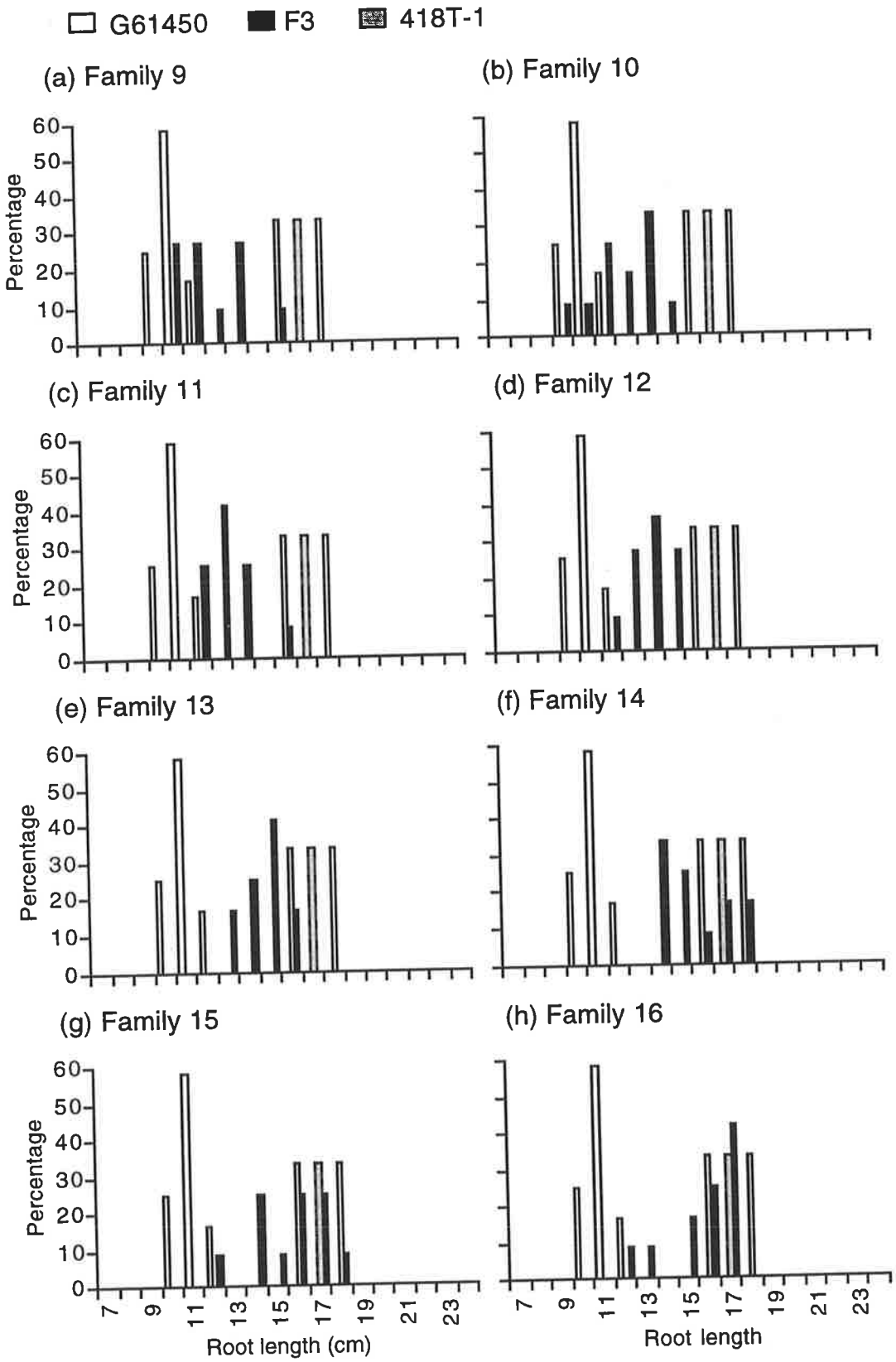
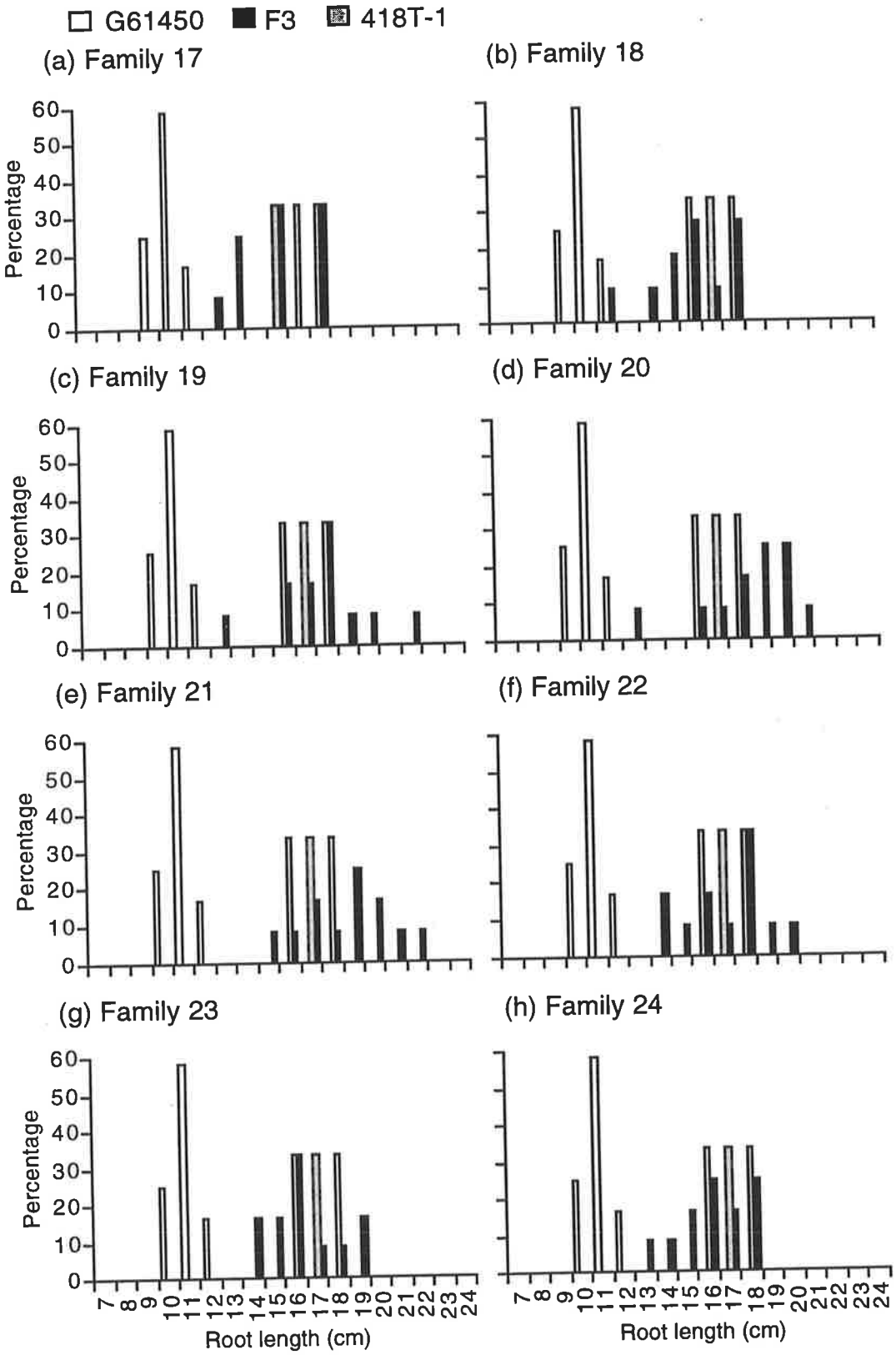

G61450
F3
418T-1
(a) Family 9
(b) Family 10
(c) Family 11
(d) Family 12
(e) Family 13
(f) Family 14
(g) Family 15
(h) Family 16
Percentage
0
10
20
30
40
50
60
7
9
11
13
15
17
19
21
23
Root length (cm)
Root length

Figure 5.16 Response of individual plants within F_3 families of (418T-1 x G61450) (Families 17-24) at B100 in comparison with individual plants of the two parents.


G61450
F3
418T-1
(a) Family 17
(b) Family 18
(c) Family 19
(d) Family 20
(e) Family 21
(f) Family 22
(g) Family 23
(h) Family 24
Percentage
0
10
20
30
40
50
60
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
Root length (cm)

of the sensitive and tolerant parents. Fourteen F_3 families of (418T-1 x Halberd) with a mean root length within the range of 8.1-9.4 cm and variance 0.1-3.0 were similar to the sensitive parent and were classified as homozygous sensitive (Figure 5.13b). There were also two families with means of lower than the confidence interval of the sensitive parent, however, these families were classified as sensitive because their variances were close to that of the sensitive parent (Figure 5.13b). The 22 families that fell within the confidence interval of the tolerant parent (11.5-14.7 cm) with variances close to that of the tolerant parent (1.4) were classified as homozygous tolerant. There were also three families which means of greater than the confidence interval of the tolerant parent but these families was classified as tolerant because the variances of these families were close to that of the tolerant parent (Figure 5.13b).

There were twenty-two families of (418T-1 x Halberd) which could not be obviously classified into the above categories. So, the performance of the individual plants of five families of these twenty-two families was investigated. Root lengths of the plants within families 1-4 overlapped the two parents (Figure 5.17) and therefore the families were classified as segregating. The root lengths of all plants within family 5 fell in the range of the tolerant parent and so the family was classified as tolerant (Figure 5.17). Chi-square analysis indicates that the segregation of (418T-1 x Halberd) was consistent with the monogenic ratios of 1 homozygous tolerant : 2 segregating : 1 homozygous sensitive (Table 5.4).

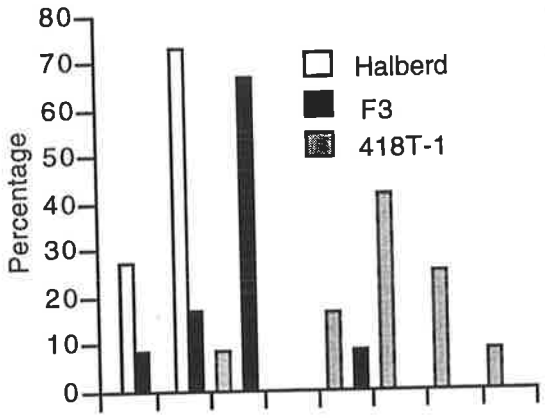
The result of the comparison between the observed and expected variances of the F_3 population and those of the parents (Table 5.5) indicated that a single gene was responsible for boron tolerance of (418T-1 x Halberd) (Table 5.5).

(c) Populations expected to be segregating at two genes

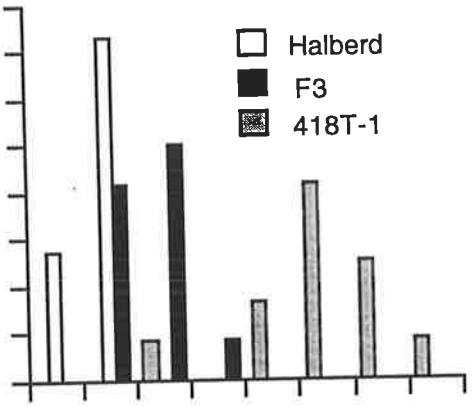
There were continuous distributions for the F_2 derived F_3 populations of (418T-1 x Schomburgk) (Figure 5.9a) and (418T-1 x 442S-1) (Figure 5.9b). Since the LSD of the parental variances of the two crosses was much higher than the variances of the two parents (Figure 5.18), it was not used to differentiate between the homozygous and

Figure 5.17 Response of individual plants within Five F_3 families of (418T-1 x Halberd) at B100 in comparison with individual plants of the two parents.

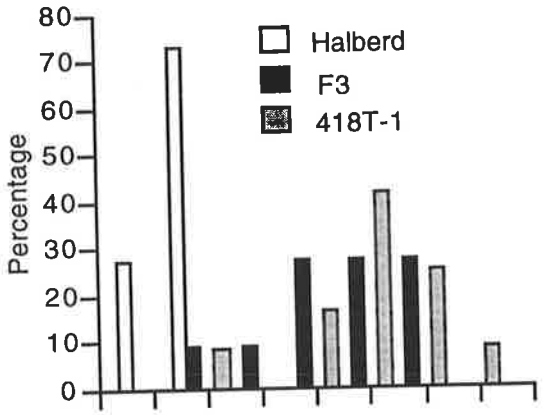
(a) Family 1



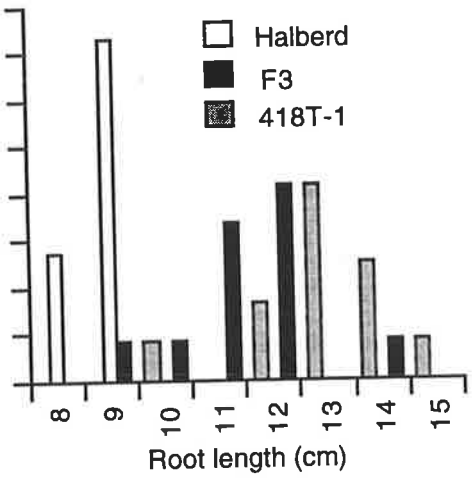
(b) Family 2



(c) Family 3



(d) Family 4



(e) Family 5

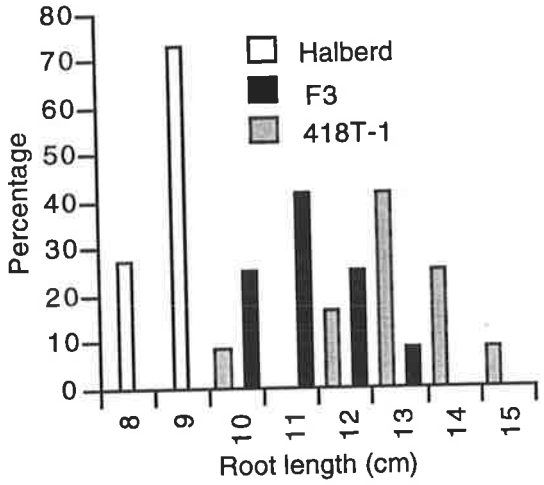


Figure 5.18 Means and variances of root length of 103 and 100 F_3 families of the crosses (418T-1 x Schomburgk) and (418T-1 x 442S-1), respectively, with parental lines, tested in filter paper at B100.

(a) (418T-1 x Schomburgk),

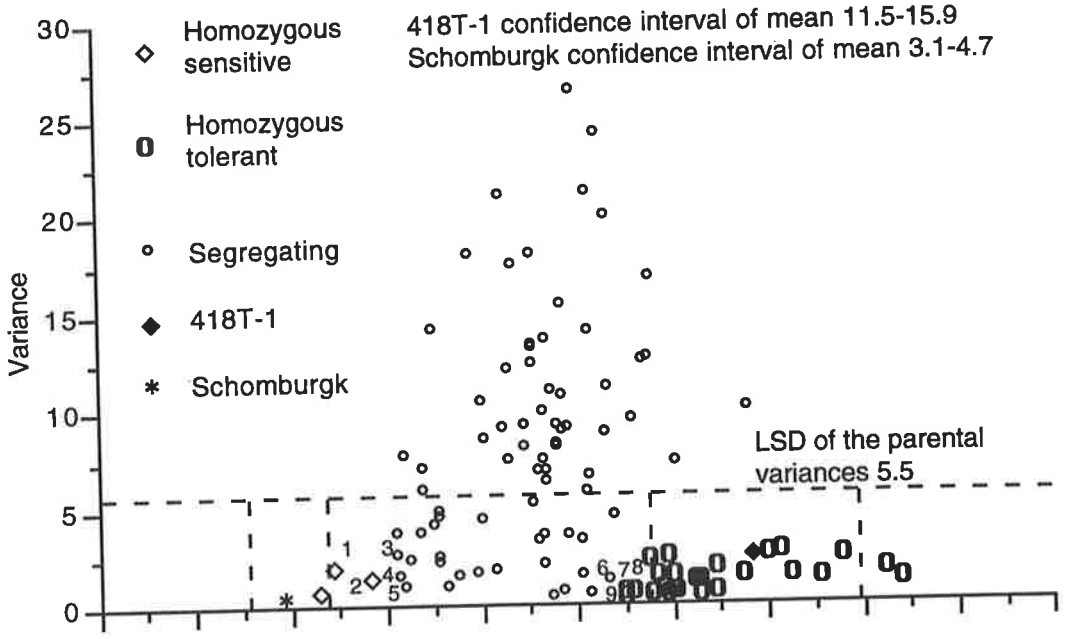
(b) (418T-1 x 442S-1)

Note: The horizontal line is the LSD of the parental variances.

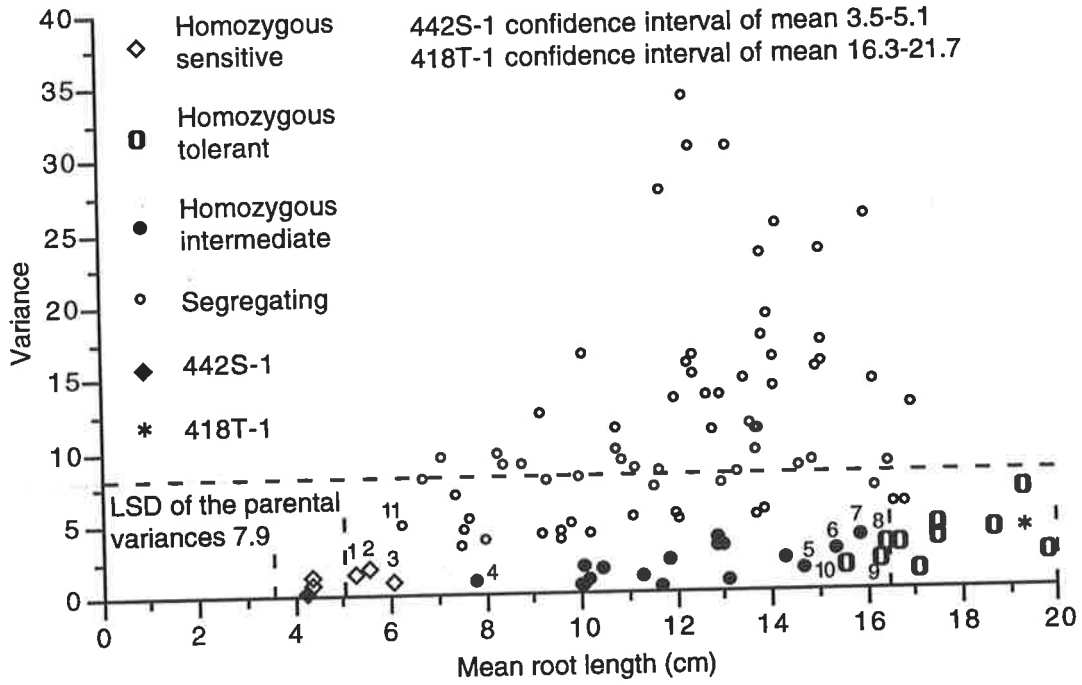
The vertical lines are the confidence intervals of means of the two parents.

The numbers on the diagram refer to the families which are depicted in Figures 5.19 and 5.20.

(a) (418T-1 x Schomburgk)



(b) (418T-1 x 442S-1)



segregating families. To estimate the number of genes controlling boron tolerance for these crosses, the mean and variance of each F_3 family was compared to those of the parents (Figures 5.18a and 5.18b).

(418T-1 x Schomburgk)

There were approximately thirty-three families of (418T-1 x Schomburgk) which could not be categorised because their means were intermediate to the two parents but with variances not significantly greater than those of the two parents (Figure 5.18a). Thus, the individual plants of nine of these families were investigated (Figure 5.19). As almost all of the plants of Families 1-2 and 7-9 were within the range of the sensitive and tolerant parent, respectively, and the variation in root lengths within these families was low, they were classified as homozygous sensitive and tolerant (Figure 5.19), respectively. The variation in root lengths within Families 3-6 was also low but there were many plants within these families outside the range of the parents, therefore they were classified as homozygous intermediate (Figure 5.19). Chi-square analysis indicated segregation of (418T-1 x Schomburgk) was consistent with the digenic ratios of 15 tolerant-segregating : 1 homozygous sensitive but not 1 homozygous tolerant : 14 homozygous intermediate-segregating : 1 homozygous sensitive (Tables 5.4 and 5.6), with the deviation from the expected ratio being in large part due to a very low frequency of homozygous sensitive families and a high frequency of homozygous tolerant families.

Both the expected variances for one and two gene models of (418T-1 x Schomburgk) were outside the confidence interval of the observed variance but were closer to that expected for the segregation at two genes (Table 5.5).

(418T-1 x 442S-1)

There were twenty-three families of (418T-1 x 442S-1) which could not be classified into specific categories because their means were intermediate to the two parents but with variances not significantly greater than those of the two parents (Figure 5.18b). Families 1-3 and 8-10 were classified as homozygous sensitive and homozygous tolerant

Figure 5.19 Response of the individual plants within nine F_3 families of (418T-1 x Schomburgk) at B100 in comparison with individual plants of the two parents.

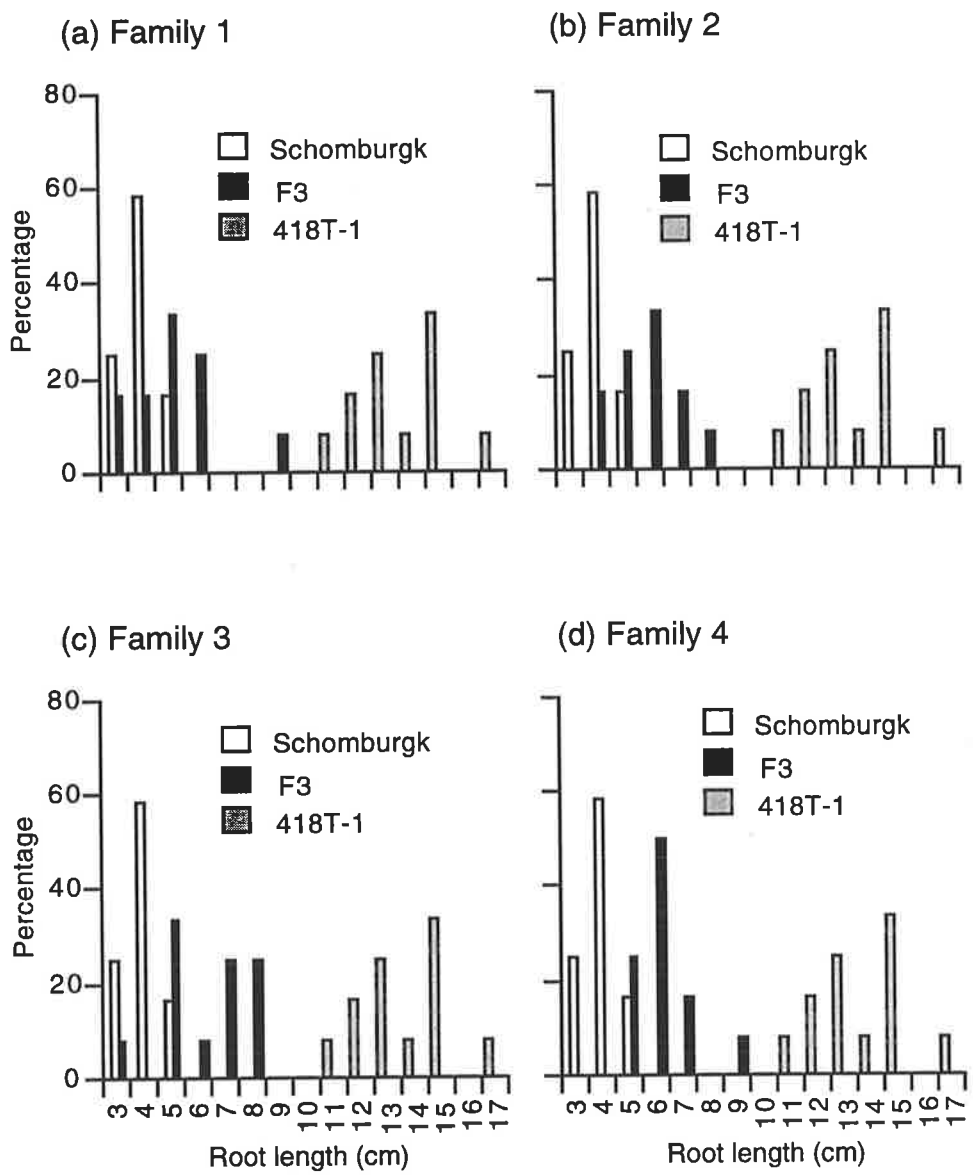


Figure 5.19 (continued).

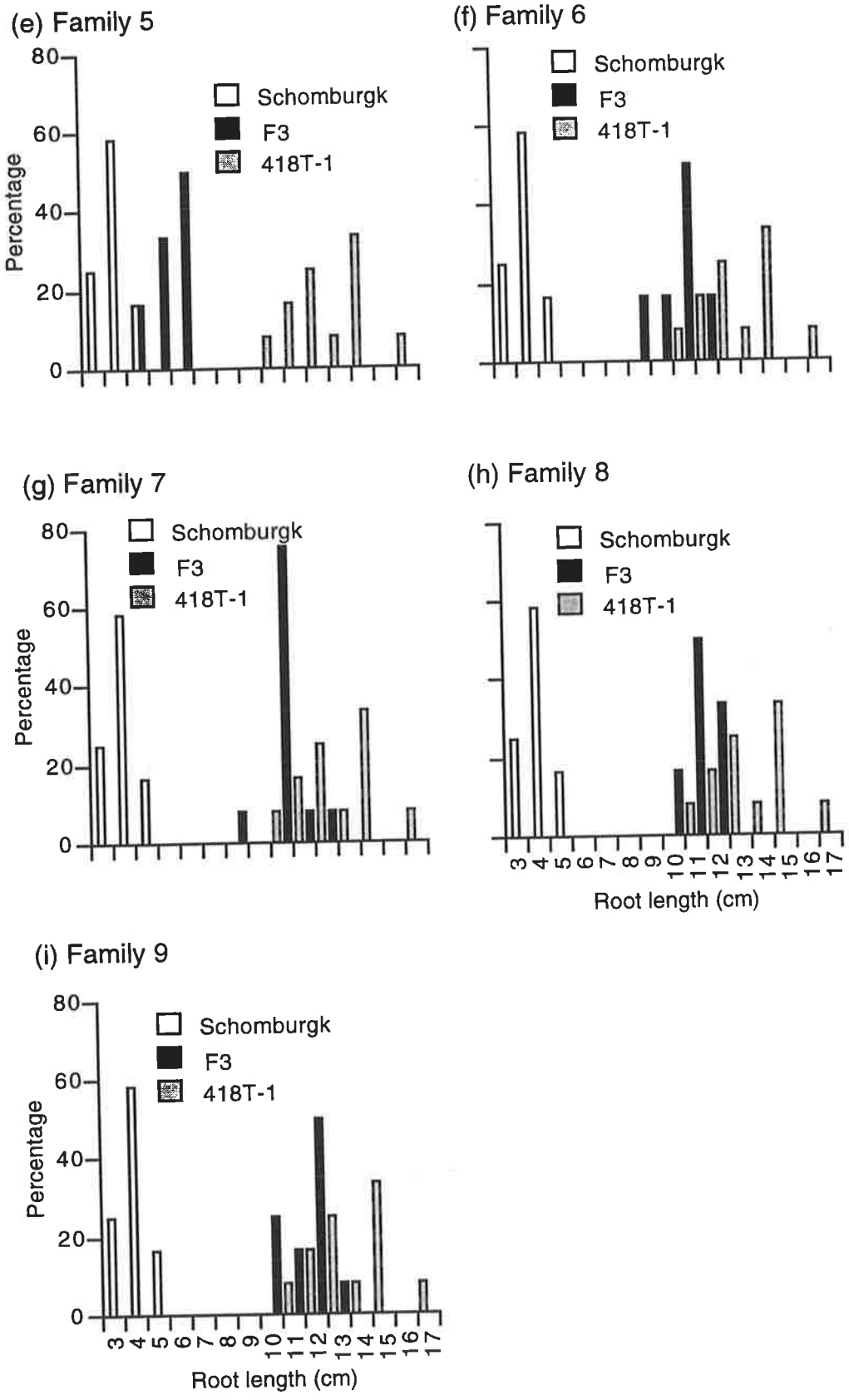


Table 5.6 Chi-square analysis of the observed and expected segregation ratios of F_2 derived families obtained for two populations, (418T-1 x Schomburgk) and (418T-1 x 442S-1), expected to be segregating at two genes, tested in filter papers at B100.

Population (F_2 derived)		Model	Frequency				
			Homo tol[a]	Homo Int-seg[b]	Homo sen[c]	χ^2_2	
(418T-1 x Schomburgk)	Obs[d]		23	77	3		
	Exp[e]	1 : 14 : 1	6.44	90.12	6.44	46.33	
(418T-1 x 442S-1)	Obs		10	85	5		
	Exp	1 : 14 : 1	6.25	87.5	6.25	2.57	
			Homo tol	Homo Int	Homo sen	Seg	χ^2_3
(418T-1 x 442S-1)	Obs		10	17	5	68	
	Exp	1 : 2 : 1 : 12	6.25	12.5	6.25	75	4.77

Probability (P) of chi-square at 2 and 3 degrees of freedom.

P	0.50	0.25	0.05	0.01
χ^2_2	1.39	2.77	5.99	9.21
χ^2_3	2.37	4.11	7.81	11.34

[a] Homozygous tolerant, [b] Homozygous intermediate-segregating, [c] Homozygous sensitive, [d] Observed value, [e] Expected value

(Figure 5.20), respectively, because their variation in root lengths was low and most of the plants of these families fell within or very close to the range of the parents. All plants of Family 4 were outside the range of the two parents and many plants in Family 5 were outside the range of the tolerant parent (Figure 5.20); therefore, these two families were classified as homozygous intermediate. Families 6 and 7 had means and variances close to the tolerant parent but there was greater overlap between the plants within these families and the two parents in comparison to those of the homozygous tolerant families (Families 8-10) (Figure 5.20), so these two families were classified as homozygous intermediate. Family 11 was classified as segregating because of the high variation in root length of the plants within this family (Figure 5.20). Chi-square analysis indicated that the segregation of (418T-1 x 442S-1) was consistent with all of the three digenic ratios of 15 segregating-tolerant : 1 homozygous sensitive (Table 5.4), 1 homozygous tolerant : 14 homozygous intermediate-segregating : 1 homozygous sensitive (Table 5.6) and 1 homozygous tolerant : 2 homozygous intermediate : 1 homozygous sensitive : 12 segregating (Table 5.6).

Both the expected variances for one and two gene models of (418T-1 x 442S-1) were outside the confidence interval of the observed variance but were closer to that expected for the segregation at two genes (Table 5.5). Examples of homozygous tolerant (AABB), homozygous intermediate (AAbb or aaBB), segregating and homozygous sensitive (aabb) F_2 derived families for 418T-1 x 442S-1 are presented in Plates 5.2 and 5.3.

5.4 Discussion

Overall conclusion

The F_2 and F_2 derived F_3 populations of six crosses were tested for tolerance to high concentrations of boron using a filter paper technique. The number of genes controlling tolerance to boron of the F_2 and F_3 populations of the six crosses were estimated from the segregation data using three methods. The first was to classify the F_2 seedlings and F_3 families into two categories (tolerant-intermediate and sensitive) according to the distribution of the seedling root length of the individual F_2 plants and the mean root

Figure 5.20 Response of the individual plants within 11 F_3 families of (418T-1 x 442S-1) at B100 in comparison with individual plants of the two parents.

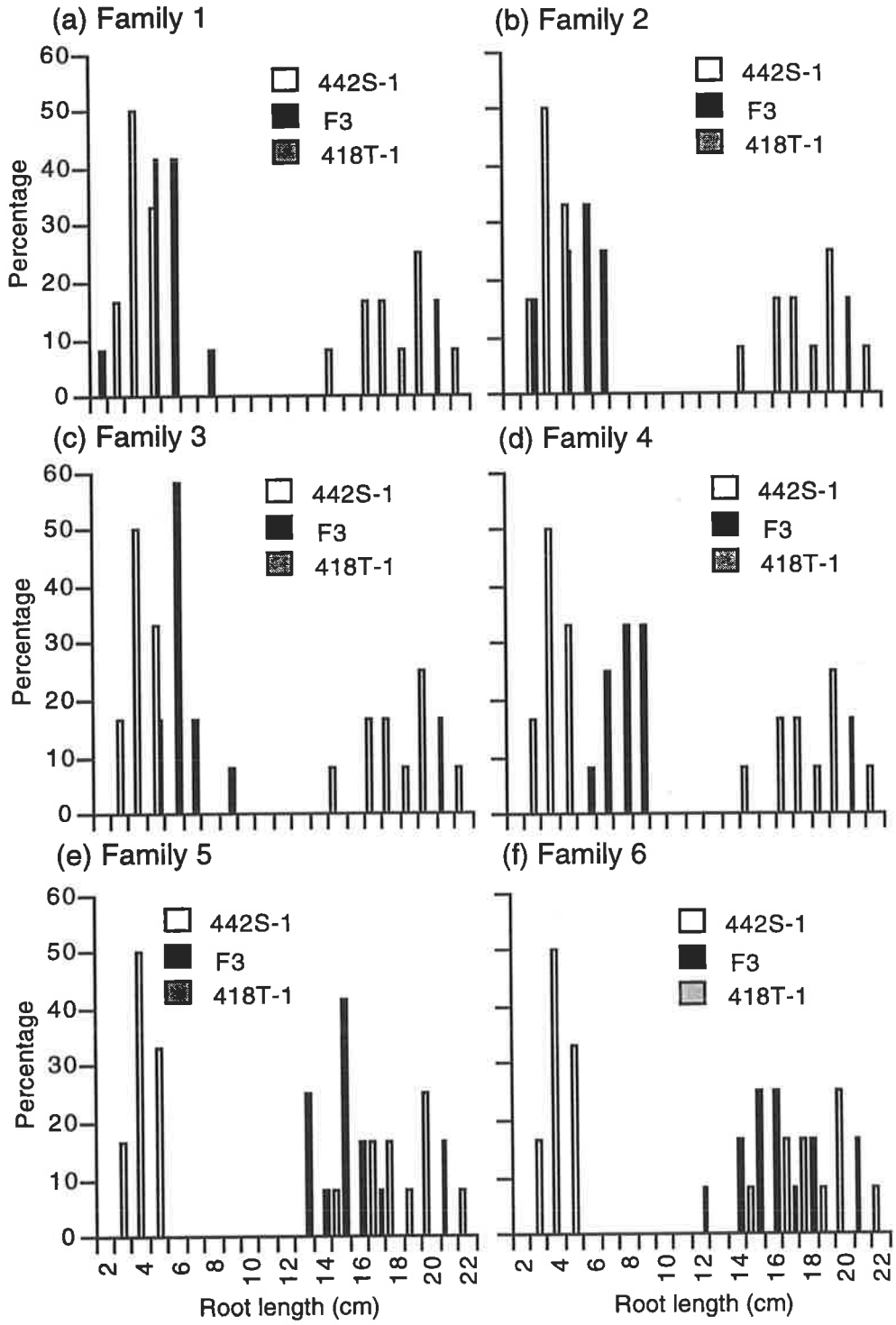


Figure 5.20 (continued).

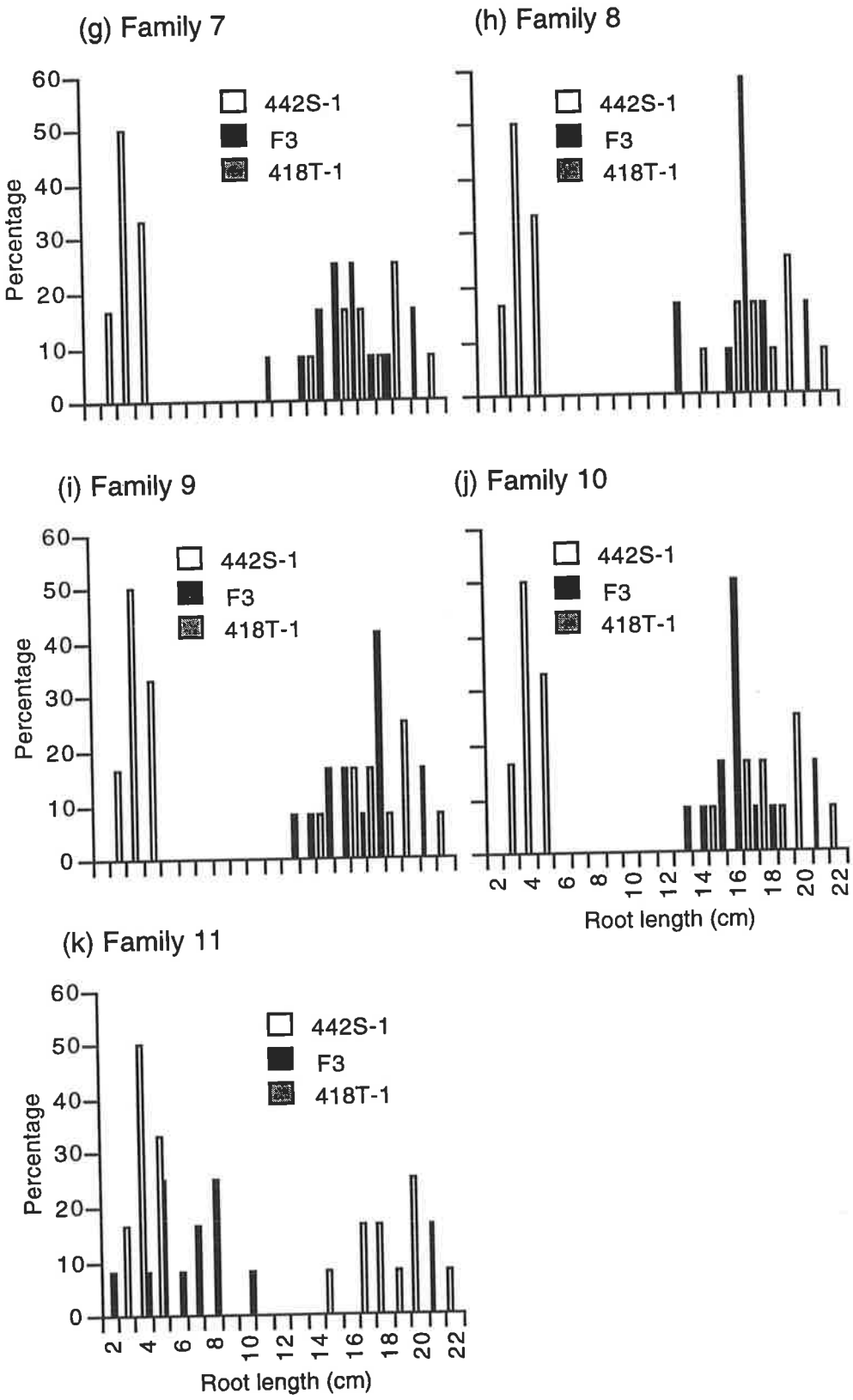


Plate 5.2 Response of F_2 derived families of (418T-1 x 442S-1) to the B100 treatment in comparison with the two parents.

(a) a homozygous tolerant family

(b) a homozygous intermediate family

(a)


Boron response of [(442-1-2-1xi416-3-1-2)F2] derived F2 families at B100
416-3-1-2
Tolerant
442-1-2-1

(b)

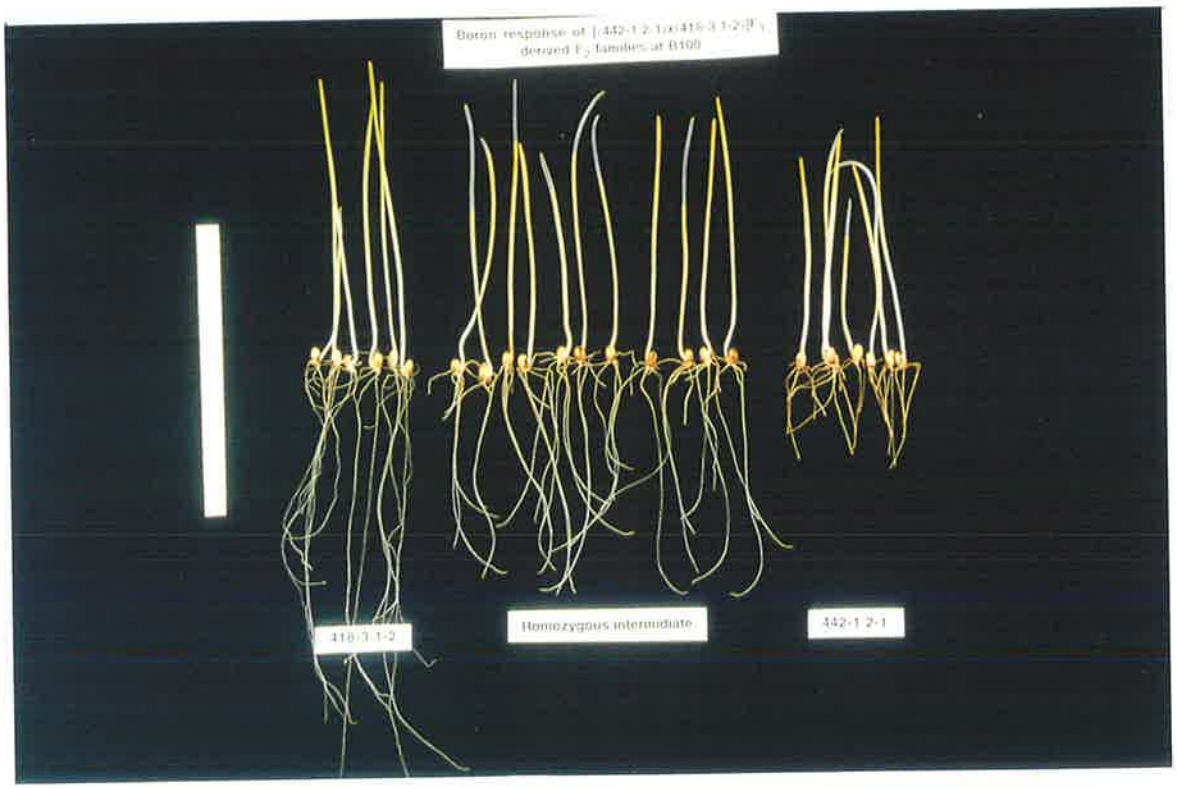
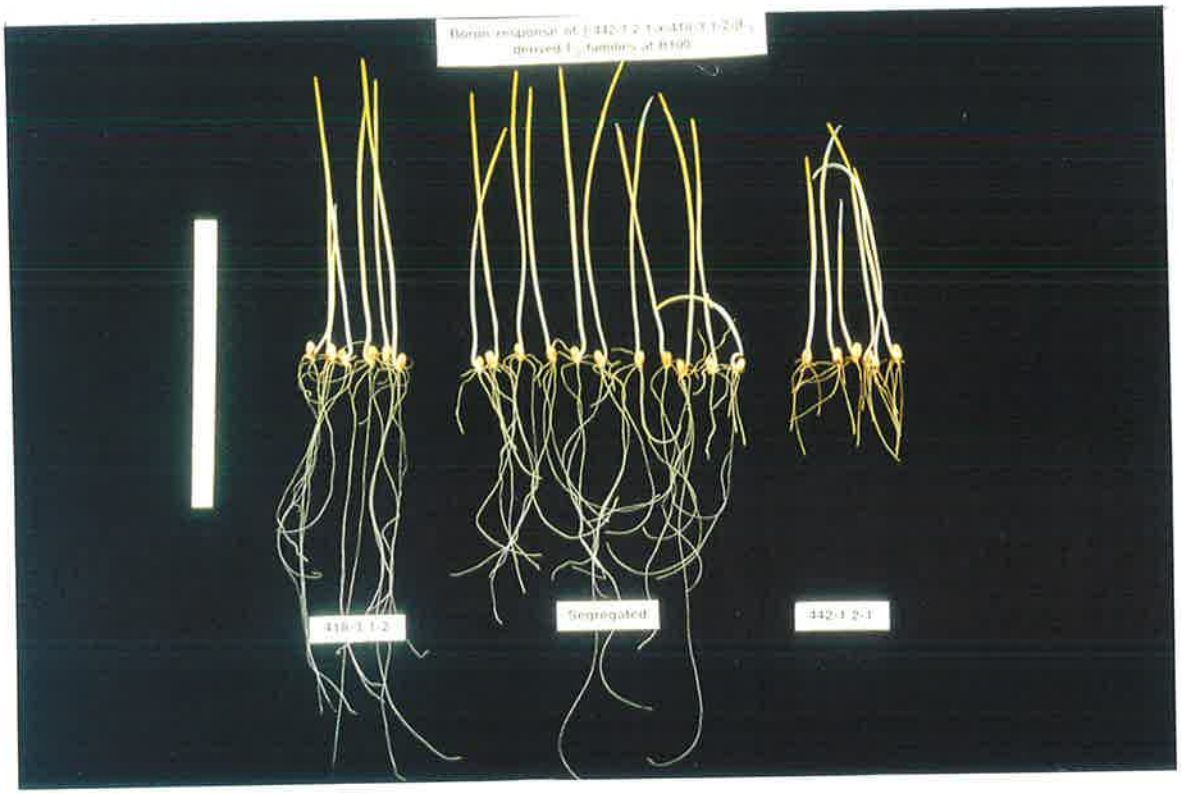

Boron response of [(442-1-2-1xi416-3-1-2)F2] derived F2 families at B100
416-3-1-2
Homozygous intermediate
442-1-2-1

Plate 5.3 Response of F_2 derived families of (418T-1 x 442S-1) to the B100 treatment in comparison with the two parents.

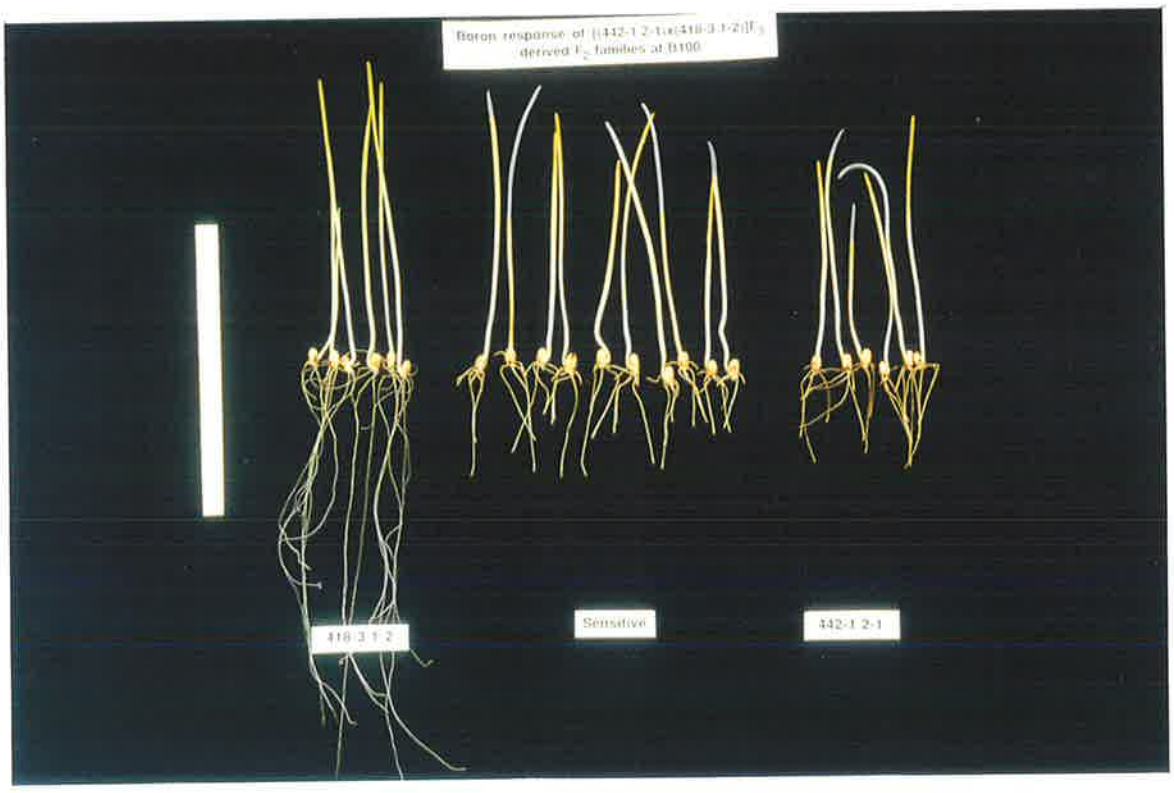
(a) a segregating family

(b) a homozygous sensitive family

(a)


Boron response of [(442-1 2-1 x 416-3 1-2)F2] derived F3 families at B100
416-3 1-2
Segregated
442-1 2-1

(b)


Boron response of [(442-1 2-1 x 416-3 1-2)F2] derived F3 families at B100
416-3 1-2
Sensitive
442-1 2-1

length of F_3 families, respectively. The second was to classify the F_3 families into three categories (homozygous tolerant, segregating and homozygous sensitive) using the comparison between the means and variances of the families and those of the two parents. Chi-square analysis was used in both methods to test for the goodness of fit of the observed segregation ratios to the ratios expected for monogenic or digenic segregation. The third method was to compare the variances observed for individual populations with the expected variances for alternative genetic models as described in Chapter 3. While the first method could only be used when the distribution of the populations was bimodal or trimodal, the second and the third could be used regardless of the distribution pattern of the populations.

Paull et al. (1991b) defined Halberd as *Bo1Bo1Bo2Bo2Bo3Bo3* with variation at the *Bo1* locus accounting for differences in response between Halberd and Warigal, a variety derived from the same parents as Schomburgk and closely related to Condor. The non-segregating F_2 populations and F_2 derived families of the crosses (442S-1 x Schomburgk) (Figures 5.2a and 5.6a) and (Schomburgk x Condor) (Figures 5.2b and 5.6b) and the non-significant difference in the seedling root lengths of 442S-1 and Schomburgk (Figure 5.1 and Plate 5.1) indicate that these three genotypes are similar in response to high boron concentration (Figure 5.13), and are of the genotype *bo1bo1Bo2Bo2Bo3Bo3*.

Based on the results observed in this experiment, a fourth locus *Bo4*, is proposed with the genotypes of Halberd and G61450 being *Bo1Bo1Bo2Bo2Bo3Bo3bo4bo4* and *bo1bo1Bo2Bo2Bo3Bo3Bo4Bo4*, respectively (Figure 5.21). When the *Bo1* and *Bo4* alleles were combined in the one line, such as 418T-1, a higher level of tolerance than either parent was expressed. On the other hand, when both the *Bo1* and *Bo4* alleles were absent, more sensitive lines, such as 442S-1 were observed.

The summary of the evidence for estimating the number of genes controlling tolerance to boron for the F_2 and F_2 derived populations of all crosses tested in this experiment is demonstrated in Table 5.7. This summary indicates the relationship between the tested lines as described in Figure 5.21. There was no segregation in F_2 and F_2 derived populations of the crosses (442S-1 x Schomburgk) and (Schomburgk x Condor) which was

Figure 5.21 Hypothetical relationship, genotypes and number of genes controlling boron tolerance for six lines of wheat (442S-1, 418T-1, Halberd, G61450, Schomburgk and Condor).

Note: 1 = 1 gene, 2 = 2 genes

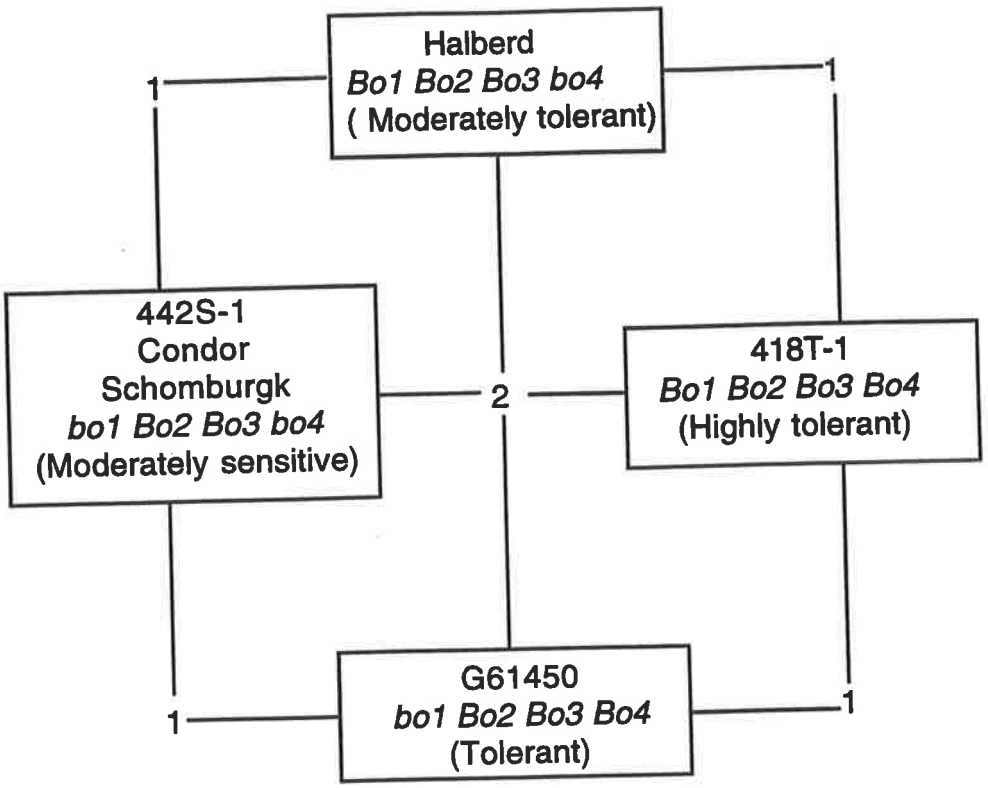

Halberd
Bo1 Bo2 Bo3 bo4
(Moderately tolerant)
1
1
442S-1
Condor
Schomburgk
bo1 Bo2 Bo3 bo4
(Moderately sensitive)
2
418T-1
Bo1 Bo2 Bo3 Bo4
(Highly tolerant)
G61450
bo1 Bo2 Bo3 Bo4
(Tolerant)
1
1

Table 5.7 A summary of evidence from F_2 and F_2 derived populations of eight crosses for estimating the number of genes controlling tolerance to boron between six lines.

Cross	Hypo[a]	Evidence					
		F_2			F_2 derived		
		Distribution	Seg ratio[b]	No of eff[c]	Distribution	Seg ratio	No of eff
(442S-1xSchomburgk)	no seg[d]	continuous	no seg	similar to parents	continuous	no seg	similar to parents
(SchomburgkxCondor)	no seg	continuous	no seg	similar to parents	continuous	no seg	similar to parents
(442S-1xG61450)	1 gene	trimodal	3 : 1	1	bimodal	3:1, 1:2:1	1
(442S-1xHalberd)	1 gene	continuous	unclassified	1 or 2	bimodal	3:1, 1:2:1	2
(418T-1xG61450)	1 gene	continuous	unclassified	2	continuous	neither 3:1 nor 1:2:1	2
(418T-1xHalberd)	1 gene	continuous	unclassified	1	continuous	1:2:1	1
(418T-1xSchomburgk)	2 genes	continuous	unclassified	2	continuous	15:1	2
(418T-1x442S-1)	2 genes	continuous	unclassified	1	continuous	15:1, 1:14:1, 1:2:1:12	2

[a] Hypothesis, [b] Segregation ratio that fit to the population [c] Number of effective factors based on the comparison between the observed and expected variances, [d] no segregation.

consistent with the hypothesis. For the populations expected to be segregating at a single gene, the F_2 and F_2 derived populations of (442S-1 x G61450) segregated at monogenic ratios and the observed variances of these populations were also similar to the variances expected for a one gene model. For (442S-1 x Halberd) and (418T-1 x Halberd), the segregation of their F_2 derived families was consistent with the monogenic ratio. The observed variances for both F_2 and F_2 derived populations of (418T-1 x Halberd) were also consistent with the variances expected for a one gene model but (442S-1 x Halberd) was not. In contrast to the hypothesis, the segregation of F_2 derived families of (418T-1 x G61450) was not consistent with the monogenic ratio and the observed variance of this cross was similar to the variances expected for a two gene model and there was no evidence from this cross to support a single gene segregation.

For the populations expected to be segregating at two genes, The segregation of the F_2 derived populations of (418T-1 x Schomburgk) and (418T-1 x 442S-1) was consistent with the digenic ratio and the observed variances of these populations were also similar to the expected variance of a two genes model.

The segregation ratios obtained for the F_2 derived families are more reliable than those of the F_2 generation (Table 5.7). Because 12-15 F_3 seeds were tested per F_2 derived family, it was possible to assign a genotype to the F_2 plant from which the family was derived, whereas the F_2 generation phenotypes were based on the response of single plants. For five of the six crosses in this experiment, the segregation ratios were consistent with the overall hypothesis, only one cross demonstrated a deficiency of the intolerant segregants.

Anomalous evidence

In a number of instances, the concentration at B100 may be not have been enough to discriminate between the families and the higher concentration of boron, such as B150, could have assisted in the discrimination. For example, chi-square analysis indicated that the segregation of the F_2 population of (442S-1 x G61450) was consistent with the monogenic ratio of 3 intermediate-tolerant : 1 sensitive but not with 1 tolerant : 2

intermediate : 1 sensitive (Table 5.2) apparently as a result of misclassification. The misclassification of sensitive as tolerant-segregating families was also observed for the F_3 populations of the crosses (418T-1 x G61450), (418T-1 x Halberd) and (418T-1 x Schomburgk). Chi-square analysis indicates that the segregation of (418T-1 x G61450) and (418T-1 x Halberd) was not consistent with the ratio of 3 tolerant-segregating : 1 sensitive (Table 5.4). This misclassification is because of the lack of bimodal distribution possibly due to low boron concentration.

In (418T-1 x G61450), there were only 8 families classified as sensitive in comparison to the expected frequency of 24, while there were 88 families classified as tolerant-segregating in comparison to the expected frequency of 72 (Table 5.4). This indicated a general deficiency of the sensitive segregants for the crosses having 418T-1 as a parent. Since 418T-1 was selected as the most tolerant line in response to boron (Figure 5.1), there were possibly effects of minor genes other than the genes *Bo1* and *Bo4* in the response to boron of this line. Thus, the homozygous sensitive families derived from the crosses having 418T-1 as a parent, (for example, (418T-1 x G61450), (418T-1 x Halberd) and (418T-1 x Schomburgk)), were slightly more tolerant than the sensitive parent and were therefore misclassified as being tolerant-segregating in these three crosses. For example, Families 5-8 of (418T-1 x G61450) (Figure 5.13a) and Families 1 and 2 of (418T-1 x Schomburgk) (Figure 5.18a) classified as homozygous sensitive were slightly more tolerant than the sensitive parents.

For the cross (418T-1 x G61450), segregation of the F_3 families was not consistent with the monogenic ratio and this was mainly due to a low frequency of sensitive families and a high frequency of segregating families, possibly because of the misclassification as described above. However, the frequency of the families classified as homozygous tolerant (19) was very close to the frequency (24) expected for this category (Table 5.4) and chi-square analysis indicated segregation of the F_3 families of this cross was consistent with the monogenic ratio of 3 sensitive-segregating : 1 homozygous tolerant ($\chi^2_1 = 1.39$, $0.05 < P < 0.25$) (data were not shown).

The use of the LSD of the parental variances to differentiate between the families was not appropriated in the case where the variances of the parents were comparatively high. A high variance of the parent will increase the LSD. In (442S-1 x Halberd), the variances of 442S-1 and Halberd were 1.2 and 2.7, respectively, and the LSD of the parental variances was 7.2 (Figure 5.10b) which was high in comparison to that of 4.6 of (442S-1 x G61450) where the variances of 442S-1 and G61450 were 1.0 and 1.4 (Figure 5.10a), respectively. The LSD of the parental variances are, in general, subject to a high level of uncertainty.

The use of the confidence interval of the mean root length of the two parents for classification of the families into categories must also be applied with caution because of the errors from over (Error Type II) and under (Error Type I) estimation (Chapter 3). For example, in F_3 populations of (418T-1 x Halberd) and (418T-1 x Schomburgk), the number of the families classified as tolerant-segregating were over-estimated in comparison to the expected frequency, whereas the number of the families classified as sensitive were under-estimated (Table 5.4). Hence, a few families (about 5%) are expected to be beyond the confidence interval of the parents (Figure 5.18a).

The expected variance for one gene was likely to be over-estimated in some instances due to the high value of *d* (the departure of one of a pair of corresponding homozygotes from their mid-point or mid-parent (*m*). For example, the *d* values of the F_2 and F_3 populations of (418T-1 x G61450) (Table 5.3 and 5.5) were higher than expected due to the high mean of the parental line 418T-1 compared to its value with other populations. The mean root length of 418T-1 was 14.4 and 17 cm, respectively, for F_2 and F_3 populations of (418T-1 x G61450) (Figures 5.4a and 5.8a) in comparison to 12.2 and 13.7 cm of 418T-1 for the F_2 and F_3 of (418T-1 x Schomburgk) (Figures 5.5a and 5.9a) and 13.1 and 13.7 cm for (418T-1 x Halberd) (Figures 5.8b and 5.4b). If the means of 418T-1 had been about 12 and 15 cm, respectively, for the F_2 and F_3 populations of (418T-1 x G61450) (Figures 5.4a and 5.8a), the observed variances would be similar to the expected variances for the one gene model.

The expected variances of the F_2 population of (418T-1 x G61450) (Table 5.3) and F_3 population of (442S-1 x Halberd) (Table 5.5) may also be over-estimated due to the high value of the environmental variance (*E*) calculated from the variances of parents. For example, the variances of the parents 442S-1 and Halberd of F_3 population of (442S-1 x Halberd) (Table 5.5) were higher than expected in comparison to the same parents with other crosses and thus increasing the expected variances of this population.

There were also examples of the expected variance being lower than expected on the basis of the hypothesis and this may be due to low values of *d* and *E* . For example, the expected variance for two genes for the F_2 generation of (418T-1 x 442S-1) may have been low due to the low value of *d*. The value of *d* for the F_2 generation was 4.1 (Table 5.3), compared to a value of 7.4 for the F_3 generation (Table 5.4).

This method of estimation of the number of genes should be used cautiously, especially when the difference between mean of the parents is either very large or very small.

Potential for breeding

The Greek line G61450 (here confirmed as being of the genotype *bo1bo1Bo2Bo2Bo3Bo3Bo4Bo4*) and Australian variety Halberd (*Bo1Bo1Bo2Bo2Bo3Bo3bo4bo4*) have been classified as tolerant and moderately tolerant, respectively (Moody et al., 1988). The greater level of tolerance exhibited by G61450 compared to Halberd (Figure 5.1) indicates that the *Bo4* allele is more potent than *Bo1*. For example, the mean root length of G61450 and Halberd in this experiment were approximately 9.0 and 10.5 cm, respectively. The gene product of *Bo4* would therefore appear either to be expressed at a higher level than *Bo1* or to affect a different step in the exclusion of boron from plants.

The results of this experiment indicate that when the *Bo1* and *Bo4* alleles are combined in the one line, such as 418T-1, a higher level of tolerance than either parent is expressed (Figures 5.1 and Plate 5.1). This was demonstrated by the mean root length of 418T-1 in this experiment (about 13.5 cm) being longer than those of G61450 (10.5 cm)

and Halberd (9.0 cm). Therefore varieties more tolerant than G61450 could be bred by transferring the two genes *Bo1* and *Bo4* to well adapted varieties using the backcrossing method.

Chapter 6

Chromosomal location of genes controlling tolerance to a high concentration of boron

6.1 Introduction

Identifying chromosomes controlling boron tolerance would assist in

(a) the prediction of parental varieties which, when crossed, will show transgressive segregation. These varieties could have different chromosomes controlling boron tolerance. Transgressive segregation indicates at least two genes involved in controlling the character, but, while it is possible that more than one gene can be located on the one chromosome, it is more likely that the segregation will be independent and separate chromosomes be involved. When the number of genes are defined, precise breeding methods, such as backcrossing can be employed. If accurate techniques for the screening of those characters are devised, selection for transgressive segregation is likely to be rapid and effective.

(b) determination of linkage between the character of interest and other readily identified factors. For example, the use of linkage between brown glumes and stripe rust resistance enhanced the rapid selection for stripe rust resistance and resulted in the release of the wheat variety Angas. While selection for stripe rust resistance is not possible in summer in southern Australia, it is feasible to select for the brown glumes strongly linked to the stripe rust resistance. Establishing linkage maps, including the molecular markers of considerable current interest, allows the identification of closely linked markers which could be used for selection.

(c) prediction of the situation in other species based on Vavilov's law of homoeologous variation. For example, the homoeologous chromosomes, 1A, 1B, 1D of wheat (*T. aestivum*) and 1R of rye carry similar homoeologous genes such as the structural genes for seed storage proteins (Wang et al., 1992). Genetic maps of the homoeologous group two chromosomes indicates that gene orders are highly conserved in the genomes of wheat, barley and rye, except for the distal ends of chromosome arms 2BS and 2RS, which have

been involved in interchromosomal translocations (Devos et al., 1993). From analogy with wheat, barley will also have genes controlling boron tolerance on chromosome groups four and seven. Therefore, to identify the chromosomal location of the gene in barley, chromosomes four and seven should be the first to be studied.

Paull's (1990) results with reciprocal monosomic and monosomic analysis using aneuploid stocks of Chinese Spring and Federation were equivocal, suggesting that chromosomes 7B and 7D were the most probable locations of genes for tolerance to boron of Federation and G61450, respectively. Several chromosomes in the reciprocal monosomic analysis of Federation responded in the manner expected of a critical chromosome. Of these, 7B appears to be the most probable, however, other chromosomes possibly implicated include 3A, 3B, 5B and 2B. For the monosomic analysis of G61450, chromosomes 7D and 4A were the most probable locations of the genes. However, there was some uncertainty on the classification as sensitive of some of the $F_3^{substitution}$ lines of chromosomes 7D and 4A. This occurred as a result of the small difference in response between parents for symptoms of boron toxicity on the leaves and the relatively large environmental effects. The experiments described in this chapter attempted to locate the genes controlling tolerance to boron by monosomic analysis but using response as determined by seedling root length under high boron conditions in filter papers, instead of the leaf symptoms as used by Paull (1990).

The experiments described in this chapter were conducted to

(a) determine whether the responses of plants to boron is modified when chromosomes are present in the monosomic condition in comparison to disomics for the 21 monosomics of Condor and, if so, to identify the chromosomes involved.

(b) Since Halberd and G61450 were found to be more tolerant than Condor (Chapter 4) and to differ in genetic control of boron tolerance (Chapter 5), closer investigation of the genetic control of these varieties was needed. The F_2 monosomic analysis (Sears, 1953) with Condor monosomics as aneuploid stocks was used to identify the chromosomal locations of genes in these two varieties.

Monosomic analysis

Monosomic series, the set of 21 aneuploids (2n = 41) with single missing chromosome, can be manipulated to determine the chromosome carrying a particular gene (Sears, 1953; Kuspira and Unrau, 1959).

All of the 21 possible monosomics and trisomics (2n = 43), together with their nullisomic (2n = 40) and tetrasomic (2n = 44) derivatives, were developed in the bread wheat variety Chinese Spring (Sears, 1954). The essential genotypes for the development of the monosomic series were either the haploid or nullisomic 3B of Chinese Spring which have reduced chromosome pairing at meiosis. Backcrosses with these genotypes produced a large number of monosomics with the complete set of 21 monosomics eventually being derived by selection among these back-cross progenies (Law and Worland, 1973). The monosomic series of Condor were developed from the crosses between the 21 monosomic series of Chinese Spring as female and CSP44 (Condor Single plant Selection 44) as a male donor variety. The F_1 monosomic plants of each cross were cytologically identified and backcrossed seven times to the donor variety CSP44 (R. A. McIntosh, pers. comm.).

Monosomic analysis consists of crossing each of the twenty one different monosomics as the female with a variety carrying a character to be analysed (Figure 6.1). Hemizygous (unpaired) chromosomes identified cytologically in the F_1 must derive from the male donor. Monosomic analysis has been widely used in mapping genes for both quantitative (Larson, 1966) and qualitative characters (Law and Worland, 1973). For example, the monosomic analysis was used to identify the chromosomal locations of genes controlling plant height (Petrovic, 1979; Worland et al., 1988), glume pubescence (Sridevi et al., 1989), culm length (Allan and Vogel, 1963), awns (Ganeva and Bochev, 1988) and stripe rust (*Puccinia striiformis*) resistance (Macer, 1966; Chen et al., 1991).

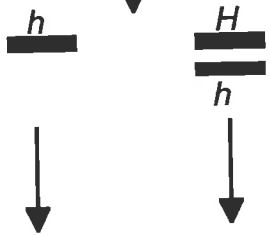
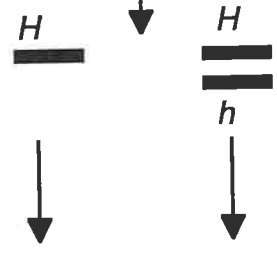
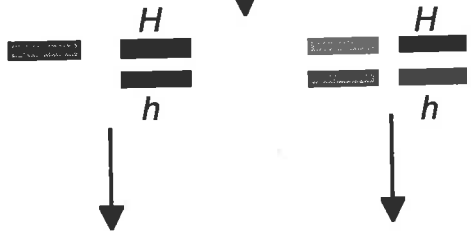
If the allele in the donor variety of the gene being analysed is recessive to the allele carried by the recipient monosomic variety, the monosomic plants of the critical F_1 families (for example in Figure 6.1, monosomic 1A is the critical chromosome) will all have the recessive phenotype. All the twenty other families will have the dominant phenotype. In this case it is not necessary to examine the F_2. However, if the F_2 population of the

Figure 6.1 Procedure for monosomic analysis in F_1 and F_2 generations for the analysis of the chromosomal location of the gene for glume pubescence on 1A. Derived from K. W. Shepherd (pers. comm.).

Note: P = Parents, *H* = a dominant gene controlling glume pubescence, *h* = a recessive gene controlling non-pubescent glumes

▬ indicates a non critical chromosome (does not carrying the gene)

▬ indicates the critical chromosome

Generation	Crosses with monosomic 1A (critical cross of the chromosome 1A carrying the gene)				Crosses with other 20 monosomics (non critical crosses)	
	recessive		dominant			
P						
F1						
F2 Ratio	**all h**	3H:1h	**97H:3h**	3H:1h	3H:1h	3H:1h
Disomic (~ 24 %)	hh	3H:1h	HH	3H:1h	3H:1h	3H:1h
Monosomic (~73 %)	h -		H -		3H:1h	
Nullisomic (~3 %)	-- (hh)		-- (hh)		3H:1h	

critical family is examined, all of the plants, disomic, monosomic and nullisomic plants, will again have recessive phenotypes because there is only the recessive allele on the hemizygous chromosome in the F_1 (Figure 6.1).

When the allele in the donor variety is dominant, all the F_1 offspring of all the 21 families express the dominant phenotype. In the F_2 generation, the differences between the families derived from different F_1 monosomics will appear. All the F_2 monosomic families, except one, will segregate as three dominant to one recessive phenotype. For the critical monosomic family, almost all the progeny will have the dominant phenotype, with only a small proportion of segregants, depending upon the differences in the transmission frequency of the univalent chromosome between male and female gametes, will have the recessive phenotype. For the monosomic plants, approximately 75% and 4% of the functioning female and male gametes have twenty chromosomes, thus the progeny from selfing will include disomics, monosomics and nullisomics in an approximate ratio of 24 : 73 : 3 percent, respectively (Sears, 1944, 1953) (Table 6.1). Of these, only the nullisomic progeny will lack the dominant allele and have the recessive phenotype. A ratio of 97 dominant : 3 recessive (nullisomics) (Table 6.1 and Figure 6.1) should be obtained in this particular case. Thus, the critical chromosome can be identified by deviation from the usual 3 : 1 segregation ratio (Sears, 1953).

For a check population, the F_2 disomic segregants from the cross between the variety being investigated and the variety used to generate the aneuploid stocks may be used (Kuspira and Unrau, 1959). For example, in the experiment reported here, the F_2 populations of (Halberd x Condor disomic) and (G61450 x Condor disomic) were used as the check for the F_2 populations.

Monosomic analysis may also detect critical chromosomes for two or more genes and for different types of gene action and interaction (Sears, 1953; Kuspira and Unrau, 1959).

Table 6.1 The frequency of disomics, monosomics, and nullisomic progenies derived from selfing of a monosomic plant. Derived from Kuspira and Unrau (1959).

Female gamete	Male gamete	
	96% (n)	4% (n-1)
25% (n)	24% (disomic)	1% (monosomic)
75% (n-1)	72% (monosomic)	3% (nullisomic)

n = haploid number of chromosome = 21

6.2 Materials and methods

Genetic Materials

Seeds of the monosomic series of Condor (selection P44) were kindly provided by Dr. R. A. McIntosh, Plant Breeding Institute, University of Sydney. The twenty-one monosomic families of Condor were multiplied in standard potting mix in a glasshouse. The plants were cytologically examined at meiosis to identify monosomics using the methods described in chapter 3. The seeds of each monosomic family were harvested from the identified monosomic plants.

Halberd and G61450 were derived from stocks selected from single plants by Paull (1990).

Boron response of Condor monosomic families

The twenty-one monosomic families and the disomic of Condor were tested for response to boron using the filter paper technique (Chapter 3). The experiment consisted of a split plot design with fifteen seeds of each family per filter paper, two replicates and three boron treatments B0, B50 and B100.

The experimental procedures, including the pre-treatment and treatment of seeds and the measurement of the roots, were as described in Chapter 4. The analysis of variance was calculated using the MSTAT microcomputer program (Chapter 3).

F_2 monosomic analysis

The twenty-one monosomic families of Condor were sown and selected plants, identified as monosomics by cytological examination of pollen mother cells at metaphase I (Chapter 3), were crossed as female parents with Halberd and G61450. F_1 hybrids were also examined for their chromosome complement at metaphase I of meiosis and monosomic plants selected.

F_2 seeds from the F_1 monosomic plants of each cross were tested for segregation in response to boron and compared to the control disomic F_2 of the crosses (Condor x Halberd) and (Condor x G61450).

Approximately one hundred and ten seeds of each of the 21 F_2 populations of the monosomic crosses and the corresponding disomic F_2 were tested for each of the two donor parents. Eleven seeds of each cross plus two seeds of each parent (Condor and Halberd or G61450) were placed in a filter paper, treated with 100 mgB l^{-1}. The experimental procedure included the pre-treatment and treatment of seeds and the measurement of the roots followed the methods described in Chapter 4.

The following methods were used to differentiate between the segregation patterns of the F_2 populations of the monosomic crosses and the disomic population.

(a) If the distributions of the populations were bimodal the F_2 seedlings were classified into two categories (tolerant-intermediate and homozygous sensitive) according to the distribution patterns. Chi-square analysis was then used to test for the goodness of fit of the observed segregation ratio to monogenic (3 tolerant-intermediate : 1 sensitive) and digenic ratios (15 tolerant-intermediate : 1 sensitive). The F_2 population derived from the monosomic of the critical chromosome would be expected to have a segregation pattern deviating from the disomic F_2 population.

(b) When the distributions of the populations were continuous, it was not possible to classify the F_2 seedlings into distinct categories. In these populations, the variance of each of the 21 F_2 populations derived from the monosomics was compared with that of the disomic population. The variance of the F_2 population with the critical chromosome is

expected to be lower than that of the disomic population with the latter expected to be close to those of the other F_2 populations based on non-critical chromosomes.

The mean length of roots of the F_2 population derived from the monosomic of the critical chromosome is also expected to be longer than those of populations derived from non-critical chromosomes and the disomic F_2 population. This is because the segregation ratio for the critical F_2 population is 97 tolerant : 3 sensitive in comparison with 3 tolerant : 1 sensitive for the disomic and the non-critical F_2 populations.

6.3 Results

Boron response of monosomic families

A highly significant interaction between monosomic families and boron treatments indicates that there was variation in response to boron treatments among the monosomic families. At B0, the root lengths of some monosomic families (1B, 2B, 3A, 3B, 3D, 4A, 5A, 5B and 7A) were significantly shorter than those of the disomic (Figure 6.2). However, the non-significant variation of the means and standard deviations of root length among the families of the monosomics and the disomic at both of the boron treatments B50 and B100 demonstrated that the genes in the hemizygous condition had no effect on the response to boron in the monosomic families (Figure 6.2). Examples of monosomic families for chromosomes groups 4 and 7, the most probable chromosomal locations of genes conferring tolerance to boron in wheat (Paull, 1990), at B0 and B50 are presented in Plate 6.2.

F_2 monosomic analysis

Halberd populations

In the combinations between the monosomics of Condor and Halberd, the F_2 populations showed a continuous distribution in response to high boron (Figure 6.3). Thus, it was not possible to classify the F_2 seedlings into distinct categories. The variance of each F_2 population was compared with that of the disomic F_2. The variance of population derived from monosomic 7B was significantly less than those of the disomic F_2

Figure 6.2 Seedling root length (cm) of 21 Condor monosomic families in comparison with the Condor disomic variety when tested in filter papers at B0, B50 and B100.

Note: The vertical bars attached to the histograms represent standard deviations of the means. An unattached vertical bar represents the LSD (0.05) for the genotype x treatment interaction.

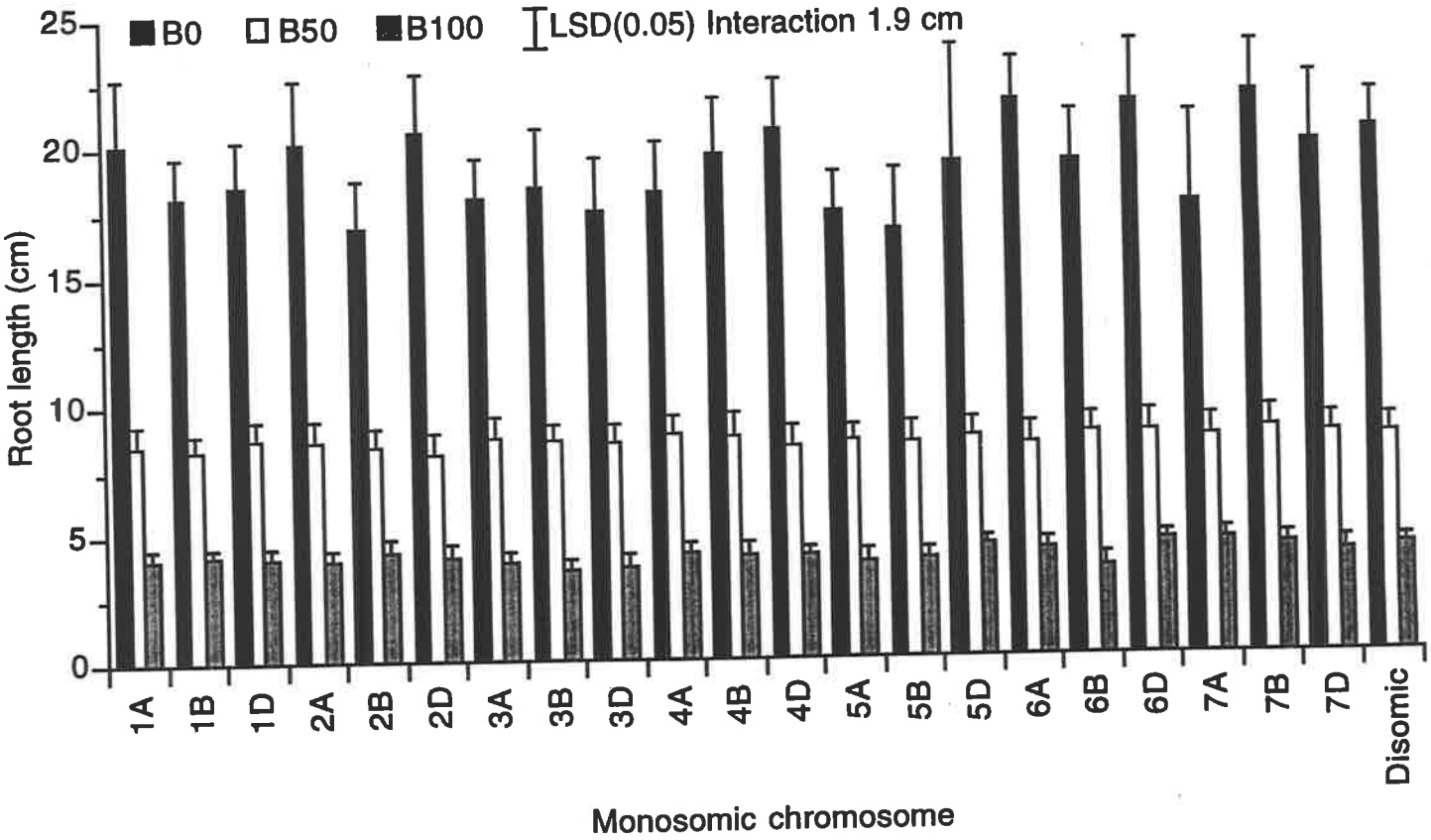

B0
B50
B100
LSD(0.05) Interaction 1.9 cm
Root length (cm)
25
20
15
10
5
0
1A
1B
1D
2A
2B
2D
3A
3B
3D
4A
4B
4D
5A
5B
5D
6A
6B
6D
7A
7B
7D
Disomic
Monosomic chromosome

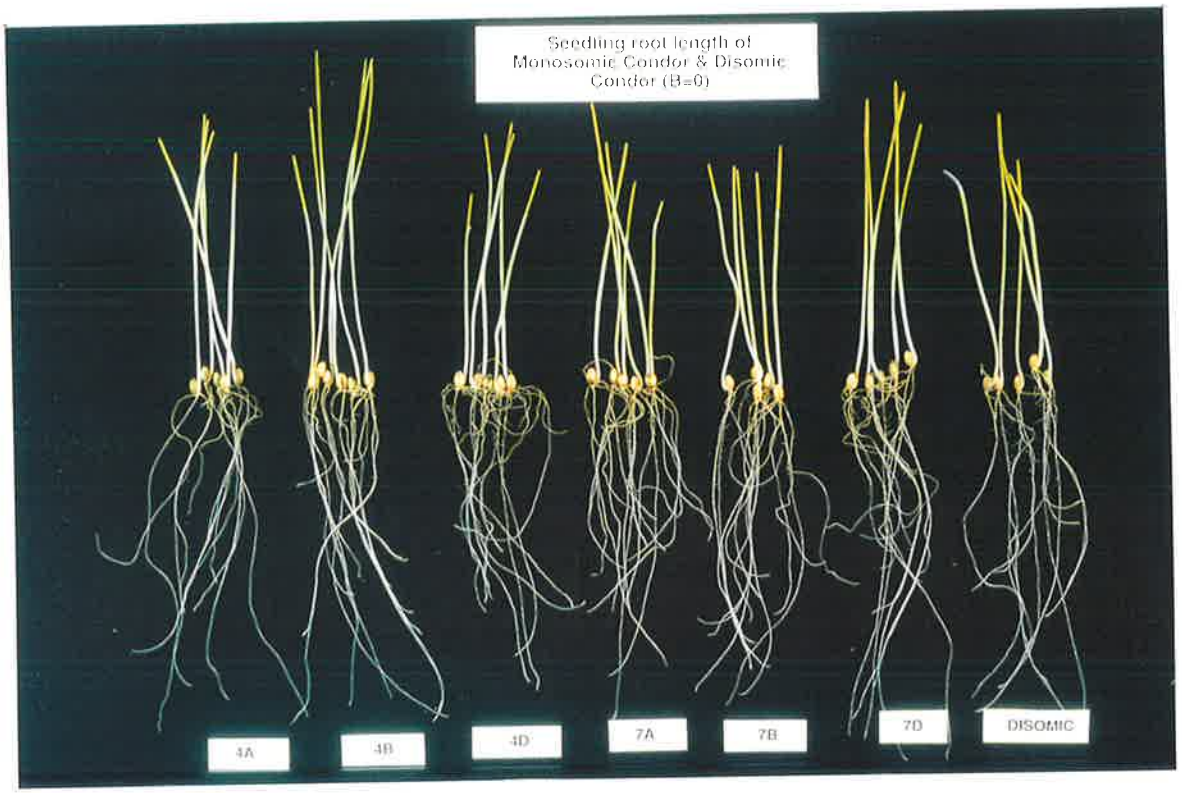
Plate 6.1 Comparison of the response of the Condor monosomic families for chromosomes of homoeologous groups 4 and 7 and the disomic when tested at two boron levels.

(a) B0

(b) B50

Note: Left to right; Condor monosomic families of chromosomes 4A, 4B, 4D, 7A, 7B, 7D and the disomic of Condor

(a)


Seedling root length of
Monosomic Condor & Disomic
Condor (B=0)
4A
4B
4D
7A
7B
7D
DISOMIC

(b)

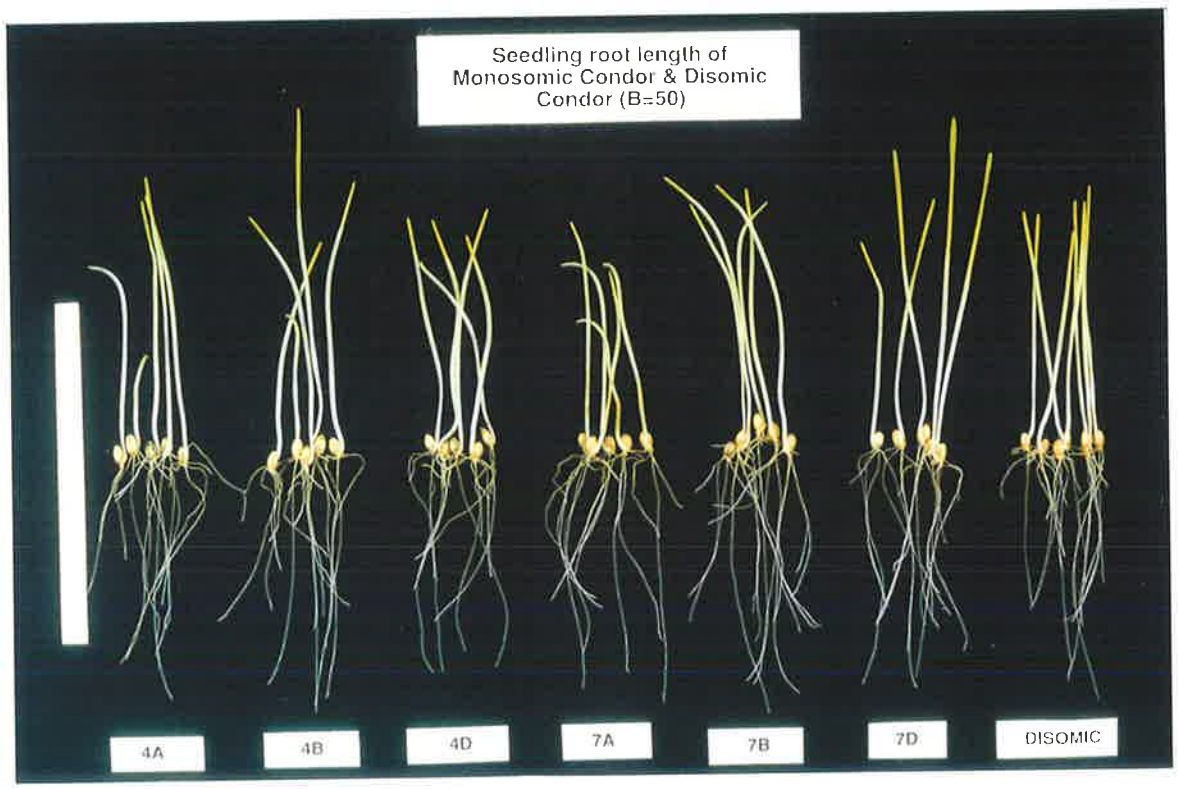

Seedling root length of
Monosomic Condor & Disomic
Condor (B=50)
4A
4B
4D
7A
7B
7D
DISOMIC

Figure 6.3 Percentage distribution for seedling root length (cm) of the 21 F_2 monosomic families derived from the crosses between the monosomic Condor series and Halberd and the two parents when tested in filter papers at B100.

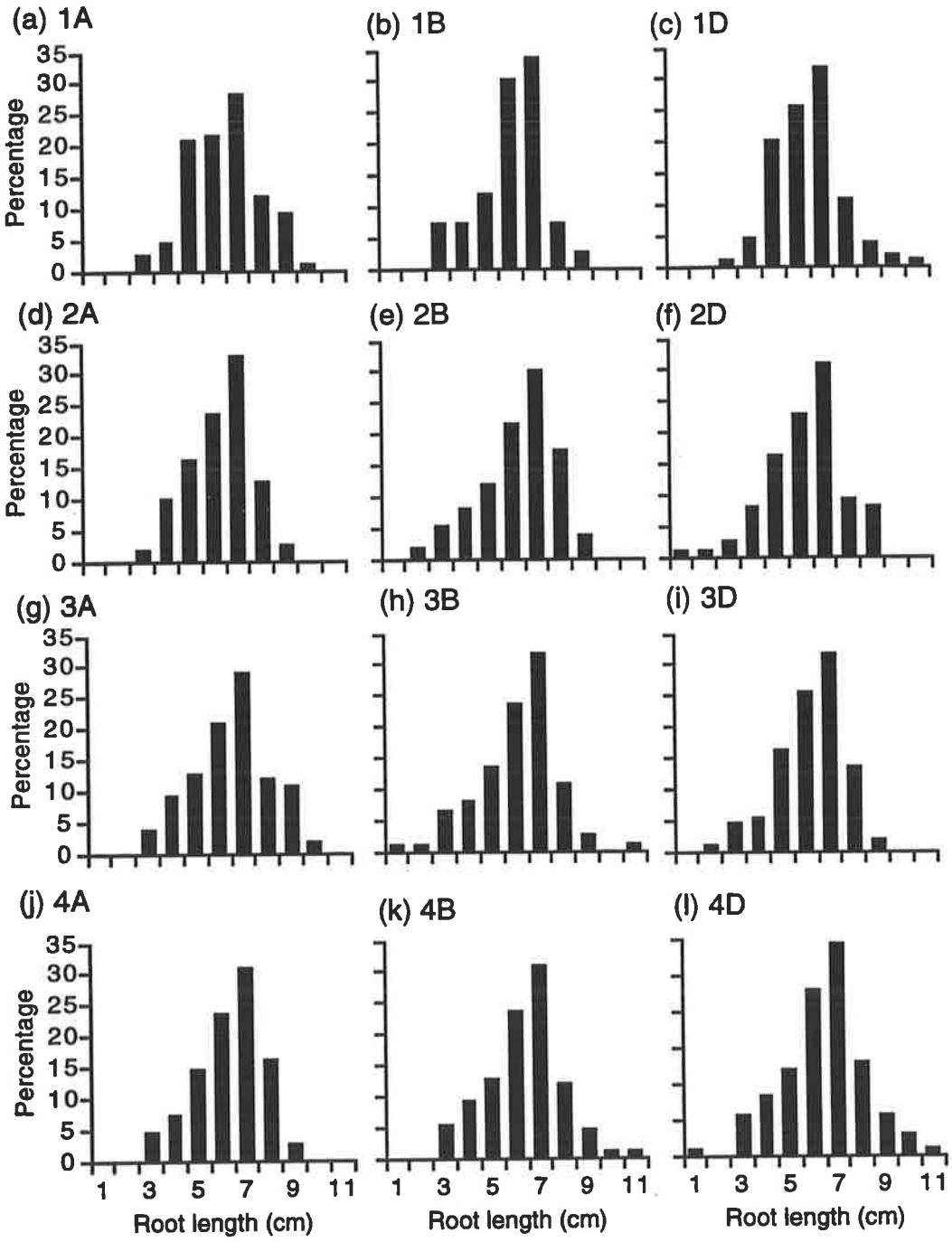
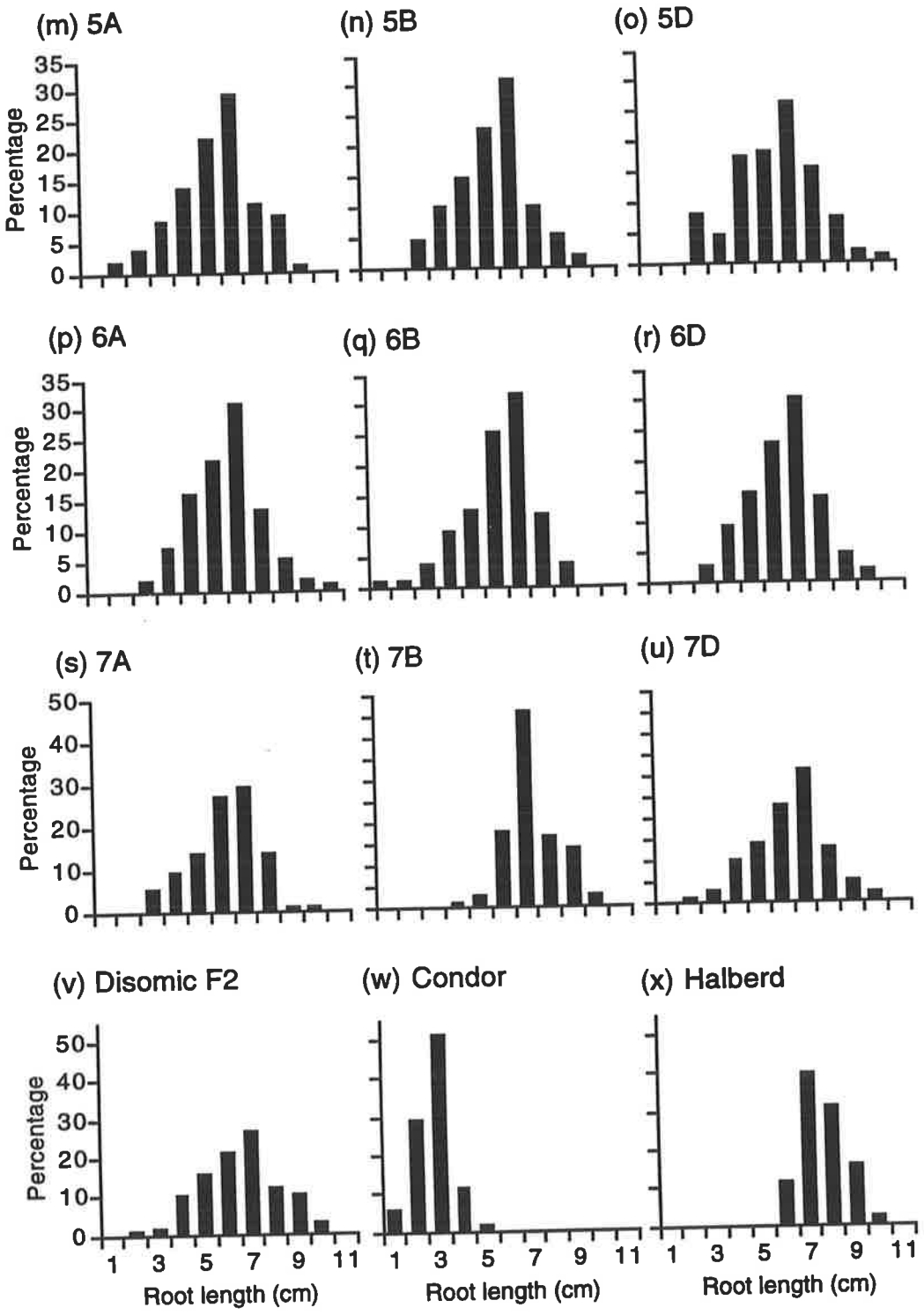


Figure 6.3 (continued).



and other populations (Table 6.2) although the populations derived from monosomics 1A, 1B, 1D, 2A, 3D, 4A, 6A, 6D and 7A also had low variances. However, the mean root length of the F_2 population derived from monosomic 7B was longer than that of the disomic F_2 and the other populations (Table 6.2). The low variance and high mean for the monosomic 7B population is consistent with a very high frequency of tolerant and very low frequency of sensitive plants as is expected for the critical chromosome in monosomic analysis. Examples of the response for F_2 population derived from monosomic 7B and the disomic F_2 control population in comparison with their parents Halberd and Condor are presented in Plate 6.1. This result indicates that the boron tolerance of Halberd, relative to Condor, is under the control of a gene located on chromosome 7B.

G61450 populations

The distributions of F_2 disomic population of (Condor x G61450) and all of the F_2 populations derived from the crosses between the monosomic families of Condor and G61450 showed bimodal distribution (Figure 6.4).

A cut-off point at 6 cm of the disomic population was used for all of the other populations. Thus, the F_2 seedlings with a root length less than or equal to 6 cm were classified as sensitive and the F_2 seedlings with a root length more than 6 cm were classified as tolerant-intermediate. Chi-square analysis indicated that the F_2 disomic control population segregated in the monogenic ratio of 3 tolerant : 1 sensitive (78 : 32, $\chi_1^2 = 0.98$, $0.5 < P < 0.25$) (Table 6.3). The segregation ratios of all the F_2 populations except for those derived from monosomics 1D, 2A, 2B, 3A, 3B, 3D, 4A, 4B and 5A, were consistent with the monogenic segregation ratio of 3 tolerant-intermediate : 1 sensitive (Table 6.3). The F_2 population derived from monosomic 4A had the fewest seedlings with short roots (Figure 6.5) and the segregation ratio of root length (> 6 cm : ≤ 6 cm) (96 : 14, $\chi_1^2 = 8.84$, $P < 0.001$) was highly significantly different from the 3 tolerant : 1 sensitive ratio (Table 6.3 and Figure 6.4j). Examples of the F_2 population derived from monosomic 4A and the disomic F_2 control population in comparison with their parents are presented in Plate 6.3. The other significant deviations of the segregation ratios for monosomics 1D, 2A, 2B, 3A,

Table 6.2 Minimum, maximum and mean root length (cm) and variance of the root length of 21 F_2 monosomic families derived from crosses between the monosomic Condor series and Halberd compared to those of the disomic cross and the parental varieties.

Chromosome	Min	Max	Total	Mean	Variance	F-test
1A	2.8	9.7	110	6.5	2.0	1.7**
1B	2.9	9.5	110	6.0	1.7	1.4*
1D	3.3	10.7	110	6.4	1.7	1.4*
2A	2.9	8.8	110	6.1	1.7	1.4*
2B	2.2	9.4	110	6.2	2.3	1.9**
2D	1.2	9.5	110	6.2	2.6	2.2**
3A	3.0	10.0	110	6.5	2.6	2.2**
3B	1.1	10.5	110	6.0	2.6	2.2**
3D	2.1	9.3	110	6.2	2.0	1.8**
4A	3.1	9.2	110	6.2	2.0	1.8**
4B	2.5	10.9	110	6.4	2.9	2.4**
4D	1.4	10.6	110	6.5	2.9	2.4**
5A	2.0	9.6	109	6.5	2.9	2.4**
5B	2.5	10.0	110	6.3	2.6	2.2**
5D	2.6	10.9	110	6.4	3.2	2.7**
6A	3.1	10.6	110	6.4	2.0	1.7**
6B	1.5	9.0	110	6.1	2.3	1.9**
6D	2.7	9.7	109	6.3	2.0	1.7**
7A	2.9	9.9	110	6.2	2.0	1.7**
7B	4.2	9.9	109	7.3	1.2	
7D	2.0	10.3	110	6.3	2.3	1.9**
Control F_2	1.7	10.0	110	6.4	2.9	2.4**
Halberd	6.1	10.1	215	7.6	0.8	
Condor	0.9	5.1	212	2.7	0.6	

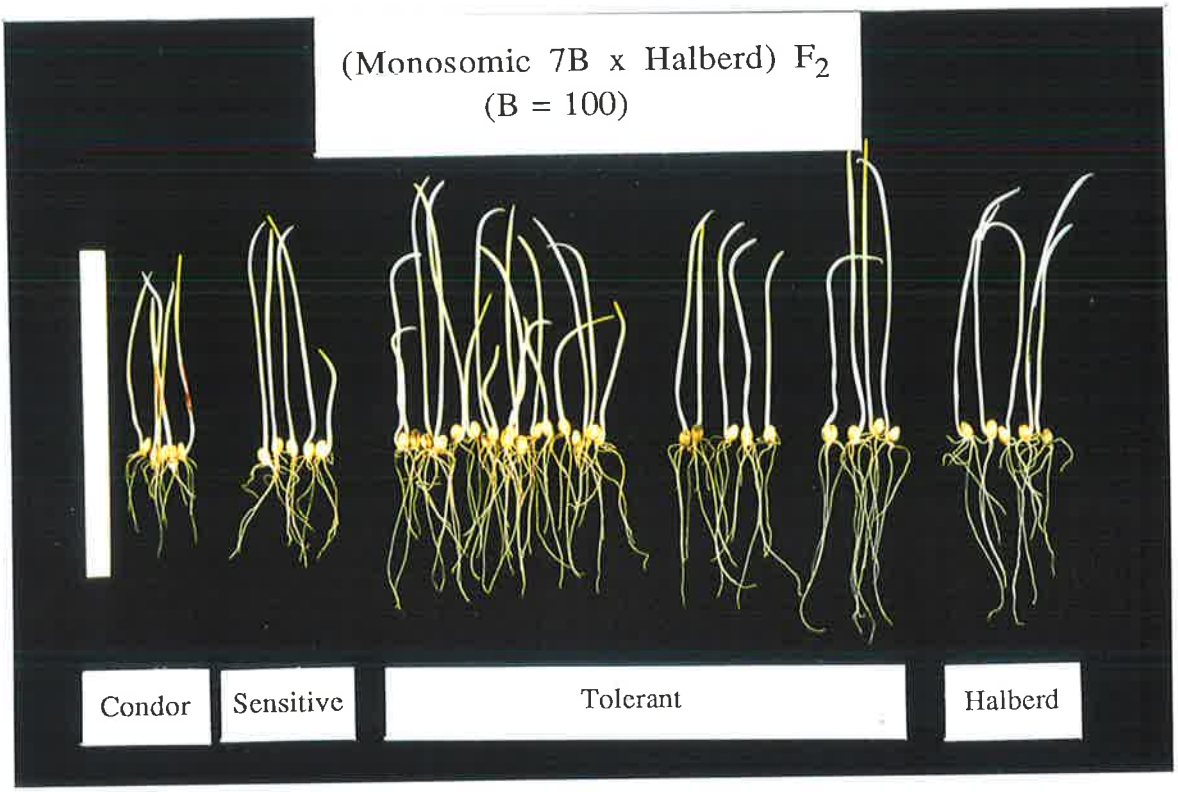
*, ** different from the variance of F_2 monosomic 7B at the 0.05 and 0.01 significance levels, respectively.

Plate 6.2 Response of F_2 populations to boron and the two parents Halberd and Condor when tested in filter papers at the B100.

(a) F_2 population derived from (monosomic 7B x Halberd)

(b) disomic F_2 control population

(a)


(Monosomic 7B x Halberd) F_2
(B = 100)
Condor
Sensitive
Tolerant
Halberd

(b)

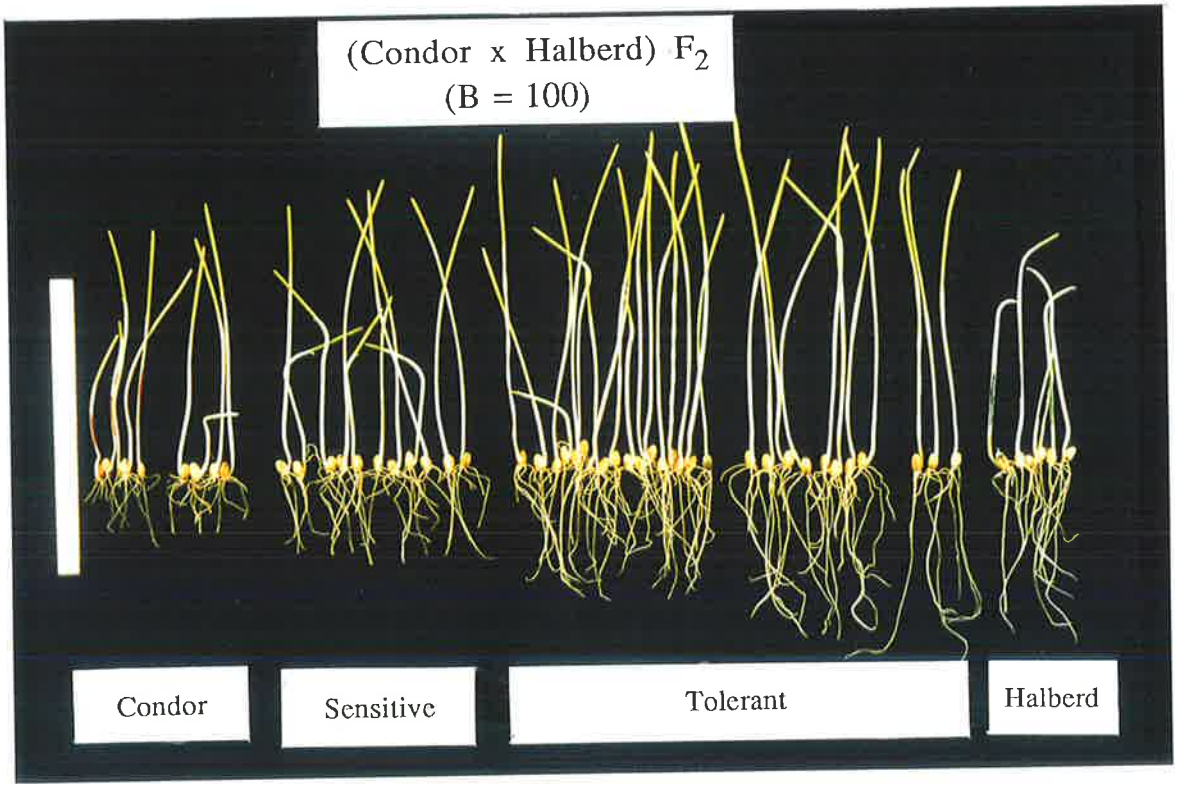

(Condor x Halberd) F_2
(B = 100)
Condor
Sensitive
Tolerant
Halberd

Figure 6.4 Frequency distribution for seedling root length (cm) of the 21 F_2 monosomic families derived from crosses between the monosomic Condor series and G61450 and the two parents when tested in filter papers at B100. The arrows indicate the cut-off points (at 6 cm) between sensitive and tolerant seedlings.

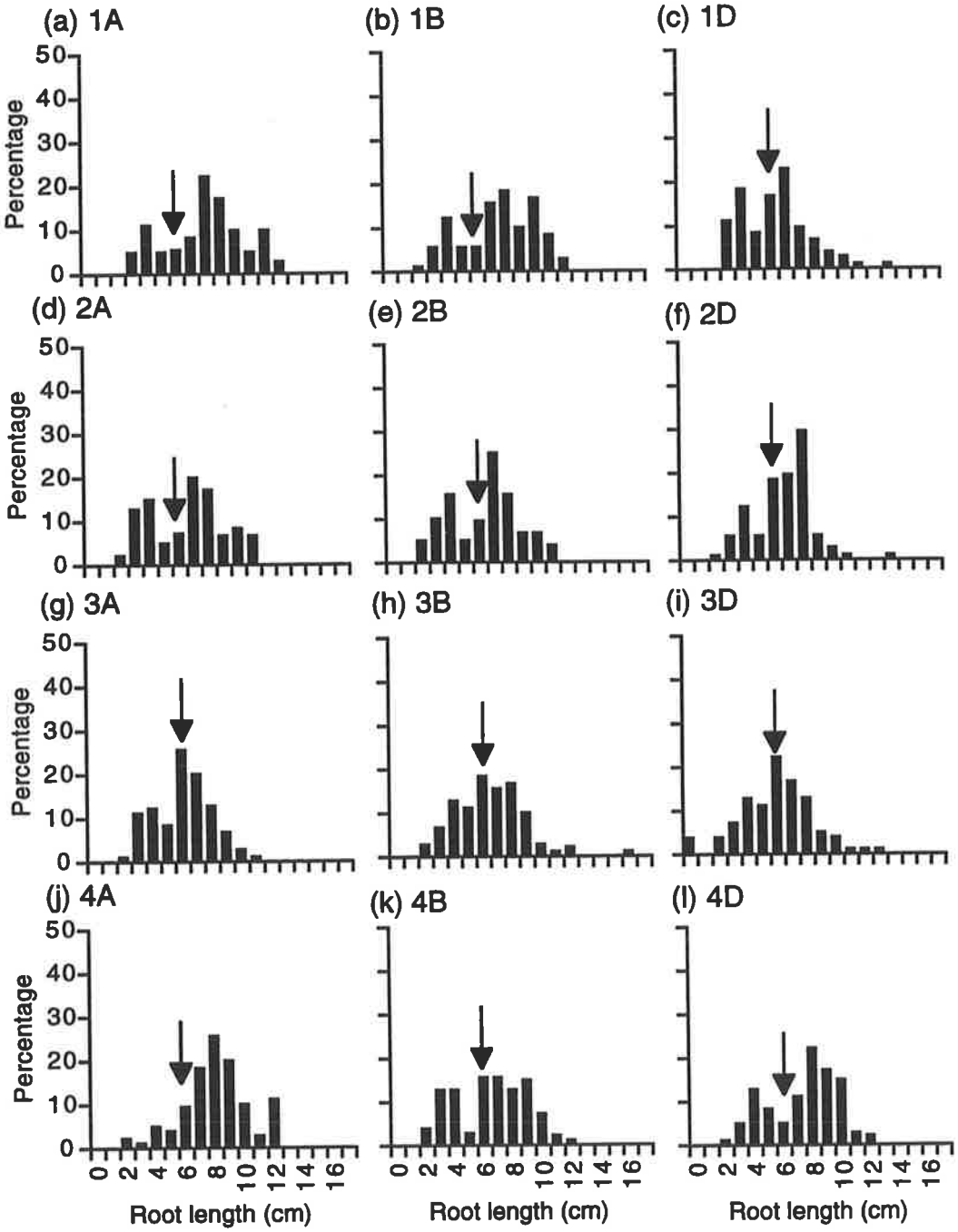


Figure 6.4 (continued).

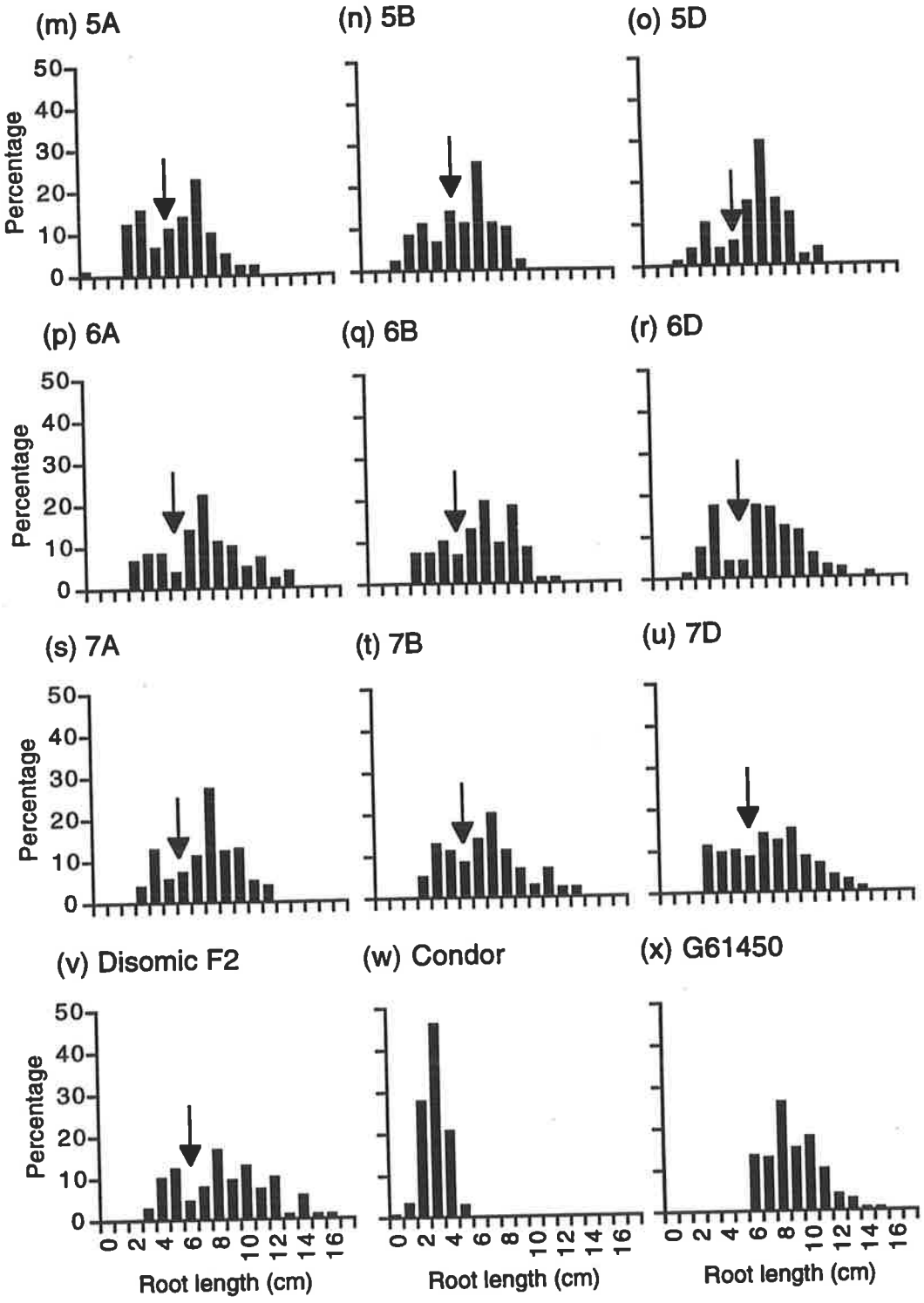


Table 6.3 Segregation for response to boron of 21 F_2 monosomic families derived from crosses between the monosomic Condor series and G61450.

Chromosome	Sensitive (≤ 6 cm)	Tolerant (> 6 cm)	Total number of seedlings	χ_1^2 for 3:1 ratio
1A	25	85	110	2.05
1B	28	82	110	0.01
1D	59	51	110	48.11**
2A	45	64	109	15.41**
2B	41	69	110	8.84**
2D	34	76	110	2.05
3A	49	61	110	22.41**
3B	47	62	109	19.08**
3D	66	44	110	71.87**
4A	14	96	110	8.84**
4B	41	69	110	8.84**
4D	32	78	110	0.98
5A	42	68	110	10.19**
5B	34	76	110	2.05
5D	25	85	110	0.30
6A	26	84	110	0.12
6B	31	79	110	0.59
6D	32	78	110	0.98
7A	29	81	110	0.12
7B	35	75	110	2.73
7D	36	74	110	3.50
Control F_2	32	78	110	0.98

Significance of differences: ** $P < 0.01$

Figure 6.5 Percentage of short roots (≤ 6 cm) of (Condor monosomic x G61450) F_2 families and the check disomic F_2, measured on 110 seedlings of each family at B100.

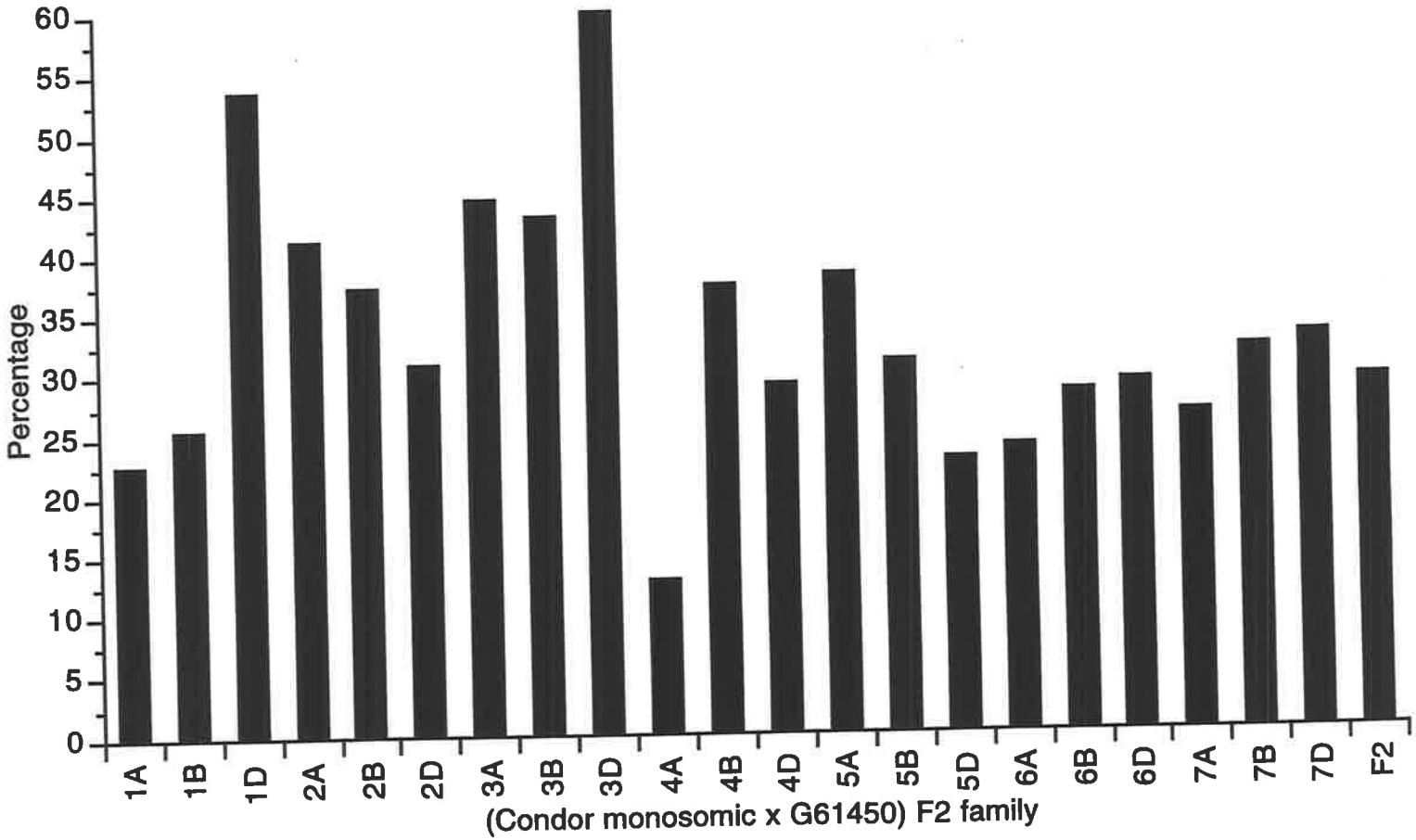
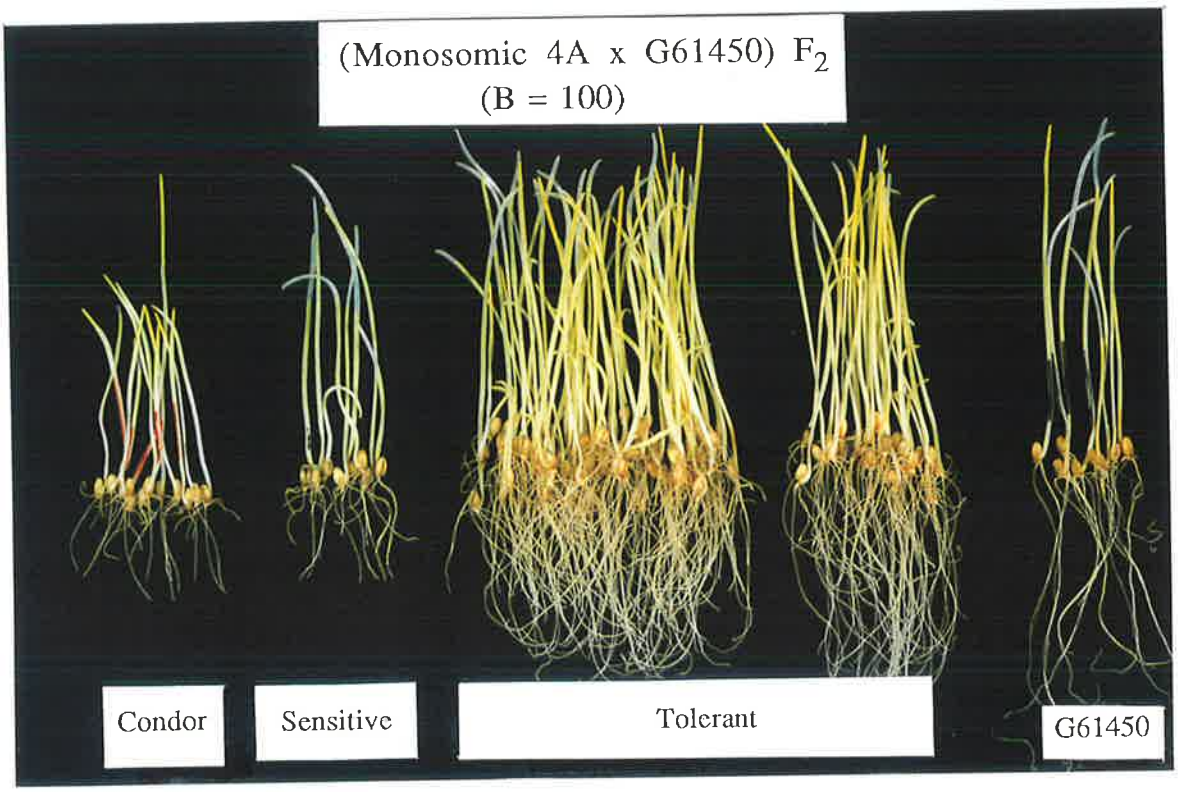

Percentage
60
55
50
45
40
35
30
25
20
15
10
5
0
1A
1B
1D
2A
2B
2D
3A
3B
3D
4A
4B
4D
5A
5B
5D
6A
6B
6D
7A
7B
7D
F2
(Condor monosomic x G61450) F2 family

Plate 6.3 Response of F_2 populations to boron and the two parents G61450 and Condor when tested in filter papers at the B100.

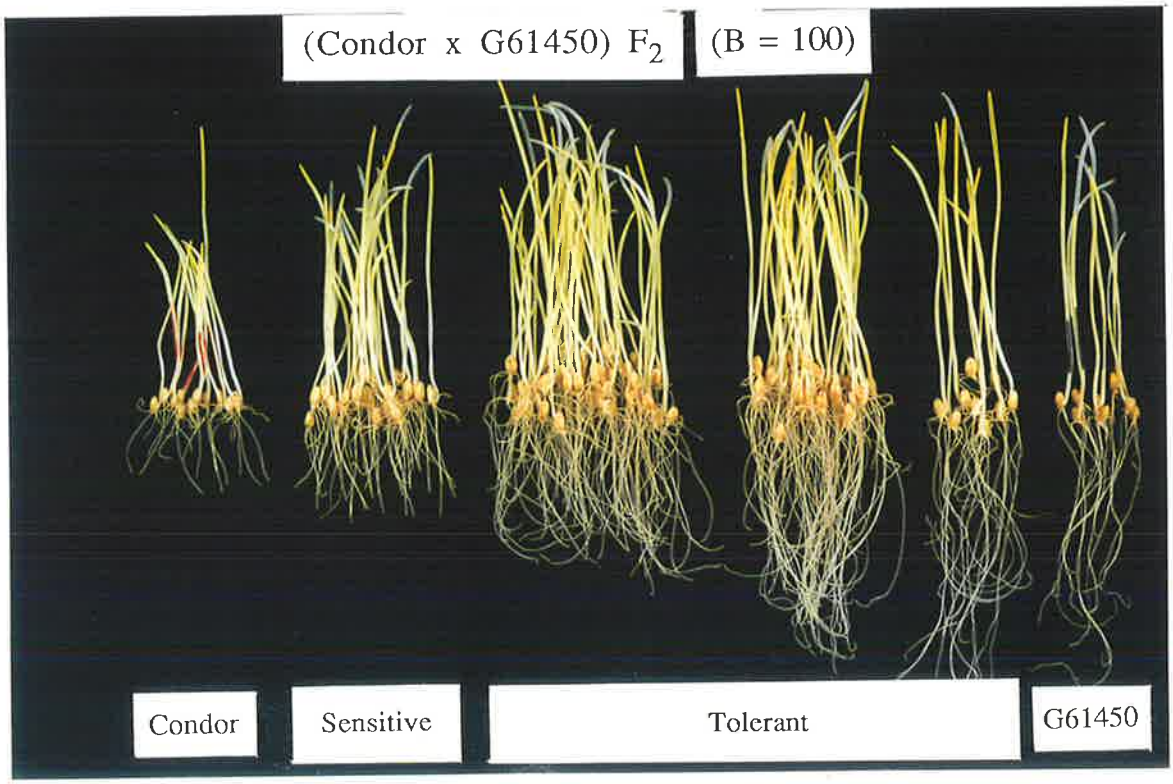
(a) F_2 population derived from monosomic 4A

(b) disomic F_2 control population

(a)


(Monosomic 4A x G61450) F_2
(B = 100)
Condor
Sensitive
Tolerant
G61450

(b)


(Condor x G61450) F_2
(B = 100)
Condor
Sensitive
Tolerant
G61450

3B, 3D, 4B and 5A from the 3 : 1 ratio were due to an excess of sensitive plants, probably resulting from the misclassification of poorly germinated seeds or low vigor seedlings as sensitive genotypes.

The mean root length of the F_2 population derived from the monosomic 4A was longer than those of the other F_2 populations, except those from monosomics 1A, 6A and the disomic F_2 population (Table 6.4). The maximum and minimum root lengths of these three latter populations were longer than those of the population derived from the monosomic 4A (Table 6.4), indicating that the three populations are more vigorous than that population derived from monosomic 4A. The variance of the F_2 population derived from monosomic 4A was the lowest for all of the F_2 populations, except those derived from 2D and 3A. As the chi-square analysis indicates that the F_2 populations derived from monosomics 1A, 2D, and 6A segregated in the monogenic ratio and were not different from the disomic F_2 population (Table 6.3) and that there was a high frequency of sensitive plants in the F_2 population from 3A (Table 6.3), these results indicate that chromosome 4A is responsible for boron tolerance in G61450.

6.4 Discussion

Although the disomic F_2 population of (Condor x Halberd) and all of its F_2 monosomic populations showed continuous distribution (Figure 6.3), thereby invalidating the segregation ratios as a means of identifying the critical chromosome, the variances and means of the F_2 populations indicated that monosomic 7B was the critical chromosome (Table 6.2). The low variance and high mean was consistent with a very high frequency of tolerant and very low frequency of sensitive plants as is expected for the critical chromosome. This result indicated that, relative to Condor, chromosome 7B is responsible for boron tolerance in Halberd.

In the monosomic analysis of G61450, all of the F_2 populations had bimodal distributions (Figure 6.4), and the comparison between the segregation ratios identified 4A as the critical chromosome (Table 6.3). This indicated that the boron tolerance of G61450, relative to Condor, is under the control of a gene located on chromosome 4A.

Table 6.4 Minimum, maximum, mean root length (cm) and variance the root length of 21 F_2 monosomic families derived from crosses between the monosomic Condor series and G61450 compared to those of the disomic cross and the parental varieties.

Chromosome	Min	Max	Total	Mean	Variance	F-test
1A	2.9	13.3	110	8.1	7.3	2.0**
1B	2.3	12.3	110	7.6	6.3	1.8**
1D	2.9	13.5	110	6.2	5.3	1.5*
2A	2.4	11.2	109	6.6	6.3	1.8**
2B	1.5	10.8	110	6.4	5.3	1.5*
2D	2.2	13.6	110	6.6	3.6	1.0
3A	2.5	10.7	110	6.1	3.6	1.0
3B	2.0	15.8	109	6.5	5.3	1.5*
3D	0.3	13.0	110	5.9	5.3	1.5*
4A	2.2	11.8	110	7.8	3.6	
4B	2.4	11.5	110	6.5	5.8	1.6**
4D	2.5	12.1	110	7.3	5.3	1.5*
5A	0.5	11.6	110	6.5	5.8	1.6**
5B	2.2	11.2	110	6.9	5.3	1.5*
5D	2.5	12.5	110	7.7	5.3	1.5*
6A	2.8	14.3	110	8.0	7.8	2.2**
6B	2.9	13.8	110	7.6	6.3	1.8**
6D	2.3	15.4	110	7.3	7.3	2.0**
7A	2.8	12.3	110	7.5	5.3	1.5*
7B	2.6	14.3	110	7.4	7.3	2.0**
7D	2.7	15.2	110	7.3	7.8	2.2**
Control F_2	3.0	16.5	110	8.4	9.6	2.7**
G 61450	6.0	15.2	322	8.8	0.6	
Condor	0.5	4.9	407	2.9	3.6	

*, ** different from the variance of F_2 monosomic 4A at the 0.05 and 0.01 significance levels, respectively.

Paull et al. (1991b) described three genes controlling tolerance to boron and the gene at which Halberd and Warigal differ with respect to boron tolerance was *Bo1*. As the pedigree of Warigal and Schomburgk are (WW-15 x Raven) and (((W3589 x Oxley) x Warigal #2) x Aroona #2), respectively, and Aroona has the same pedigree as Warigal, Schomburgk is virtually a sister line to Warigal. Schomburgk has the *Sr22* gene on chromosome 7A, effective against all pathotypes of stem rust in Australia, incorporated from the donor parent W3589 (Paull et al., 1994). Condor with a pedigree of (WW-15 x WW80) is also related to Warigal and Schomburgk because WW-15 is a common parent of the three varieties. As Condor, Schomburgk (Chapter 4) and Warigal (Paull et al., 1991b), exhibit a similar level of response to boron, and segregation studies (Chapter 5) indicated Condor and Schomburgk are genetically identical, it can be assumed that Warigal and Condor are the same with respect to tolerance to boron. It can therefore be concluded that the segregation observed between Halberd and Condor resulted from allelic variation at the *Bo1* locus shown here to be located on chromosome 7B.

The result of monosomic analysis of this Chapter is consistent with the report of Paull (1990) in that chromosome 7B is the location of a gene controlling boron tolerance of Federation, an ancestor of Halberd, relative to Chinese Spring. The other chromosomes he implicated, including 2B, 3A, 3B and 5B, in this monosomic analysis had F_2 populations with variances not significantly different from the variance of the disomic F_2 population (Table 6.2). The mean root lengths of these four F_2 populations were also lower than that of the F_2 population derived from monosomic 4A (Table 6.2) indicating that chromosomes 2B, 3A, 3B and 5B are not involved in the control of tolerance to boron in Halberd, relative to Condor.

The result of monosomic analysis of G61450, which indicates that, relative to Condor, chromosome 4A is responsible for tolerance to boron in G61450, is also consistent with the suggestion of Paull (1990). His alternative hypothesis, chromosome 7D, was not supported here. In this work, the segregation ratio of the F_2 population derived from monosomic 7D was consistent with the 3 tolerant-intermediate : 1 sensitive ratio (Table 6.3), indicating that chromosome 7D is not responsible for tolerance to boron

in G61450. Nor is there any evidence here for the hypothesis that more than one gene controls tolerance in G61450, relative to Chinese Spring (Paull, 1990). In the monosomic analysis in this Chapter, there is one gene difference between G61450 and Condor, therefore only chromosome 4A is responsible for difference between G61450 and Condor.

The difference in the critical chromosomes between G61450 and Halberd is consistent with the results of Chapter 5 showing that there were two alternative genes controlling response to high boron conditions between G61450 and Halberd, explaining the transgressive segregation observed in response to high boron concentrations for their F_2s (Paull et al., 1991b). Cross between parents having separate boron tolerance genes on chromosomes 7B and 4A would make it possible to select for more tolerant segregants in a breeding program for sowing in areas where high levels of boron occur in the soil.

The results of these experiments will facilitate further study, for example the establishment of linkage maps between the genes controlling boron tolerance and other marker genes. Linkage maps using DNA markers, restriction fragment length polymorphisms (RFLP), have been developed for the chromosomes of homoeologous group seven (Chao et al., 1989). As these markers show a high degree of polymorphism they could be applied to families segregating for response to boron to determine which of the markers are linked to boron tolerance. Paull et al. (1993) tested 110 F_7 derived lines of G61450 x KF for segregation with 43 RFLPs. Linkage on chromosome 4AL between *XksuG010* locus and the boron tolerance gene of G61450 (Paull et al., 1993) is consistent with the result of the monosomic analysis in this Chapter. The linkage of boron tolerance genes and markers was also studied for chromosomes group 7 using F_3 derived lines of Halberd x Warigal (J. G. Paull, pers. comm.). Approximately eighty probes known to map to group 7 were tested. As there was a very low level of polymorphism between the two parents, no tight linkage between the RFLP markers and the *Bo1* gene was established (J. G. Paull, pers. comm.).

There is evidence of chromosomal translocation between chromosome groups four and seven of wheat. A segment of chromosome 7BS was found to be translocated to chromosome 4AL (Naranjo et al., 1987) and a segment of chromosome 4AL translocated to

5AL (Sharp et al., 1989). Homoeoloci for the seed peroxidase gene, *Per-B4*, are found on chromosomes 4B, 7A and 7D (Kobrehel and Feillet, 1975) and four cDNA clones which hybridized with 7AS and 7DS, but not 7BS, hybridized with 4BL (Chao et al., 1989). On the basis of translocations, it is possible that the genes controlling tolerance to boron observed on chromosomes 4A and 7B of G61450 and Halberd, respectively, as described in this Chapter, were originally located on chromosome 7B and later transferred to 4A by the evolutionary translocation. This would suggest that there were two loci of boron tolerance gene on group 7, unless there is a translocational difference between G61450 and Halberd. Since there is evidence of homoeology between the chromosome of wheat, barley and rye (Wang et al., 1992), it is possible that the genes conferring tolerance to boron in barley and rye may be located on chromosomes of homoeologous group seven.

Chapter 7

Chromosomal variations for boron tolerance in exotic germplasms

7.1 Introduction

Genetic diversity of crop species is important because it provides the variation for improving traits of economic importance, and adaptation to new regions and farming methods. For instance, useful agronomic traits have been transferred from the wild *Pennisetum* gene pools into cultivated pear millet (*Pennisetum typhoides*). These included rust resistance, cytoplasmic male sterility and fertility restorer genes from *P. glaucum* sp. *monodii*, and high forage yield and quality, and firm straw from *P. purpureum* (Hanna et al., 1985; Marchais and Pernes, 1985).

Genetic diversity may be originate from two major sources.

(a) the parental wild species

Polyploid wheats can be allocated into two evolutionary lineages. One lineage comprises *Triticum turgidum* (L.) Thell. (AAB^eB^e) and common wheat *T. aestivum* (L.) Thell. (AAB^eB^eDD) and the other *T. timopheevi* Zhuk. (AAB^tB^t) and *T. zhukovskyi* Men. et Er. (AAB^tB^tAA) (Dvorak, 1988). The results of RFLPs suggest that the domesticated diploid wheat *T. monococcum* ssp. *monococcum* was domesticated from *T. m.* ssp. *aegilopoides* (syn. *T. boeoticum*) but that the A genomes of both *T. turgidum* and *T. timopheevi* were contributed by *T. urartu* (Dvorak et al., 1988). From morphological studies, Sarkar and Stebbins (1956) concluded that the source of the B genome was *T. speltoides* (Tausch) Gren. or a close relative. The D genome was contributed by *T. tauschii* (Coss.) Schmal. (McFadden and Sears, 1946).

There is overwhelming evidence from cytology and molecular genetics indicating that the chromosomes of hexaploid wheat and its ancestors are homoeologous, so the ancestral species can be used as a source of new germplasm of benefit for breeding

programs. For example, *T. tauschii*, the donor of D genome in bread wheat, is being used as a source of resistance to cereal cyst nematode (CCN) (Eastwood et al., 1994), leaf blotch disease (*Septoria tritici*), stem rust and adaptation to saline soil (Lagudah and Appels, 1993). Linkage mapping of the isozyme loci *Got3, Adh1, Adh2,* and *Got2* to DNA markers on chromosomes three, four and six, respectively of *T. tauschii* confirmed their paralogous relationship to the wheat genome (Hart, 1987) as well as their homoeology to the barley (*Hordeum vulgare*) genome (Lagudah et al., 1991). The order of the marker loci on chromosome group one of *T. tauschii* was the same as that for 1D of bread wheat (Payne 1987). Gene *Sr22*, which is effective against all pathotypes of stem rust in Australia, was originally identified in A genome diploid wheat species *Triticum boeoticum* (Gerechter-Amitai et al., 1971) and *T. monococcum* L. (Kerber and Dyck, 1973). This gene was incorporated in the released wheat variety Schomburgk (Rathjen et al., 1987).

Evidence of homoeologywas first demonstrated by the ability of chromosomes within *T. aestivum* to compensate for one another in nullisomic-tetrasomic combinations (Sears, 1954, 1966). Later, more evidence was assembled from induced intergenomic pairing and recombination both within hexaploid wheat (Riley and Chapman, 1958) and between the wheat genomes and those of related species (Naranjo, 1982; Koebner and Shepherd, 1986), and from the concurrence of chromosomal and intrachromosomal locations of marker genes, particularly biochemical and molecular loci. These are often observed to be triplicated in wheat (McIntosh et al., 1990).

In wheat, the homoeologous relationships between the chromosomes have been reported using restriction fragment length polymorphism (RFLP) analysis. Eight cDNA clones, potentially located to the wheat group two chromosomes because of their hybridization to the fragments from the groups two chromosomes of barley, rye and *Aegilops umbellulata,* were labelled and hybridized to restricted DNA isolated from seven of the Chinese Spring group two aneuploid lines (nullisomic-tetrasomics N2A-T2B, N2B-T2D, N2D-T2A, and ditelocentrics DT2AS, DT2BL, DT2DS, DT2DL) (Sharp and Soltes-Rak, 1988). A considerable number of marker homoeoloci (four and nine homoeoloci located on the long and short arms, respectively) was strong evidence for the short arms

and long arms being mutually homoeologous in this group (Sharp and Soltes-Rak, 1988). Another study of homoeology between chromosome of group seven using RFLP analysis indicated that the gene orders on each of the homoeologous chromosomes were almost identical (Chao et al., 1989). The evidence was most comprehensive for chromosome 7B and 7D where, apart from the inconsistency in the Xpsr165, Xpsr150 and Xpsr152 region, nine homoeoloci and the centromere were observed to lie in the same order and to be separated by similar map distances (Chao et al., 1989).

Since chromosome 4A and 7B were responsible for tolerance to boron in G61450 and Halberd, respectively (Chapter 6), and because of the evidence of homoeoloci between homoeologous groups of chromosomes, the chromosomes of groups four and seven were selected for determination of chromosomal location of the genes controlling boron tolerance in the exotic varieties included in the experiment described in this chapter.

(b) separate evolutionary pathways

In breeding plants for resistance to disease or tolerances to soil toxicities or deficiencies, different alleles controlling those traits could be developed under similar selection regimes. In this respect, selection within a wide geographical spread of germplasm is likely to be beneficial as this makes it more likely that different alleles will be available. The screening for boron tolerance of 1576 accessions of wheats, demonstrated that variation of tolerance to boron occurred between different geographical regions. Varieties from USA, Canada, Egypt and North West Europe were mostly sensitive, those from Argentina, Turkey and Iraq varied, while those from Afghanistan, India and Japan were predominantly tolerant. Most Australian varieties were moderately sensitive (Moody et al., 1988).

Cytogenetic studies of homologous chromosome variation for boron tolerance among exotic tolerant lines was undertaken here to indicate different genotypes which could be used in the local breeding program. To manipulate such genes, the backcross method can be used for transferring tolerant genes to high yielding but sensitive varieties. For example, the gene responsible for tolerance to boron, *Bo1,* was successfully transferred

from Halberd to Schomburgk resulting in the release of BT-Schomburgk (Moody et al., 1993).

The cytological techniques for identification of the chromosomal locations of genes include the F_2 monosomic analysis (Chapter 6), the reciprocal monosomic analysis and the backcross reciprocal monosomic analysis described below.

Backcross reciprocal monosomic analysis

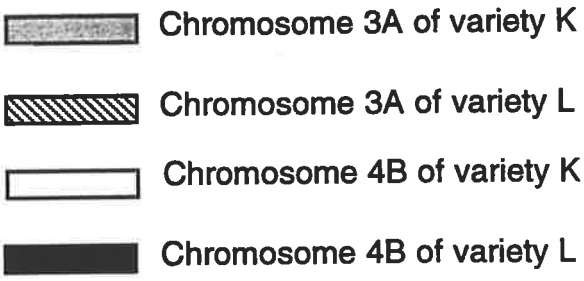
The establishment of the chromosomal location of genes controlling agronomic characters is essential for the success of intraspecific chromosome manipulation techniques in wheat improvement (Law et al., 1981). Although the F_2 monosomic method (Chapter 6) is the most commonly used method because it is easily applied and is particularly efficient (Macer, 1966), it cannot be used with characters having continuous variation where discrete phenotypes are not discernible in the F_2 because of the confounding effects of allelic variations and chromosome dosage (Snape et al., 1983). This effect was observed in Chapter 6 for the analysis of the crosses between Condor monosomic families and Halberd.

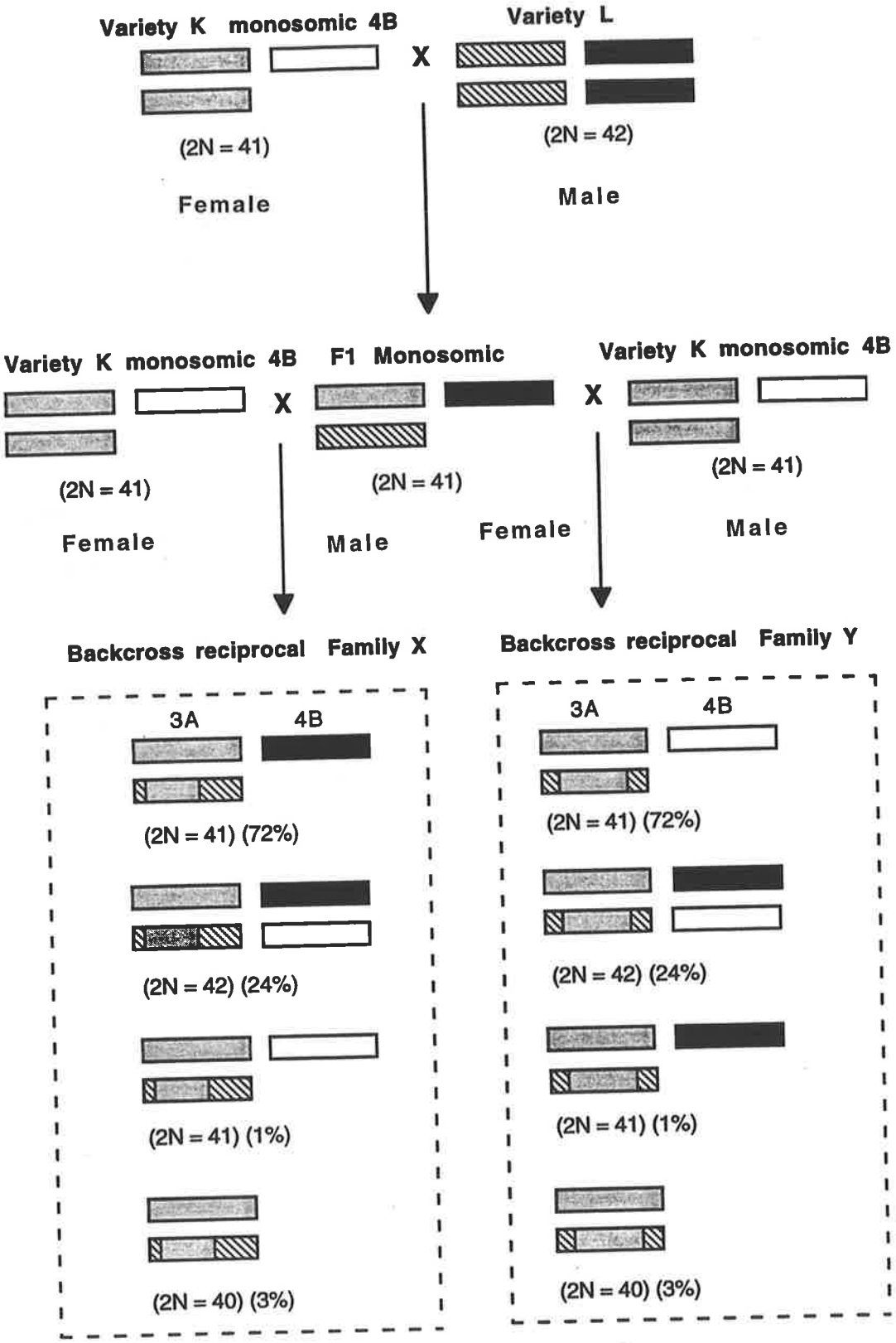
Reciprocal monosomic crossing, which allows the comparison between the two F_1 monosomics from the reciprocal crosses between the homologous monosomics of two varieties (McEwan and Kalsikes, 1970), overcomes this problem. However, the reciprocal monosomic method is limited to varieties for which a monosomic series has been developed.

A more flexible method of chromosome assay which overcomes the deficiencies of both of the above methods is the backcross reciprocal monosomic method described by Snape and Law (1980). An example of the crossing procedure for this method is illustrated in Figure 7.1 in which only two pairs of chromosomes, 3A and 4B, are demonstrated for the monosomic 4B of variety K and disomic variety L.

Monosomics for each of the chromosomes under investigation of variety K as females are crossed with L, the euploid variety. Monosomic plants are selected from the F_1 progenies and these are then used as both male and female parents in backcrosses to their original monosomic parent K. Because of differences in the transmission frequency of

Figure 7.1 Diagrammatic representation of the backcross reciprocal monosomic crossing procedure. The monosomic in variety K and disomic variety L are used to develop reciprocal families with comparable genetical backgrounds but different hemizygous chromosomes. The scheme is simplified to show only two pairs of homoeologous chromosomes 3A and 4B and the development of backcross reciprocal monosomics for chromosome 4B.




Variety K monosomic 4B
X
Variety L
(2N = 41)
Female
(2N = 42)
Male
Variety K monosomic 4B
F1 Monosomic
X
Variety K monosomic 4B
X
(2N = 41)
Female
(2N = 41)
Male
Female
(2N = 41)
Male
Backcross reciprocal Family X
Backcross reciprocal Family Y
3A
4B
(2N = 41) (72%)
(2N = 42) (24%)
(2N = 41) (1%)
(2N = 40) (3%)
3A
4B
(2N = 41) (72%)
(2N = 42) (24%)
(2N = 41) (1%)
(2N = 40) (3%)

twenty chromosomes between male (4%) and female (75%) gametes, this procedure results in contrasting backcross reciprocal (BCR) families (illustrated as families X and Y in Figure 7.1) which have equivalent genetical background but a predominance of a specific chromosome from one or other of the two parents. Approximately 72% of progenies of family X are monosomics carrying chromosome 4B from variety L (Figure 7.1). Whereas approximately 72% of the progenies of family Y are monosomics carrying chromosome 4B from variety K (Figure 7.1). The two families also have 1%, 3% and 24% of monosomic, nullisomic and disomic progenies (Figure 7.1). The disomics and nullisomics of family X are equivalent to those of family Y in genetical background and chromosomes from the two parents while the monosomics of families X and Y carry chromosomes 4B from variety K and variety L, respectively. The average difference in phenotype between the two BCR families (BC_1 F_1) X and Y (Figure 7.1) should reflect differences between genes on the chromosome 4B of the two varieties. It is necessary to undertake a randomised and replicated experiment to detect this difference.

The effects of monosomy can be excluded by selfing the backcross reciprocal monosomic plants and then selecting disomics for the comparisons of the varietal differences. However, this was not necessary for the experiment reported here because the genes in the hemizygous condition had no effect on the response to boron of the Condor monosomic families in comparison to the Condor disomic (Chapter 6).

The backcross reciprocal method makes it possible to compare a particular monosomic chromosome from one variety with same monosomic from another variety. If there is a difference between the two BCR families, either allelic differences occur between the monosomic chromosomes or the genes are identical and the differences result from interactions with different genes in their backgrounds, or a combination of both of these (Law et al., 1983). Compared to monosomic analysis, there are three advantages of using the backcross reciprocal monosomic. Firstly, the backcross reciprocal monosomic method avoids the problem of heterogeneity in the background of monosomics series. Secondly, as the crossing procedure is carried out between individual plants, the same parental monosomics and F_1 monosomics can be crossed reciprocally, thus ensuring a relative

consistency of background genotype in the reciprocals regardless of genetic diversity in the original monosomic lines. Thirdly, the homologous chromosomes of two or more varieties can be assessed simultaneously, regardless of whether the monosomics of these varieties are available.

The backcross reciprocal monosomic method has a good predictive value for the results of developing a particular single chromosome substitution line (Snape and Law, 1980). For example, when chromosome 5A of Bezostaya I was substituted into Cappelle-Desprez and the performance of the substitution line was compared with the performance predicted by the backcross reciprocal monosomic method, the results suggested that for ear emergence time, plant height, tiller number and grain weight per plant, the chromosomal difference was not influenced by hemizygosity or differential interaction with the background (Snape and Law, 1980). The method therefore provides the means of surveying homologous chromosomal effects from a range of varieties. It allows these effects to be ranked, and the chromosomes with large effects identified for use in breeding programs. This should make it possible to improve the performance of established varieties directly by using inter-varietal chromosome substitution (Snape and Law, 1980).

The results of Chapter 6 indicated that chromosomes 7B and 4A are the locations of genes controlling boron tolerance in Halberd and G61450, respectively. As it was not feasible to undertake backcross reciprocal monosomic analysis for all chromosomes for several varieties because of the time required for the cytological examination of the monosomic plants, only the effects of chromosomes of groups four and seven were tested here. The objective of this experiment was to identify whether chromosomes other than those in groups four and seven controlled boron tolerance in four other tolerant exotic varieties. Halberd and G61450 also included to the check results described in Chapter 6. In particular, chromosome 7B of Halberd needed to be reconfirmed because of the continuous distribution observed in the F_2 populations of all of the Condor monosomic families crossed with Halberd (Chapter 6).

7.2 Materials and methods

The seeds of monosomics for homoeologous groups 4 and 7 of CSP 44, a selection of Condor, were sown in standard potting mix in a glasshouse and the monosomic plants identified cytologically by determining the chromosome complements of pollen mother cells at metaphase I as described in Chapter 3. These were crossed as the female parents to six boron tolerant wheat varieties; G61450, Halberd, India 126, Benventuto Inca, Lin Calel, and AUS 4041 (Moody et al., 1988). The five tolerant exotic varieties, were selected on the basis of their being more tolerant than the Australian variety Halberd, and their genetic background being divergent from Halberd (Moody et al., 1988). Pedigree, boron response and the origin of these varieties is presented in Table 4.1.

Two monosomic F_1 plants of each cross were identified but, where possible, only one plant was used as both male and female parent in backcrosses to the Condor monosomic parental line. The second plant was crossed only if there was insufficient pollen produced by the first plant. Each pair of the 68 BCR families (BC_1F_1) [(derived from 2 groups of monosomics (groups 4 and 7)) x (3 monosomics in each group (genome A, B and D)) x (6 varieties) x (2 reciprocals) - (4 of the families lost during the experiment)] was then compared. The seeds of the backcross reciprocal families of Lin Calel and AUS 4041 for chromosome 4A were not available because their F_1 seeds were damaged by insects and not viable.

The 34 pairs of BCR families were tested for boron tolerance in a randomized complete block design with three replicates using the previously described filter paper technique with a boron treatment of 100 mg l^{-1}. The experimental procedures, included pretreatment conditions, were described in Chapter 4. Five seeds of each family of each reciprocal pair plus two seeds of each parent were included in a single filter paper. Each replicate contained 34 filter papers. The lengths of the longest root of each seedling were measured after 12 days.

The length of roots of individual families were tested by analysis of variance and the mean root length of the reciprocal lines were compared using Duncan's New Multiple Range Test (Gomez and Gomez, 1984). Variances of the seedling root lengths within

families calculated from the data from the fifteen seedlings of each family and were compared to those for the reciprocal monosomics and the disomic parents. The statistical analysis was calculated by MSTAT microcomputer program (Chapter 3).

The mean root length of the critical family, derived from (Condor monosomic x (Condor monosomic x tolerant)), in which approximately 96% of the progeny carrying the chromosome of the tolerant variety, is expected to be slightly shorter than or equal to that of the tolerant parent but longer than that of the reciprocal family ((Condor monosomic x tolerant variety) x Condor monosomic), in which only about 25% of the progeny carry the chromosome of the tolerant variety, which in turn is expected to be longer than that of Condor. The variance of the critical family, with the excess proportion of the tolerant chromosome, is expected to be higher than those of the parents but lower than that of its reciprocal family and lower than those of the non-critical families. The difference between means and variances of the critical family for the chromosome of the tolerant variety and those of its reciprocal family arises from the difference in frequency of the tolerant, intermediate and sensitive plants within these families due to the difference in transmission frequency of the univalent chromosome between male and female gametes of monosomic plants. For the monosomic plants, approximately 75% and 4% of the functioning female and male gametes, respectively, have twenty chromosomes (Figure 7.2).

The diagram in Figure 7.2 demonstrates the frequency of the progenies in critical families for the chromosome 4B backcross to the tolerant (N) (Figure 7.2a) and sensitive (M) (Figure 7.2b) varieties. The family from tolerant variety as male and the monosomic carrying chromosome 4B of the sensitive variety as female, consists of 24% and 72% of heterozygous (disomic) and hemizygous (monosomic) plants for the chromosome carrying boron tolerance gene (Figure 7.2a). Since the gene in the hemizygous condition had no effect on the response to boron of the Condor monosomic families in comparison to the Condor disomic (Chapter 6), the hemizygous plants are expected to be similar to the tolerant parent in response to boron while the heterozygous plants are expected to be intermediate between tolerant and sensitive parents. Approximately 3% and 1% of the progenies are monosomic and nullisomic (Figure 7.2a) for the critical chromosome of the

Figure 7.2 Diagram of reciprocal families derived from crossing between monosomics carrying critical chromosome 4B of varieties M (sensitive) and N (tolerant). The scheme is simplified to show only two pairs of homoeologous chromosomes 3A and 4B and the frequency of disomic, monosomic and nullisomic progenies derived from the reciprocal crosses.

(a) a family derived from crossing between monosomic 4B of variety M (as female) and monosomic 4B from variety N (as male),

(b) a family derived from crossing between monosomic 4B of variety N (as female) and monosomic 4B of variety M (as male)

n = haploid number of chromosome = 21; figures in brackets are percentage values.


Chromosome 3A of variety M


Chromosome 3A of variety N


Chromosome 4B of variety M


Chromosome 4B of variety N (critical chromosome carrying boron tolerance gene)

(a)

Female gamete (Variety M)	Male gamete (Variety N): 3A 4B, n (96%)	Male gamete (Variety N): 3A, n-1 (4%)
3A 4B, n (25%)	Disomic (intermediate) (24%)	Monosomic (sensitive) (1%)
3A, n-1 (75%)	Monosomic (tolerant) (72%)	Nullisomic (sensitive) (3%)

(b)

Female gamete (Variety N)	Male gamete (Variety M): 3A 4B, n (96%)	Male gamete (Variety M): 3A, n-1 (4%)
3A 4B, n (25%)	Disomic (intermediate) (24%)	Monosomic (tolerant) (1%)
3A, n-1 (75%)	Monosomic (sensitive) (72%)	Nullisomic (sensitive) (3%)

sensitive variety and are expected to be sensitive. Thus, approximately 96% and 4% of the progenies of the critical family for the chromosome from the tolerant variety are expected to be tolerant-intermediate and sensitive (Table 7.2a), respectively, indicating that tolerance to boron of this family is on average equal to or slightly less than the tolerant parent. For the critical family for the chromosome from the sensitive variety (M), approximately 25% (24% plus 1%) were heterozygous and hemizygous for the critical chromosome of the tolerant variety and 75% were hemizygous for the critical chromosome from sensitive variety plus nullisomic (Figure 7.2b). This family will be more tolerance to boron than the sensitive parent but considerably less tolerant than the critical family for the equivalent chromosome from the tolerant parent.

In non-critical families, there are no monosomic plants for the gene for boron tolerance. On the basis that tolerance to boron is controlled by a single gene, approximately 50% of the plants would be heterozygous for the gene controlling boron tolerance and the other 50% of the plants would be sensitive. Thus in average, these families will be more tolerant than the sensitive parent but less tolerant than the tolerant parent.

Since the ratio of tolerant-intermediate : sensitive progenies are 96 : 4 (Figure 7.2a), 25 : 75 (Figure 7.2b) and 50 : 50 for the critical family for the chromosome from the tolerant variety, the critical family for the chromosome from the sensitive variety and the non-critical families respectively, the variances of the critical family for the chromosome from the tolerant variety is expected to be higher than those of the parents but lower than that of its reciprocal family which in turn is lower than those of the corresponding non-critical families.

7.3 Results

The difference in root length between the BCR families for chromosomes of homoeologous groups four and seven of each tolerant variety compared to those for the Condor homologue are shown in Table 7.1 and Table 7.2. Families for chromosome 4A from three of the tolerant varieties, Benventuto Inca, G61450 and India 126 and for chromosome 7B of Halberd, exhibited increased root lengths relative to those families in

Table 7.1 Mean length of roots (cm) and differences between backcross reciprocal families for chromosomes of group 4 derived from the Condor monosomics in crosses with six tolerant genotypes.

Genotype	Root length (cm)								
	4A			4B			4D		
	Disomic[a]	BCR[b]	Diff[c]	Disomic	BCR	Diff	Disomic	BCR	Diff
G61450	9.9	8.38[d]	3.8**	9.5	6.00	0.12	8.8	5.99	1.32
Condor	4.4	4.58[e]		4.1	5.88		3.9	4.67	
Halberd	7.0	5.04	0.55	7.1	4.83	-0.01	6.5	5.18	-0.20
Condor	3.6	4.49		3.9	4.84		4.0	5.38	
India126	10.2	8.78	2.15*	9.2	6.51	0.57	11.3	7.67	1.48
Condor	3.5	6.63		3.5	5.94		3.7	6.19	
Benventuto. Inca	9.2	8.39	4.14**	9.4	5.81	-1.46	10.6	5.91	0.38
Condor	3.5	4.25		4.4	7.27		3.9	5.53	
Lin Calel	NA[f]	NA	NA	10.6	4.95	0.75	10.7	7.32	-0.28
Condor				3.8	4.20		4.4	7.60	
AUS4041	NA	NA	NA	10.1	6.35	1.06	12.5	6.32	-0.27
Condor				4.0	5.29		4.2	6.59	

[a] disomic parent [b] backcross reciprocal families [c] differences between backcross reciprocal families, significance of differences: * $P < 0.05$; ** $P < 0.01$, tested by Duncan's New Multiple Range Test, [d] mean root length of a family derived from crossing between F_1 monosomic of (Condor monosomic 4A x G61450) as male and Condor monosomic 4A as female, [e] mean root length of a family derived from crossing between F_1 monosomic of (Condor monosomic 4A x G61450) as female and Condor monosomic 4A as male, [f] NA = not available.

Table 7.2 Mean length of roots (cm) and differences between backcross reciprocal families for chromosomes of group 7 derived from the Condor monosomic in crosses with six tolerant genotypes.

Background	Root length (cm)								
	7A			7B			7D		
	Disomic[a]	BCR[b]	Diff[c]	Disomic	BCR	Diff	Disomic	BCR	Diff
G61450	8.7	5.75[d]	0.09	9.1	6.12	-0.80	10.9	5.51	0.71
Condor	3.4	5.66[e]		3.4	6.92		4.5	4.80	
Halberd	7.1	4.97	0.81	6.4	6.42	2.13*	8.1	4.93	0.48
Condor	3.8	4.16		3.4	4.29		3.7	4.45	
India126	10.2	6.62	1.18	12.8	8.13	1.28	12.7	5.52	-0.65
Condor	3.6	5.44		4.0	6.85		3.5	6.17	
Benventuto. Inca	9.8	6.88	0.44	8.3	6.36	1.60	9.9	5.82	0.54
Condor	4.2	6.44		3.5	4.76		3.8	5.28	
Lin Calel	10.4	5.51	-1.17	9.1	5.55	1.12	9.6	5.32	-0.04
Condor	4.0	6.68		4.0	4.43		4.2	5.36	
AUS4041	12.8	5.93	0.59	12.1	4.88	1.08	12.4	6.74	0.55
Condor	4.0	5.34		3.6	3.80		3.8	6.19	

[a] disomic parent [b] backcross reciprocal families [c] differences between backcross reciprocal families, significance of differences: * $P < 0.05$, tested by Duncan's New Multiple Range Test, [d] mean root length of a family derived from crossing between F_1 monosomic of (Condor monosomic 7A x G61450) as male and Condor monosomic 7A as female, [e] mean root length of a family derived from crossing between F_1 monosomic of (Condor monosomic 7A x G61450) as female and Condor monosomic 7A as male.

which most of their progenies carry the Condor homologues (Plates 7.1, 7.2, 7.3 and 7.4). The difference between the families with predominately chromosome 4A from Benventuto Inca and G61450 and that of Condor was 4.14 and 3.80 cm, respectively (Table 7.1) which was highly significant. There was also a significant difference of 2.15 cm between the chromosome 4A family of India 126 compared with that of Condor (Table 7.1). The difference between chromosome 7B family of Halberd compared with that of Condor was a significant 2.13 cm increase in length (Table 7.2). The differences between the other BCR families were all smaller and non-significant.

The relative root lengths of the critical families for the chromosome of the tolerant varieties were longer than those of the corresponding critical families of the Condor chromosome (Table 7.3). Those for chromosome 4A of Benventuto Inca, G61450 and India 126 and 7B of Halberd were 131%, 116%, 127% and 131%, respectively, in comparison with 66%, 64%, 96% and 88% for the corresponding families of the Condor chromosomes. The relative root lengths of the non-critical families were in the range of 62% to 108% (Table 7.3).

There were differences in the variances of the root lengths between the BCR families. The variances of the critical families for chromosome 4A of Benventuto Inca, G61450 and India 126 were in general lower than those of the non-critical families of the same variety (Table 7.4) and lower than those of the corresponding critical families of Condor. Anomalous results were observed for the families derived from G61450 where the variances for the non-critical families of chromosome 4B and 4D were lower than that of the critical family for chromosome 4A (Table 7.4). However, there was no significant difference in the mean root lengths of their reciprocal pairs, indicating that these two chromosomes (4B and 4D) had no effect in the response to boron of G61450. The variance of the critical family with chromosome 7B of Halberd was similar to that of chromosome 7B from Condor (Table 7.4). This is not entirely unexpected because both of the critical families with chromosome 7B from Halberd and Condor were expected to have lower variances in comparison with the non-critical families of the corresponding varieties.

Plate 7.1 Response of F_1 backcross reciprocal families in comparison with disomic parents.

(a) critical chromosome; chromosome 7B of Halberd v chromosome 7B of Condor,

7B(7B x Halberd) = backcross reciprocal family derived from crossing between F_1 monosomic of (Condor monosomic 7B x Halberd) as male and Condor monosomic 7B as female,

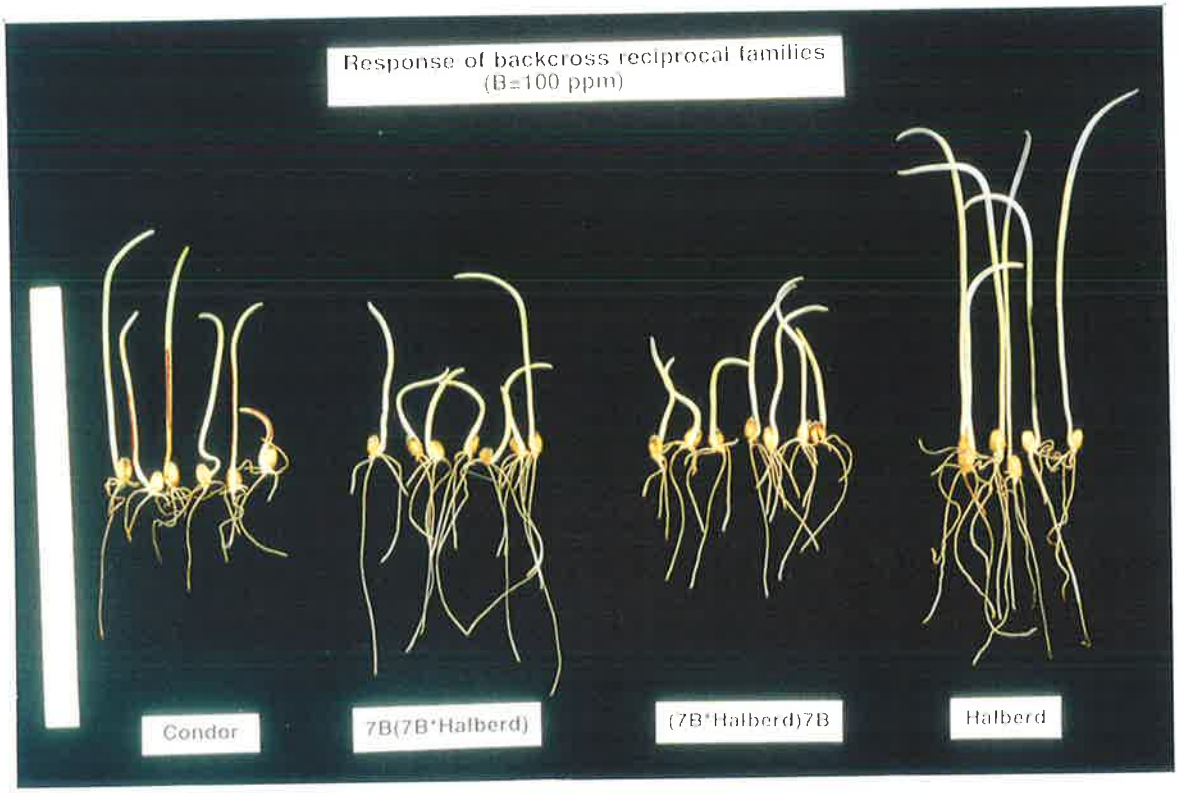
(7B x Halberd)7B = backcross reciprocal family derived from crossing between F_1 monosomic of (Condor monosomic 7B x Halberd) as female and Condor monosomic 7B as male,

(b) non-critical chromosome; chromosome 7A of Halberd v chromosome 7A of Condor.

7A(7A x Halberd) = backcross reciprocal family derived from crossing between F_1 monosomic of (Condor monosomic 7A x Halberd) as male and Condor monosomic 7A as female,

(7A x Halberd)7A = backcross reciprocal family derived from crossing between F_1 monosomic of (Condor monosomic 7A x Halberd) as female and Condor monosomic 7A as male.

(a)


Response of backcross reciprocal families
(B=100 ppm)
Condor
7B(7B*Halberd)
(7B*Halberd)7B
Halberd

(b)

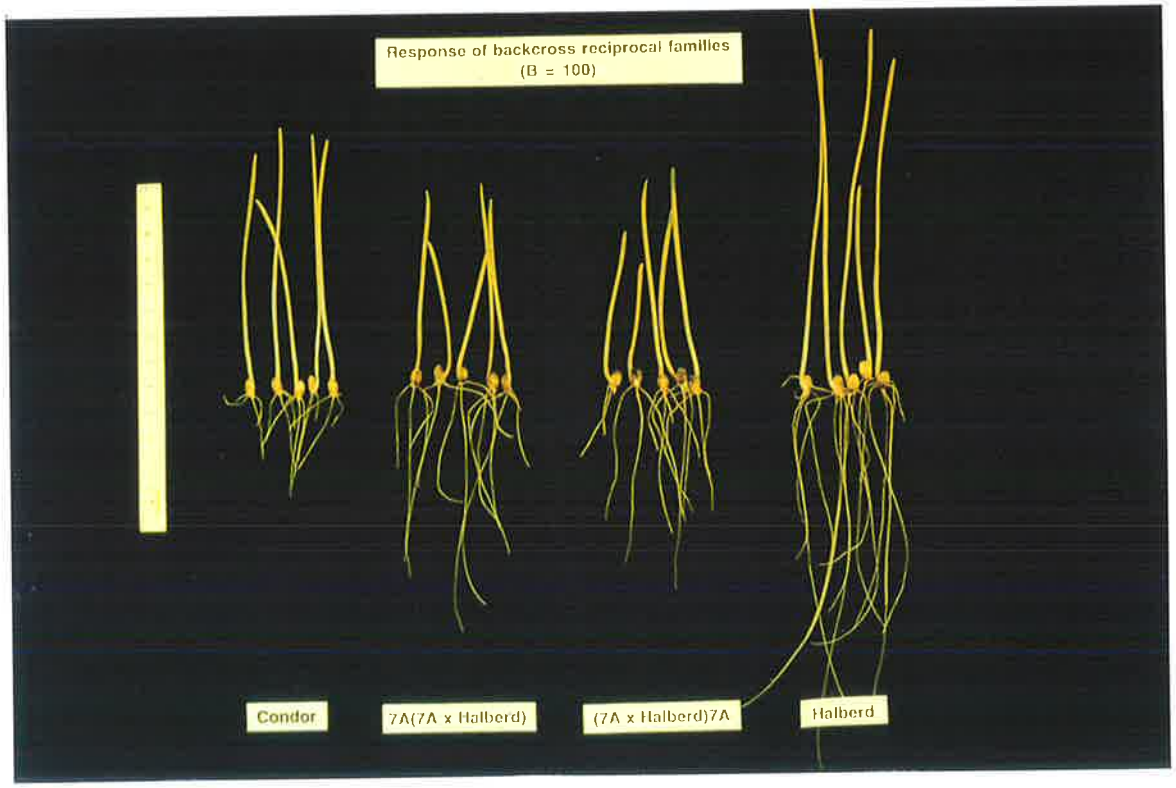

Response of backcross reciprocal families
(B = 100)
Condor
7A(7A x Halberd)
(7A x Halberd)7A
Halberd

Plate 7.2 Response of F_1 backcross reciprocal families in comparison with disomic parents.

(a) critical chromosome; chromosome 4A of G61450 v chromosome 4A of Condor,

4A(4A x G61450) = backcross reciprocal family derived from crossing between F_1 monosomic of (Condor monosomic 4A x G61450) as male and Condor monosomic 4A as female,

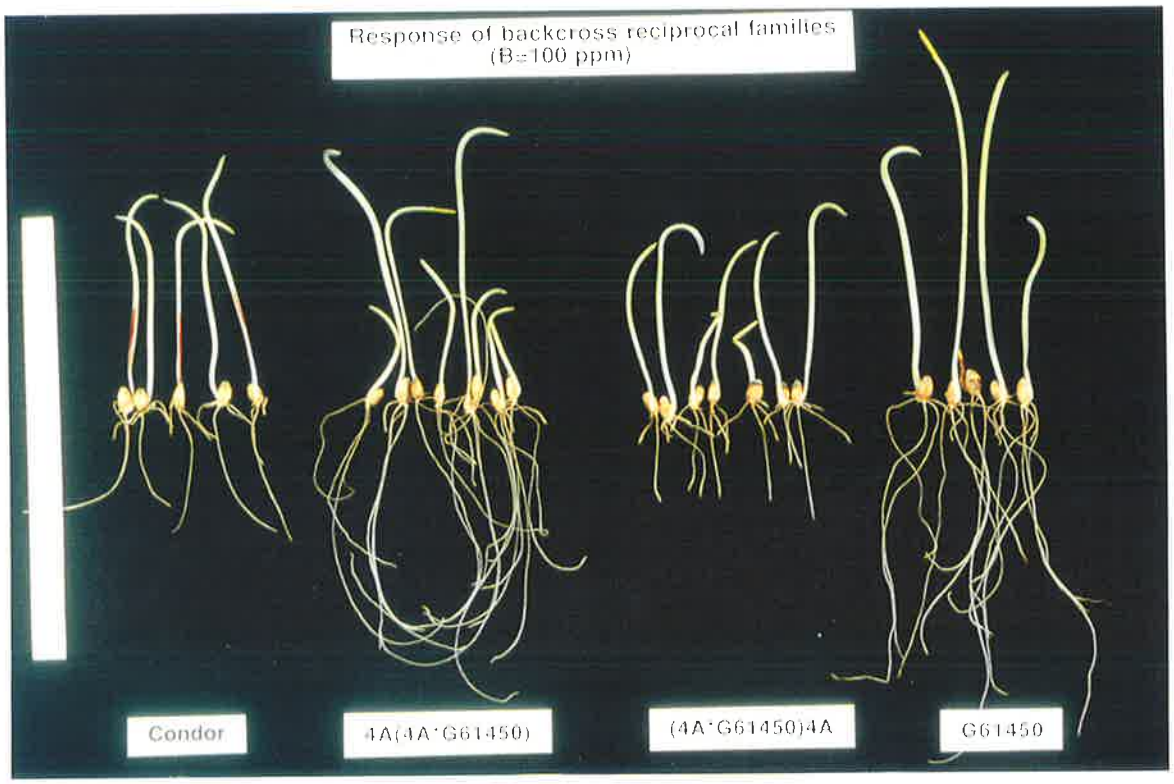
(4A x G61450)4A = backcross reciprocal family derived from crossing between F_1 monosomic of (Condor monosomic 4A x G61450) as female and Condor monosomic 4A as male,

(b) non-critical chromosome; chromosome 7A of G61450 v chromosome 7A of Condor.

7A(7A x G61450) = backcross reciprocal family derived from crossing between F_1 monosomic of (Condor monosomic 7A x G61450) as male and Condor monosomic 7A as female,

(7A x G61450)7A = backcross reciprocal family derived from crossing between F_1 monosomic of (Condor monosomic 7A x G61450) as female and Condor monosomic 7A as male.

(a)


Response of backcross reciprocal families
(B=100 ppm)
Condor
4A(4A*G61450)
(4A*G61450)4A
G61450

(b)

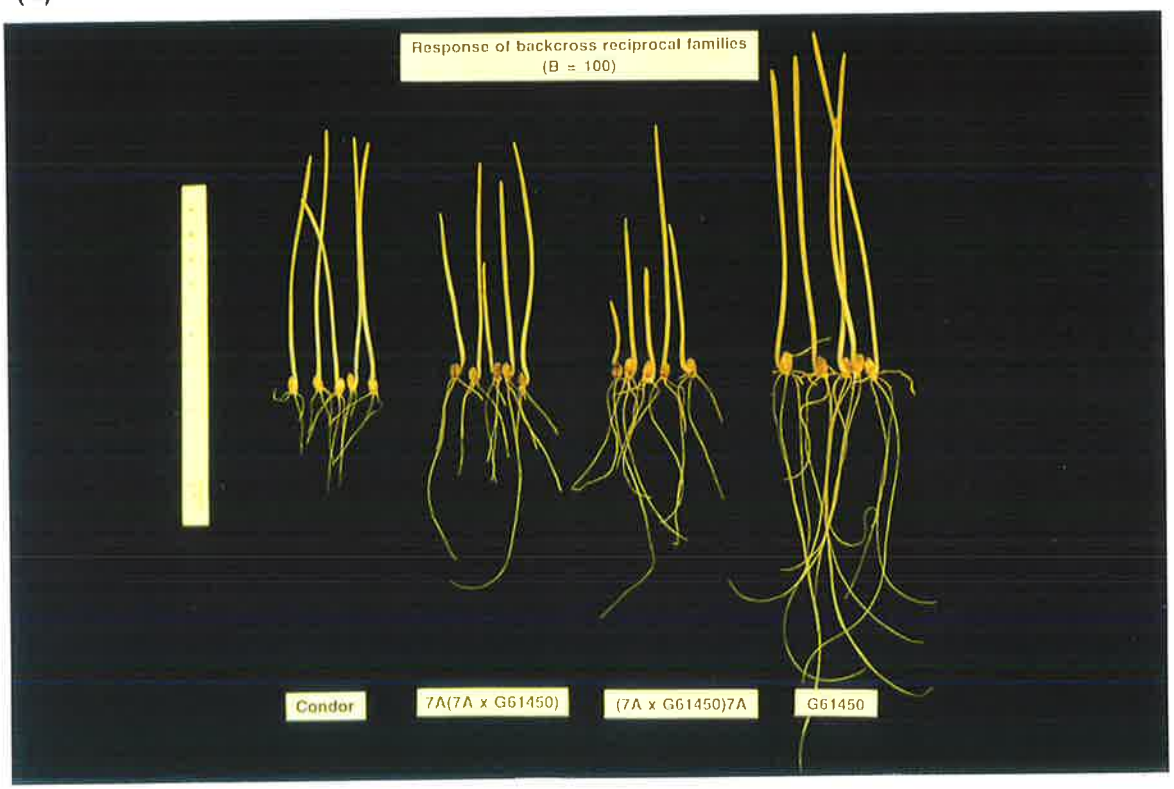

Response of backcross reciprocal families
(B = 100)
Condor
7A(7A x G61450)
(7A x G61450)7A
G61450

Plate 7.3 Response of F_1 backcross reciprocal families in comparison with disomic parents.

(a) critical chromosome; chromosome 4A of Benventuto Inca v chromosome 4A of Condor,

4A(4A x Benventuto Inca) = backcross reciprocal family derived from crossing between F_1 monosomic of (Condor monosomic 4A x Benventuto Inca) as male and Condor monosomic 4A as female,

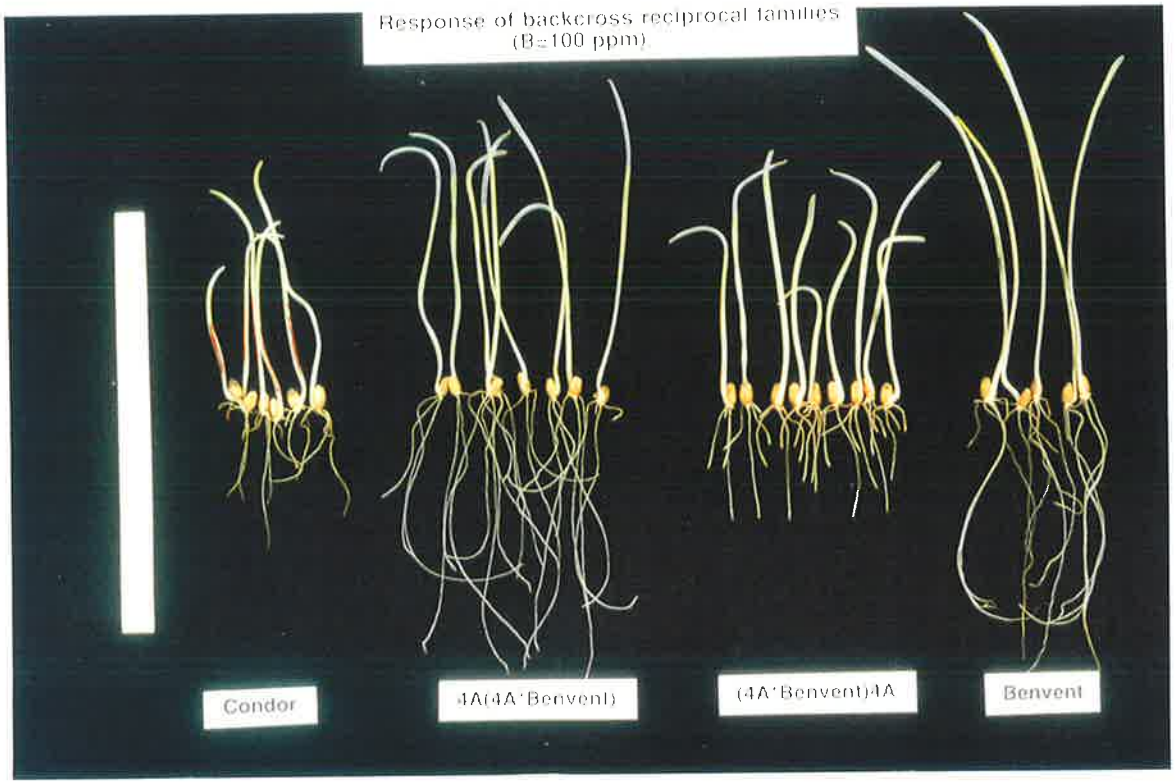
(4A x Benventuto Inca)4A = backcross reciprocal family derived from crossing between F_1 monosomic of (Condor monosomic 4A x Benventuto Inca) as female and Condor monosomic 4A as male,

(b) non-critical chromosome; chromosome 7A of Benventuto Inca v chromosome 7A of Condor.

7A(7A x Benventuto Inca) = backcross reciprocal family derived from crossing between F_1 monosomic of (Condor monosomic 7A x Benventuto Inca) as male and Condor monosomic 7A as female,

(7A x Benventuto Inca)7A = backcross reciprocal family derived from crossing between F_1 monosomic of (Condor monosomic 7A x Benventuto Inca) as female and Condor monosomic 7A as male.

(a)


Response of backcross reciprocal families
(B=100 ppm)
Condor
4A(4A'Benvent)
(4A'Benvent)4A
Benvent

(b)

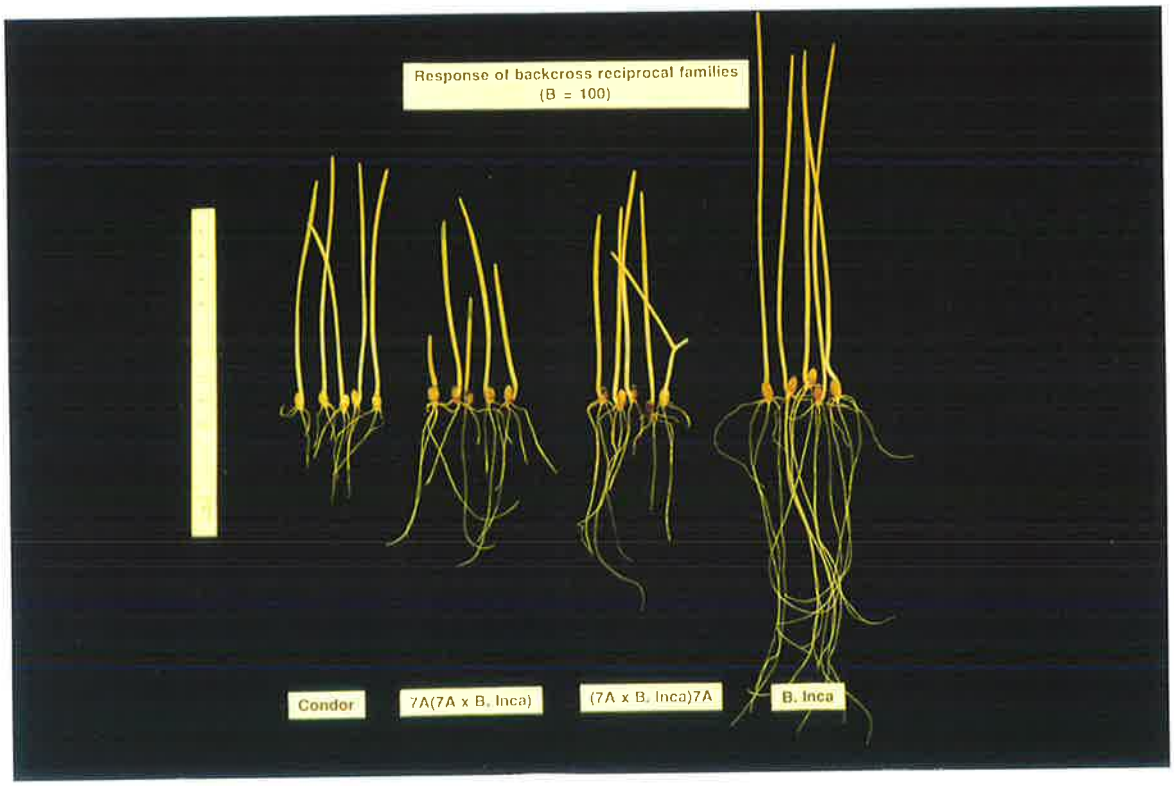

Response of backcross reciprocal families
(B = 100)
Condor
7A(7A x B. Inca)
(7A x B. Inca)7A
B. Inca

Plate 7.4 Response of F_1 backcross reciprocal monosomic families in comparison with disomic parents.

(a) critical chromosome; chromosome 4A of India 126 v chromosome 4A of Condor,

4A(4A x India 126) = backcross reciprocal family derived from crossing between F_1 monosomic of (Condor monosomic 4A x India 126) as male and Condor monosomic 4A as female,

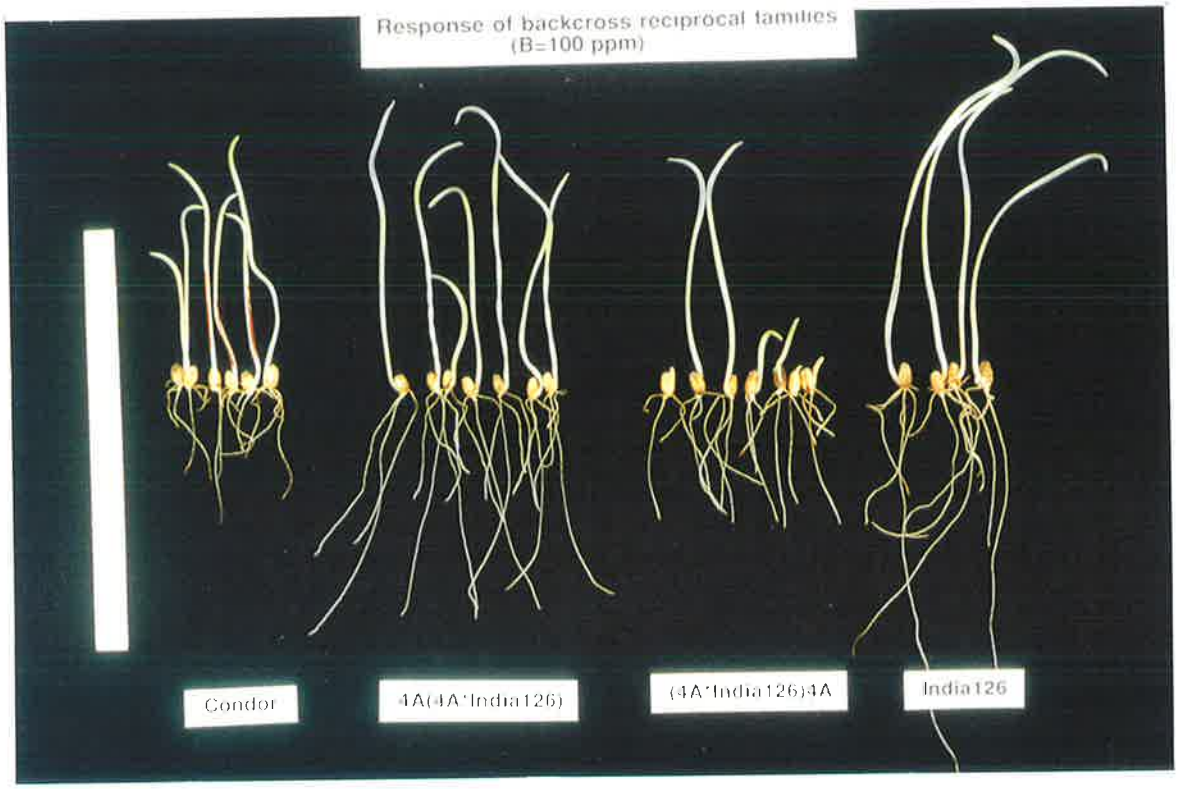
(4A x India 126)4A = backcross reciprocal family derived from crossing between F_1 monosomic of (Condor monosomic 4A x India 126) as female and Condor monosomic 4A as male,

(b) non-critical chromosome; chromosome 4B of India 126 v chromosome 4B of Condor.

4B(4B x India 126) = backcross reciprocal family derived from crossing between F_1 monosomic of (Condor monosomic 4B x India 126) as male and Condor monosomic 4B as female,

(4B x India 126)4B = backcross reciprocal family derived from crossing between F_1 monosomic of (Condor monosomic 4B x India 126) as female and Condor monosomic 4B as male.

(a)


Response of backcross reciprocal families
(B=100 ppm)
Condor
4A(4A*India126)
(4A*India126)4A
India126

(b)

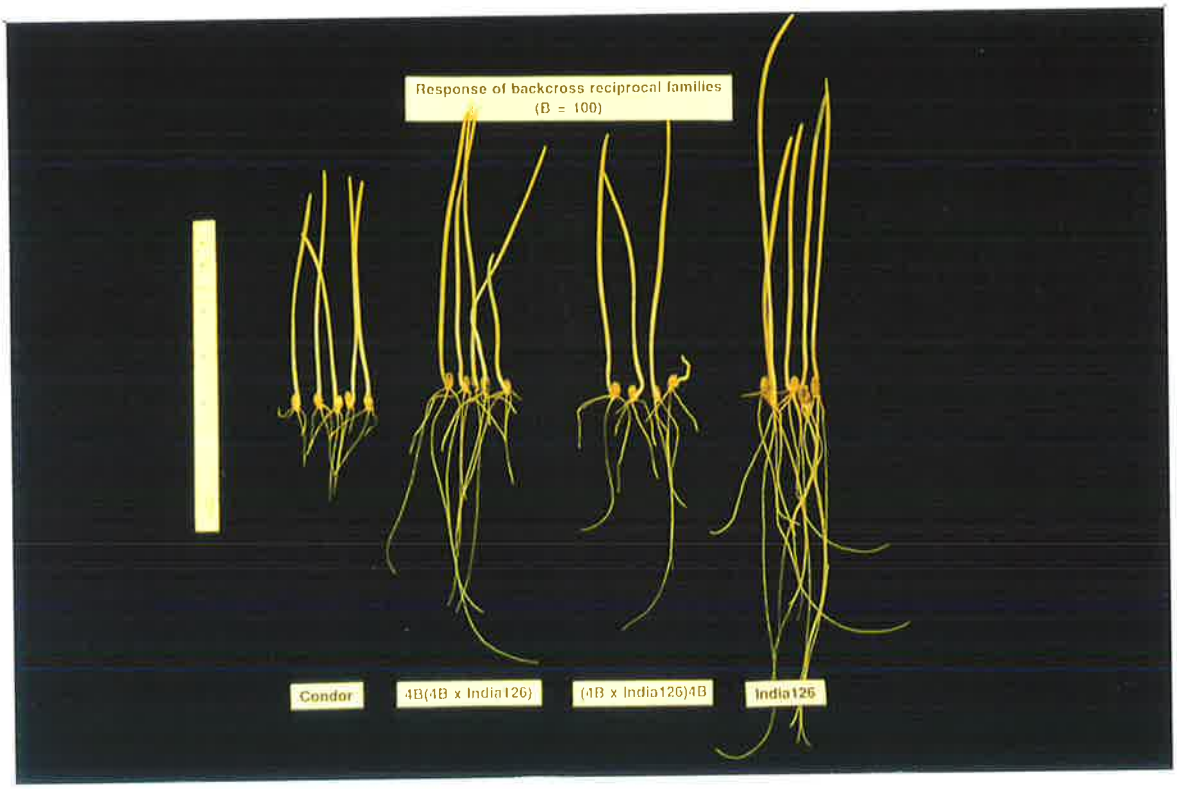

Response of backcross reciprocal families
(B = 100)
Condor
4B(4B x India126)
(4B x India126)4B
India126

Table 7.3 Relative root length (%) of backcross reciprocal families compared to the midparent for chromosomes of groups 4 and 7 employing the Condor monosomic in crosses with six tolerant genotypes.

Family	Relative root length (%)					
	4A	4B	4D	7A	7B	7D
mono[a] x (mono x G61450)	116[b]	88	94	94	97	72
(mono x G61450) x mono	64[c]	87	73	93	110	62
mono x (mono x Halberd)	95	88	98	90	131	82
(mono x Halberd) x mono	85	88	102	76	88	74
mono x (mono x India126)	127	102	102	96	97	68
(mono x India126) x mono	96	93	83	79	82	76
mono x (mono x BenventutoInca)	131	84	81	98	108	84
(mono x BenventutoInca) x mono	66	105	76	92	81	77
mono x (mono x Lin Calel)	NA[d]	69	96	77	84	77
(mono x Lin Calel) x mono	NA	58	100	93	67	78
mono x (mono x AUS4041)	NA	89	75	71	62	83
(mono x AUS4041) x mono	NA	75	78	64	48	76

[a] Condor monosomic,

[b] Relative root length of the family derived from crossing between F_1 of (Condor monosomic 4A x G61450) as male and Condor monosomic 4A as female,

[c] Relative root length of the family derived from crossing between F_1 of (Condor monosomic 4A x G61450) as female and Condor monosomic 4A as male,

[d] NA = not available

Table 7.4 Variances of seedling root lengths within backcross reciprocal families for chromosomes groups 4 and 7 derived from the Condor monosomic in crosses with six tolerant genotypes in comparison with the variance of their disomic parents.

Genotype	Variance											
	4A		4B		4D		7A		7B		7D	
	Di[a]	BCR[b]	Di	BCR	Di	BCR	Di	BCR	Di	BCR	Di	BCR
G61450	1.7	5.0[c]	1.1	3.2	0.8	2.1	5.6	6.4	1.7	6.1	4.1	5.5
Condor	0.5	10.4[d]	0.6	5.2	0.6	2.4	0.1	3.7	1.5	5.2	1.7	9.2
Halberd	0.6	1.8	0.3	1.8	0.8	0.9	3.1	1.2	0.3	0.5	0.1	1.7
Condor	1.3	4.4	1.3	2.1	1.8	2.3	0.5	0.9	0.8	0.5	1.0	1.8
India126	5.0	0.7	3.0	3.9	2.5	7.8	2.7	3.7	3.1	9.8	1.0	6.7
Condor	1.1	2.0	0.8	2.8	1.5	4.4	0.5	5.6	0.9	6.9	0.8	7.4
Benventuto. Inca	2.0	0.5	1.8	11.3	0.9	8.2	0.7	4.8	3.4	7.8	0.2	5.0
Condor	1.5	6.1	0.8	8.9	2.1	7.7	0.5	9.1	2.2	4.1	1.0	4.7
Lin Calel	NA	NA[e]	1.0	2.2	0.4	6.9	0.7	5.6	0.3	2.1	0.9	2.9
Condor	NA	NA	0.3	1.9	1.3	9.8	1.1	6.7	1.3	2.5	0.5	3.6
AUS4041	NA	NA	3.9	5.2	1.6	5.2	1.5	10.7	3.4	3.7	1.5	6.2
Condor	NA	NA	0.3	4.1	0.8	4.1	0.8	8.2	1.0	4.8	1.0	7.2

[a] disomic parent, [b] backcross reciprocal families, [c] variance of a family derived from crossing between F_1 monosomic of (Condor monosomic 4A x G61450) as male and Condor monosomic 4A as female, [d] variance of a family derived from crossing between F_1 monosomic of (Condor monosomic 4A x G61450) as female and Condor monosomic 4A as male, [e] NA = not available.

The variances of all of the disomic parents were lower than or equal to those of the corresponding families except for India 126 and Benventuto Inca for chromosomes 4A and Halberd for chromosome 7A (Table 7.4).

The non-critical families of each variety were examined individually to estimate the number of genes controlling tolerance to boron of the varieties relative to Condor. On the basis of only one gene controlling tolerance to boron between Condor and a tolerant variety, the segregation ratio of 1 intermediate : 1 sensitive was expected, whereas a segregation ratio of 3 intermediate : 1 sensitive was expected if there were two genes. This is because the segregation of the non-critical families (BC_1 F_1) is similar to that of the normal backcross (BC_1) family. For example, if there are two genes controlling tolerance to boron between a tolerant (AABB) and a sensitive (aabb), the F_1 backcross family using the sensitive variety as a recurrent parent will segregate at 3 intermediate (1 AaBb, 1 Aabb and 1 aaBb) : 1 sensitive (aabb). The chi-square analysis should be treated with a degree of caution, however, particularly for the two genes model, because of the low expected numbers of sensitive plants.

For the critical families of chromosome of the tolerant varieties and those of Condor the expected segregation ratio would be 24 tolerant-intermediate : 1 sensitive (Figure 7.2a) and 1 tolerant-intermediate : 3 sensitive (Figure 7.2b), respectively.

The root length of the individual plants within each family was compared with that of the disomic parents and classified into categories. A plant with a root length within the range of the sensitive parent was classified as sensitive. Whereas a plant with a root length longer than the range of the sensitive parent was classified as tolerant-intermediate.

Chi-square analysis indicated that the segregation of root length of the plants within the critical families for chromosomes of G61450, Halberd, India 126 and Benventuto Inca was consistent with the expected ratio of 24 tolerant-intermediate : 1 sensitive (Table 7.5). Whereas the segregation of the critical families for chromosomes of Condor was consistent with the ratio of 1 tolerant-intermediate : 3 sensitive with the exception of the critical family for chromosome 7B (Table 7.5). The deviation from the expected ratio was principally due to a deficiency of the tolerant-intermediate plants. The distribution of the

Table 7.5 Chi-square analysis of the observed and expected segregation ratio of F_1 backcross reciprocal families carrying critical chromosome of G61450, Halberd, India 126, Benventuto Inca and Condor, tested in filter paper at B100.

Family		Model	Frequency		
			Tol-int[a]	Sens[b]	χ^2_1
G61450 4A[c]	Obs[e]		13	2	
	Exp[f]	24 : 1	14.40	0.60	3.40
Condor 4A[d]	Obs		4	11	
	Exp	1 : 3	3.75	11.25	0.03
Halberd 7B	Obs		13	1	
	Exp	24 : 1	13.44	0.56	0.36
Condor 7B	Obs		0	13	
	Exp	1 : 3	3.25	9.75	4.33*
India 4A	Obs		15	0	
	Exp	24 : 1	14.4	0.60	0.63
Condor 4A	Obs		4	9	
	Exp	1 : 3	3.25	9.75	0.23
Benventuto Inca 4A	Obs		14	1	
	Exp	24 : 1	14.4	0.60	0.28
Condor 4A	Obs		3	12	
	Exp	1 : 3	3.75	11.25	0.20

[a] Tolerant-intermediate, [b] Sensitive,

[c] Family derived from crossing between the F_1 monosomic of (Condor monosomic 4A x G61450) as male and Condor monosomic 4A as female,

[d] Family derived from crossing between the F_1 monosomic of (Condor monosomic 4A x G61450) as female and Condor monosomic 4A as male,

[e] Observed value, [f] Expected value,

* Significant difference at $0.01 < P < 0.05$.

seedling root lengths of the critical and non-critical families for chromosome of G61450, Halberd, India 126 Benventuto Inca and Condor in comparison with the disomic parents are demonstrated in Figures 7.3-7.6. These results confirmed that chromosome 4A of G61450, India 126 and Benventuto Inca and 7B of Halberd were responsible for tolerance to boron relative to Condor.

For the non-critical families, the root length of the individual plant within each family was compared with that of the disomic parents and classified. A plant with a root length within the range of the sensitive parent was classified as sensitive. Whereas a plant with a root length of longer than the range of the sensitive parent was classified as intermediate. Chi-square analysis indicated that the segregation of all of the non-critical families for Halberd and Condor was consistent with the monogenic ratio of 1 intermediate : 1 sensitive (Table 7.6, Figure 7.3) but not the digenic ratio of 3 intermediate : 1 sensitive (Table 7.7) with the exception of families for chromosome 4A and 7A of Condor (Table 7.6). The consistency of the segregation ratios to the monogenic ratio of 1 intermediate : 1 sensitive indicates a single gene controlling tolerance to boron in Halberd relative to Condor, consistent with the results of Chapter 6. However, chi-square analysis indicated that the segregation ratio of total intermediate : total sensitive was not consistent with either the 1 : 1 (Table 7.6) and 3 : 1 (Table 7.7) ratios, confirming a major deficiency in the intermediate categories compared to sensitives.

The segregation of all of the non-critical families for the chromosome of G61450 (Table 7.8, Figure 7.4) and Benventuto Inca (Table 7.10, Figure 7.5) was also consistent with the monogenic ratio of 1 intermediate : 1 sensitive with the exception of chromosome 4B of G61450 and 4D of Condor (Table 7.8). The segregation of these two families also deviated from the digenic ratio of 3 intermediate : 1 sensitive. The segregation of some of the non-critical families for chromosomes of G61450 (Table 7.8, 7.9), Benventuto Inca (Table 7.10, 7.11) and Condor were consistent with both ratios of 1 intermediate : 1 sensitive and 3 intermediate : 1 sensitive. However, most of the non-critical families for the chromosomes of G61450, Benventuto Inca and their reciprocal families segregated in the monogenic ratio of 1 intermediate : 1 sensitive. The segregation of the total frequency

Table 7.6 Chi-square analysis of the observed and expected segregation ratio of 1 intermediate : 1 sensitive of F_1 backcross reciprocal families carrying non-critical chromosomes of Halberd and Condor, tested in filter paper at B100.

Family		Model	Frequency		
			Int[a]	Sens[b]	χ^2_1
Halberd 4A[c]	Obs[e]		5	7	
	Exp[f]	1 : 1	6	6	0.33
Condor 4A[d]	Obs		2	13	
	Exp	1 : 1	7.5	7.5	8.07**
Halberd 4B	Obs		4	11	
	Exp	1 : 1	7.5	7.5	3.27
Condor 4B	Obs		5	10	
	Exp	1 : 1	7.5	7.5	1.68
Halberd 4D	Obs		6	9	
	Exp	1 : 1	7.5	7.5	0.60
Condor 4D	Obs		6	9	
	Exp	1 : 1	7.5	7.5	0.60
Halberd 7A	Obs		4	10	
	Exp	1 : 1	7	7	2.57
Condor 7A	Obs		1	14	
	Exp	1 : 1	7.5	7.5	11.27**
Halberd 7D	Obs		5	10	
	Exp	1 : 1	7.5	7.5	1.68
Condor 7D	Obs		5	10	
	Exp	1 : 1	7.5	7.5	1.68
Total frequency	Obs		43	103	
	Exp	1 : 1	73	73	24.66**

[a] Intermediate, [b] Sensitive,

[c] Family derived from crossing between the F_1 monosomic of (Condor monosomic 4A x Halberd) as male and Condor monosomic 4A as female,

[d] Family derived from crossing between the F_1 monosomic of (Condor monosomic 4A x Halberd) as female and Condor monosomic 4A as male,

[e] Observed value, [f] Expected value,

** Significant difference at $P < 0.01$.

Figure 7.3 The distributions of root lengths of individual plants within the backcross reciprocal families derived from the reciprocal crosses between the F_1 monosomic of (Condor monosomic x Halberd) and Condor monosomic, in comparison with the disomic parents.
(a) 7B x (7B x Halberd) = family derived from crossing between the F_1 monosomic of (Condor monosomic 7B x Halberd) as male and Condor monosomic 7B as female.
(b) (7B x Halberd) x 7B = family derived from crossing between the F_1 monosomic of (Condor monosomic 7B x Halberd) as female and Condor monosomic 7B as male. This system also applied to the other crosses.

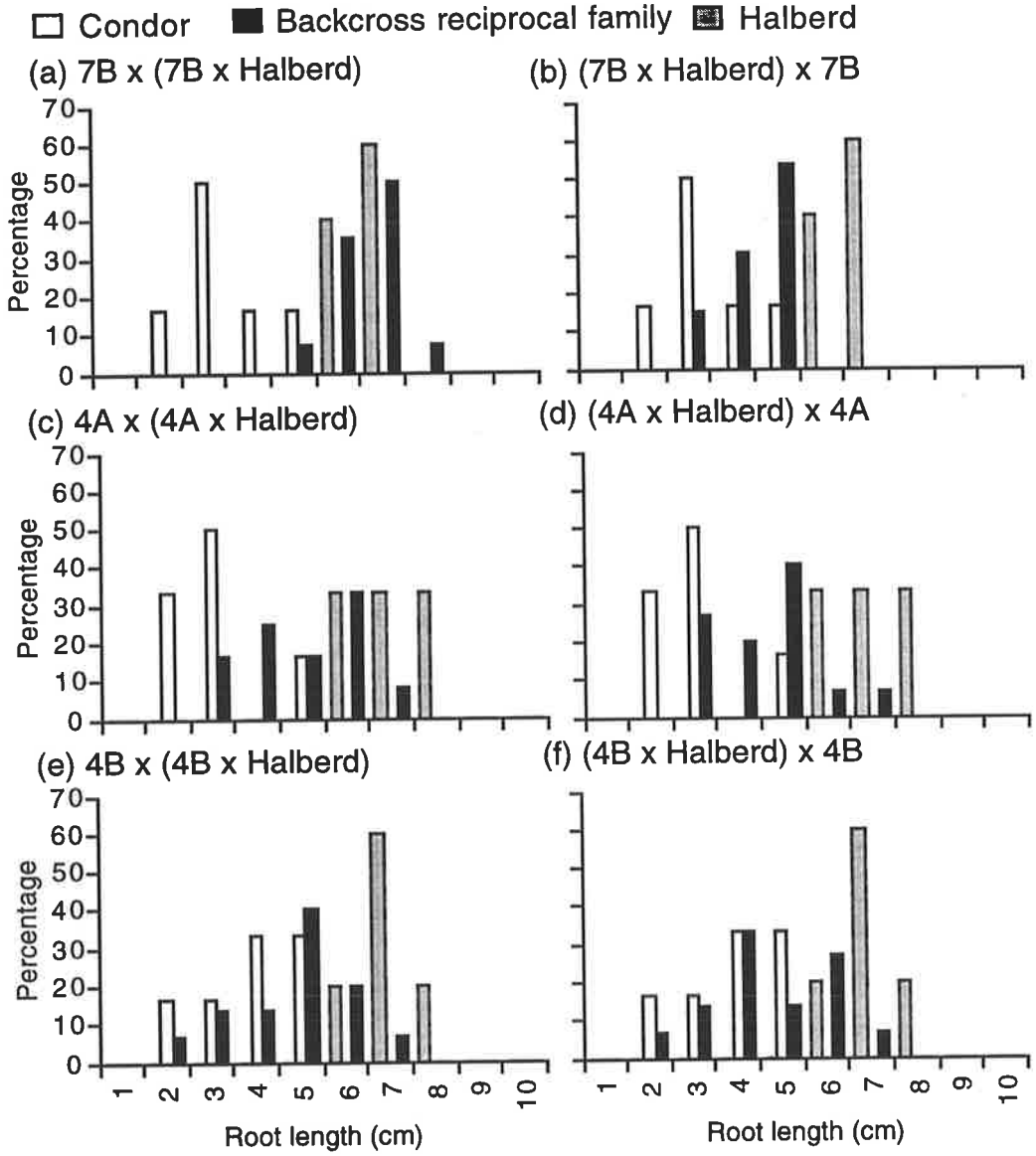


Figure 7.3 (continued).

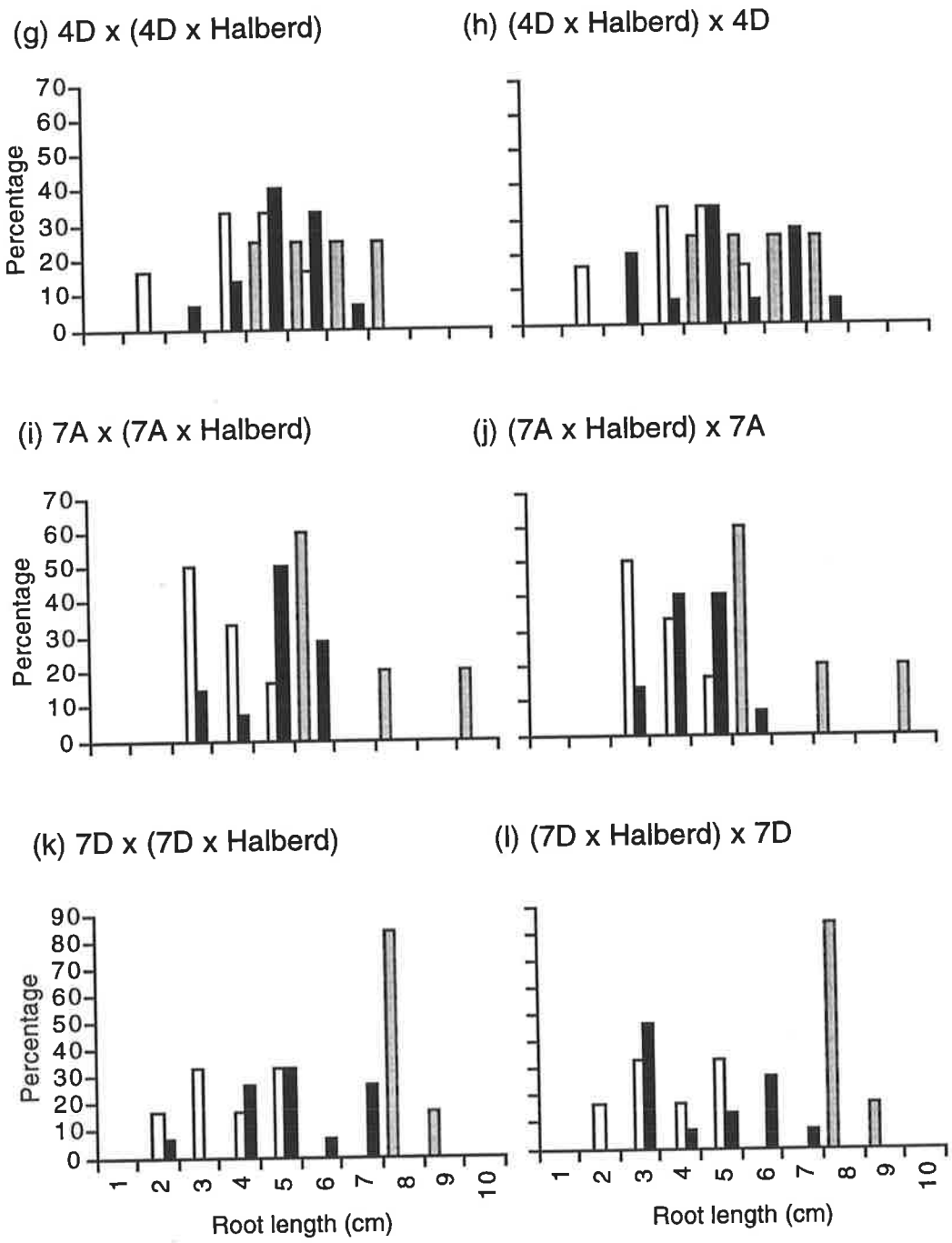


Table 7.7 Chi-square analysis of the observed and expected segregation ratio of 3 intermediate : 1 sensitive of F_1 backcross reciprocal families carrying non-critical chromosomes of Halberd and Condor, tested in filter paper at B100.

Family		Model	Frequency		
			Int[a]	Sens[b]	χ^2_1
Halberd 4A[c]	Obs[e]		5	7	
	Exp[f]	3 : 1	9	3	7.13**
Condor 4A[d]	Obs		2	13	
	Exp	3 : 1	11.25	3.75	30.42**
Halberd 4B	Obs		4	11	
	Exp	3 : 1	11.25	3.75	18.64**
Condor 4B	Obs		5	10	
	Exp	3 : 1	11.25	3.75	13.89**
Halberd 4D	Obs		6	9	
	Exp	3 : 1	11.25	3.75	9.8**
Condor 4D	Obs		6	9	
	Exp	3 : 1	11.25	3.75	9.8**
Halberd 7A	Obs		4	10	
	Exp	3 : 1	10.5	3.5	16.09**
Condor 7A	Obs		1	14	
	Exp	3 : 1	11.25	3.75	37.36**
Halberd 7D	Obs		5	10	
	Exp	3 : 1	11.25	3.75	13.89**
Condor 7D	Obs		5	10	
	Exp	3 : 1	11.25	3.75	13.89**
Total frequency	Obs		43	103	
	Exp	3 : 1	109.5	36.5	161.54**

[a] Intermediate, [b] Sensitive,

[c] Family derived from crossing between the F_1 monosomic of (Condor monosomic 4A x Halberd) as male and Condor monosomic 4A as female,

[d] Family derived from crossing between the F_1 monosomic of (Condor monosomic 4A x Halberd) as female and Condor monosomic 4A as male,

[e] Observed value, [f] Expected value,

** Significant difference at $P < 0.01$.

Table 7.8 Chi-square analysis of the observed and expected segregation ratio of 1 intermediate : 1 sensitive of F_1 backcross reciprocal families carrying non-critical chromosomes of G61450 and Condor, tested in filter paper at B100.

Family		Model	Frequency		
			Int[a]	Sens[b]	χ^2_1
G61450 4B[c]	Obs[e]		3	12	
	Exp[f]	1 : 1	7.5	7.5	5.40*
Condor 4B[d]	Obs		8	7	
	Exp	1 : 1	7.5	7.5	0.04
G61450 4D	Obs		10	5	
	Exp	1 : 1	7.5	7.5	1.68
Condor 4D	Obs		3	12	
	Exp	1 : 1	7.5	7.5	5.40*
G61450 7A	Obs		9	4	
	Exp	1 : 1	6.5	6.5	1.92
Condor 7A	Obs		8	5	
	Exp	1 : 1	6.5	6.5	0.69
G61450 7B	Obs		7	6	
	Exp	1 : 1	6.5	6.5	0.08
Condor 7B	Obs		10	5	
	Exp	1 : 1	7.5	7.5	1.68
G61450 7D	Obs		7	8	
	Exp	1 : 1	7.5	7.5	0.04
Condor 7D	Obs		5	10	
	Exp	1 : 1	7.5	7.5	1.68
Total frequency	Obs		70	74	
	Exp	1 : 1	72	72	0.11

[a] Intermediate, [b] Sensitive,

[c] Family derived from crossing between the F_1 monosomic of (Condor monosomic 4B x G61450) as male and Condor monosomic 4B as female,

[d] Family derived from crossing between the F_1 monosomic of (Condor monosomic 4B x G61450) as female and Condor monosomic 4B as male,

[e] Observed value, [f] Expected value,

* Significant difference at $0.01 < P < 0.05$.

Figure 7.4 The distributions of root lengths of individual plants within the backcross reciprocal families derived from the reciprocal crosses between the F_1 monosomic of (Condor monosomic x G61450) and Condor monosomic, in comparison with the disomic parents.
(a) 4A x (4A x G61450) = family derived from crossing between the F_1 monosomic of (Condor monosomic 4A x G61450) as male and Condor monosomic 4A as female.
(b) (4A x G61450) x 4A = family derived from crossing between the F_1 monosomic of (Condor monosomic 4A x G61450) as female and Condor monosomic 4A as male. This system also applied to the other crosses.

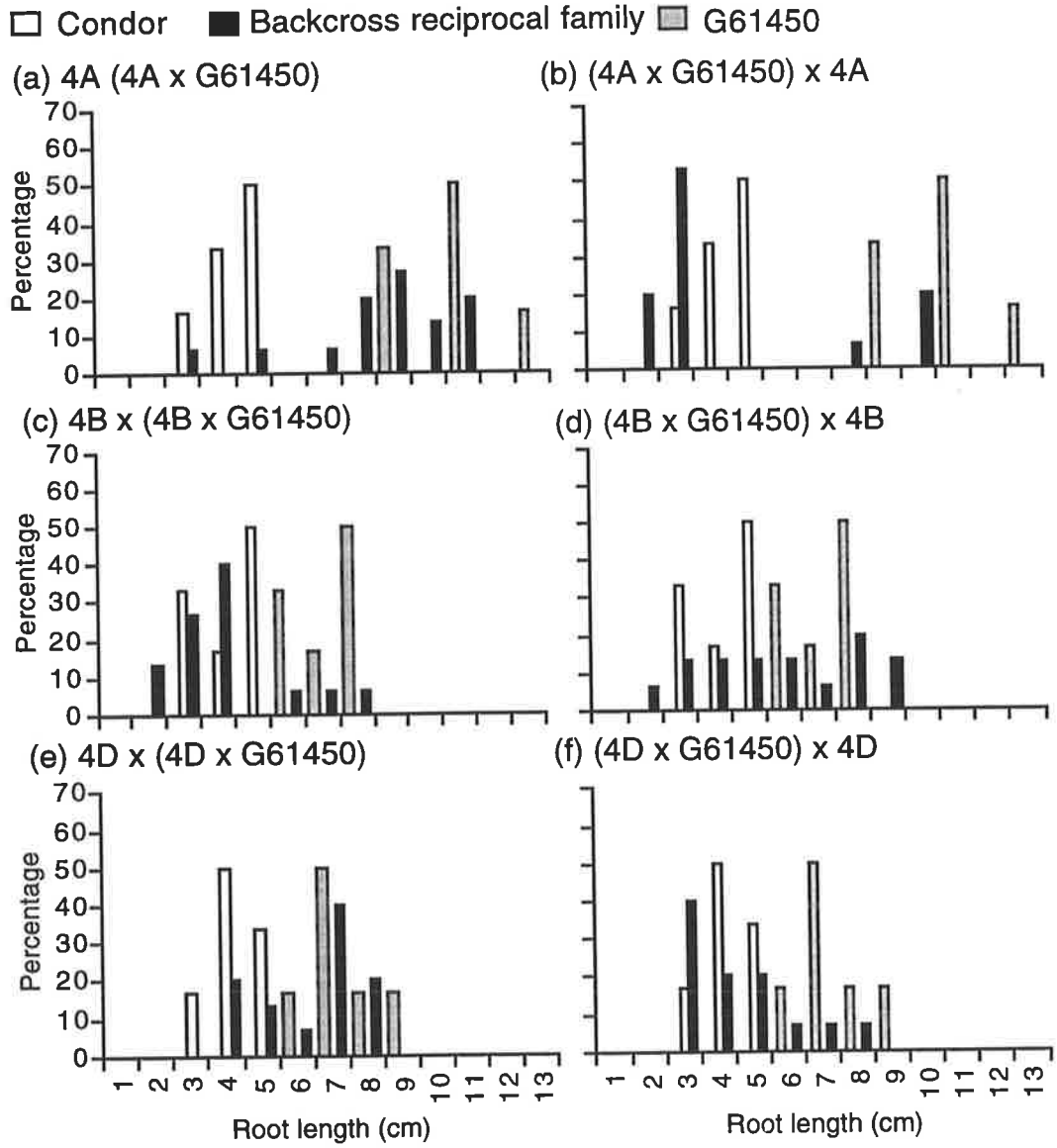


Figure 7.4 (continued).

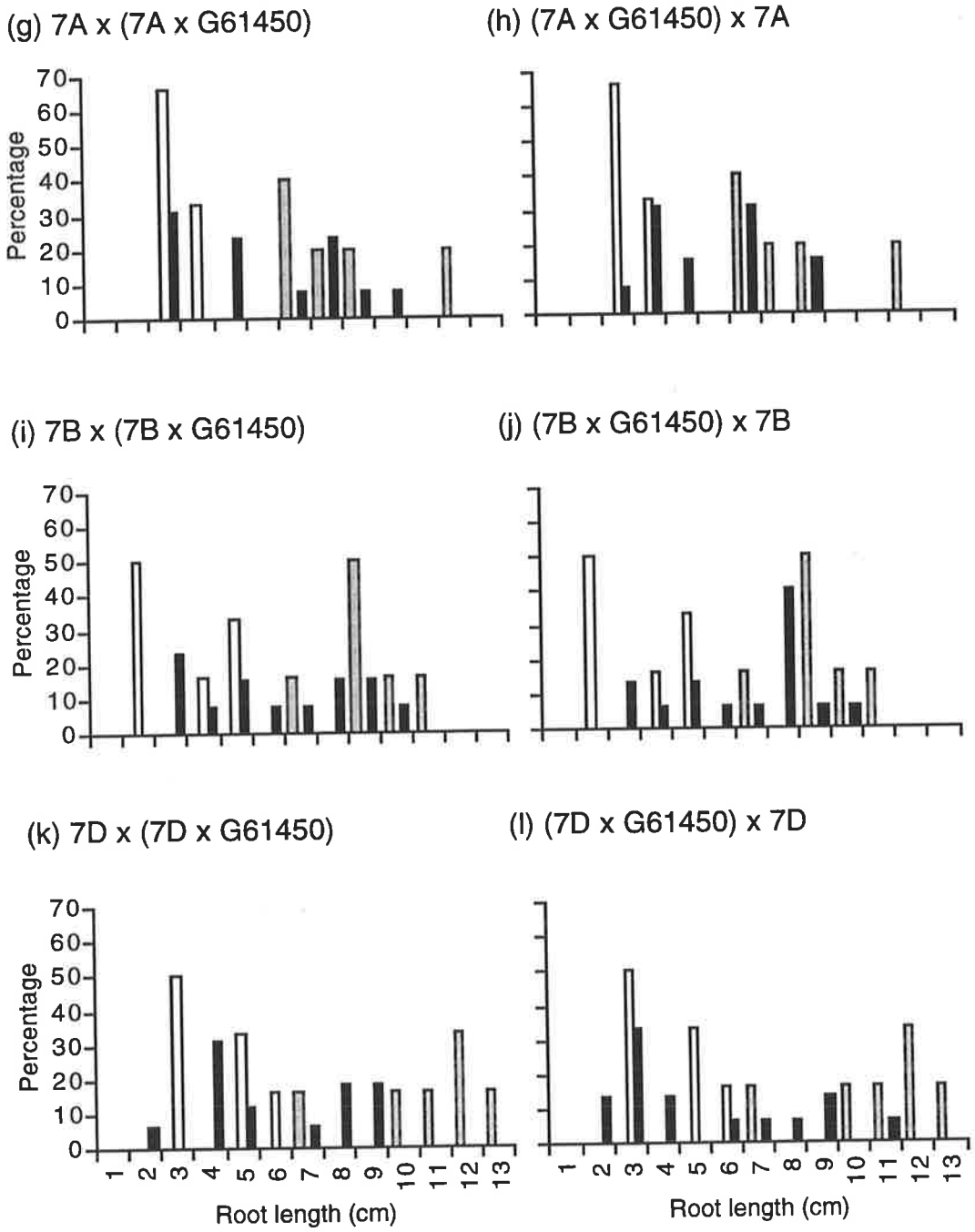


Table 7.9 Chi-square analysis of the observed and expected segregation ratio of 3 intermediate : 1 sensitive of F_1 backcross reciprocal families carrying non-critical chromosomes of G61450 and Condor, tested in filter paper at B100.

Family		Model	Frequency		
			Int[a]	Sens[b]	χ^2_1
G61450 4B[c]	Obs[e]		3	12	
	Exp[f]	3 : 1	11.25	3.75	24.20**
Condor 4B[d]	Obs		8	7	
	Exp	3 : 1	11.25	3.75	3.75
G61450 4D	Obs		10	5	
	Exp	3 : 1	11.25	3.75	0.56
Condor 4D	Obs		3	12	
	Exp	3 : 1	11.25	3.75	24.20**
G61450 7A	Obs		9	4	
	Exp	3 : 1	9.75	3.25	0.23
Condor 7A	Obs		8	5	
	Exp	3 : 1	9.75	3.25	1.26
G61450 7B	Obs		7	6	
	Exp	3 : 1	9.75	3.25	3.10
Condor 7B	Obs		10	5	
	Exp	3 : 1	11.25	3.75	0.56
G61450 7D	Obs		7	8	
	Exp	3 : 1	11.25	3.75	6.42*
Condor 7D	Obs		5	10	
	Exp	3 : 1	11.25	3.75	13.89**
Total frequency	Obs		70	74	
	Exp	3 : 1	108	36	53.48**

[a] Intermediate, [b] Sensitive,

[c] Family derived from crossing between the F_1 monosomic of (Condor monosomic 4B x G61450) as male and Condor monosomic 4B as female,

[d] Family derived from crossing between the F_1 monosomic of (Condor monosomic 4B x G61450) as female and Condor monosomic 4B as male,

[e] Observed value, [f] Expected value,

*, ** Significant difference at $0.01 < P < 0.05$ and $P < 0.01$, respectively.

Table 7.10 Chi-square analysis of the observed and expected segregation ratio of 1 intermediate : 1 sensitive of F_1 backcross reciprocal families carrying non-critical chromosomes of Benventuto Inca and Condor, tested in filter paper at B100.

Family		Model	Frequency		
			Int[a]	Sens[b]	χ^2_1
Benventuto Inca 4B[c]	Obs[e]		6	9	
	Exp[f]	1 : 1	7.5	7.5	0.60
Condor 4B[d]	Obs		10	5	
	Exp	1 : 1	7.5	7.5	1.68
Benventuto Inca 4D	Obs		5	5	
	Exp	1 : 1	5	5	0.00
Condor 4D	Obs		6	13	
	Exp	1 : 1	9.5	9.5	2.58
Benventuto Inca 7A	Obs		10	5	
	Exp	1 : 1	7.5	7.5	1.68
Condor 7A	Obs		8	7	
	Exp	1 : 1	7.5	7.5	0.04
Benventuto Inca 7B	Obs		9	6	
	Exp	1 : 1	7.5	7.5	0.60
Condor 7B	Obs		5	10	
	Exp	1 : 1	7.5	7.5	1.68
Benventuto Inca 7D	Obs		8	7	
	Exp	1 : 1	7.5	7.5	0.04
Condor 7D	Obs		6	9	
	Exp	1 : 1	7.5	7.5	0.60
Total frequency	Obs		73	76	
	Exp	1 : 1	74.5	74.5	0.06

[a] Intermediate, [b] Sensitive,

[c] Family derived from crossing between the F_1 monosomic of (Condor monosomic 4B x Benventuto Inca) as male and Condor monosomic 4B as female,

[d] Family derived from crossing between the F_1 monosomic of (Condor monosomic 4B x Benventuto Inca) as female and Condor monosomic 4B as male,

[e] Observed value, [f] Expected value,

Figure 7.5 The distributions of root lengths of individual plants within the backcross reciprocal families derived from the reciprocal crosses between the F_1 monosomic of (Condor monosomic x Benventuto Inca) and Condor monosomic, in comparison with the disomic parents.

(a) 4A x (4A x Benventuto Inca) = family derived from crossing between the F_1 monosomic of (Condor monosomic 4A x Benventuto Inca) as male and Condor monosomic 4A as female.

(b) (4A x Benventuto Inca) x 4A = family derived from crossing between the F_1 monosomic of (Condor monosomic 4A x Benventuto Inca) as female and Condor monosomic 4A as male. This system also applied to the other crosses.

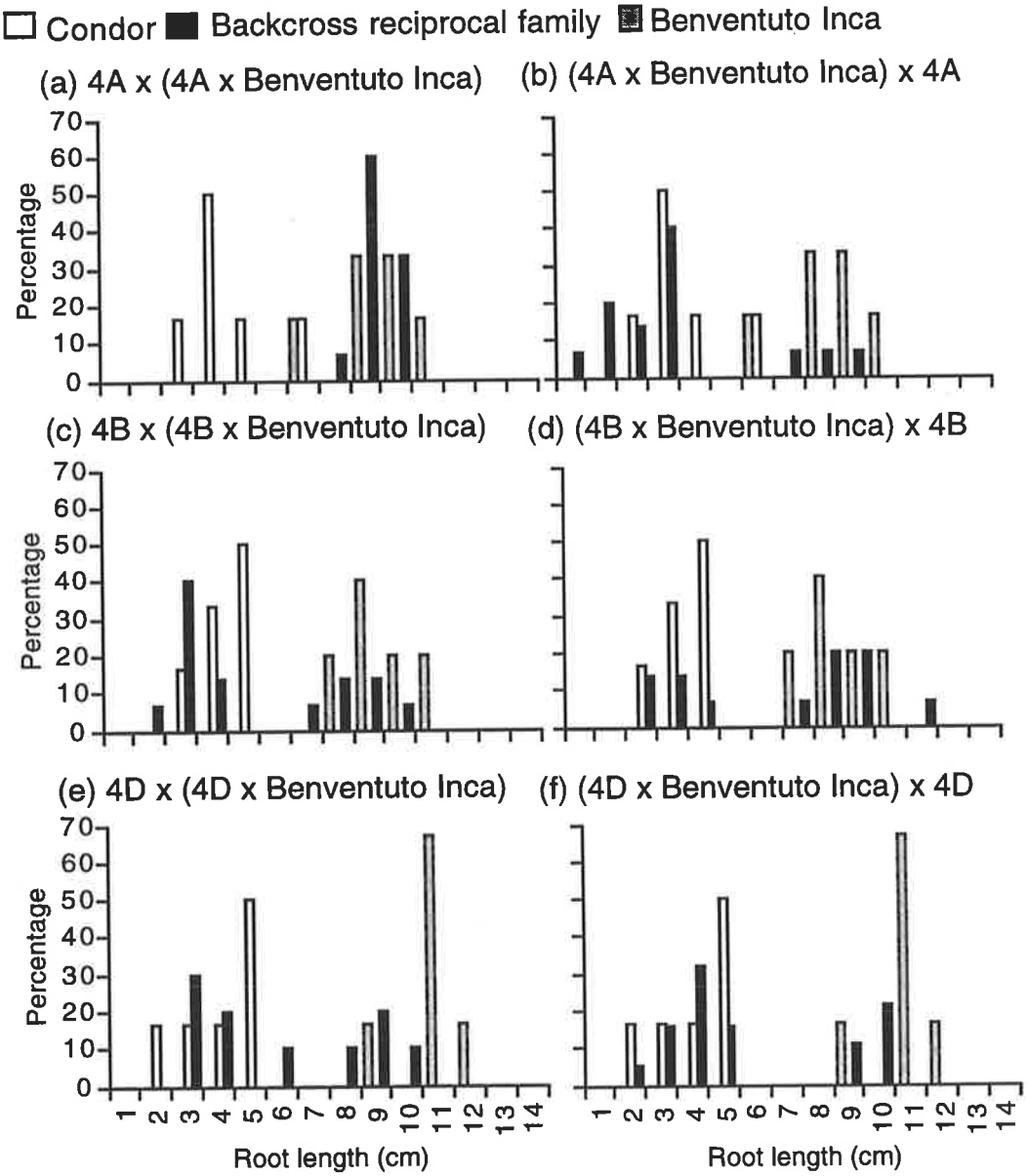


Figure 7.5 (continued).

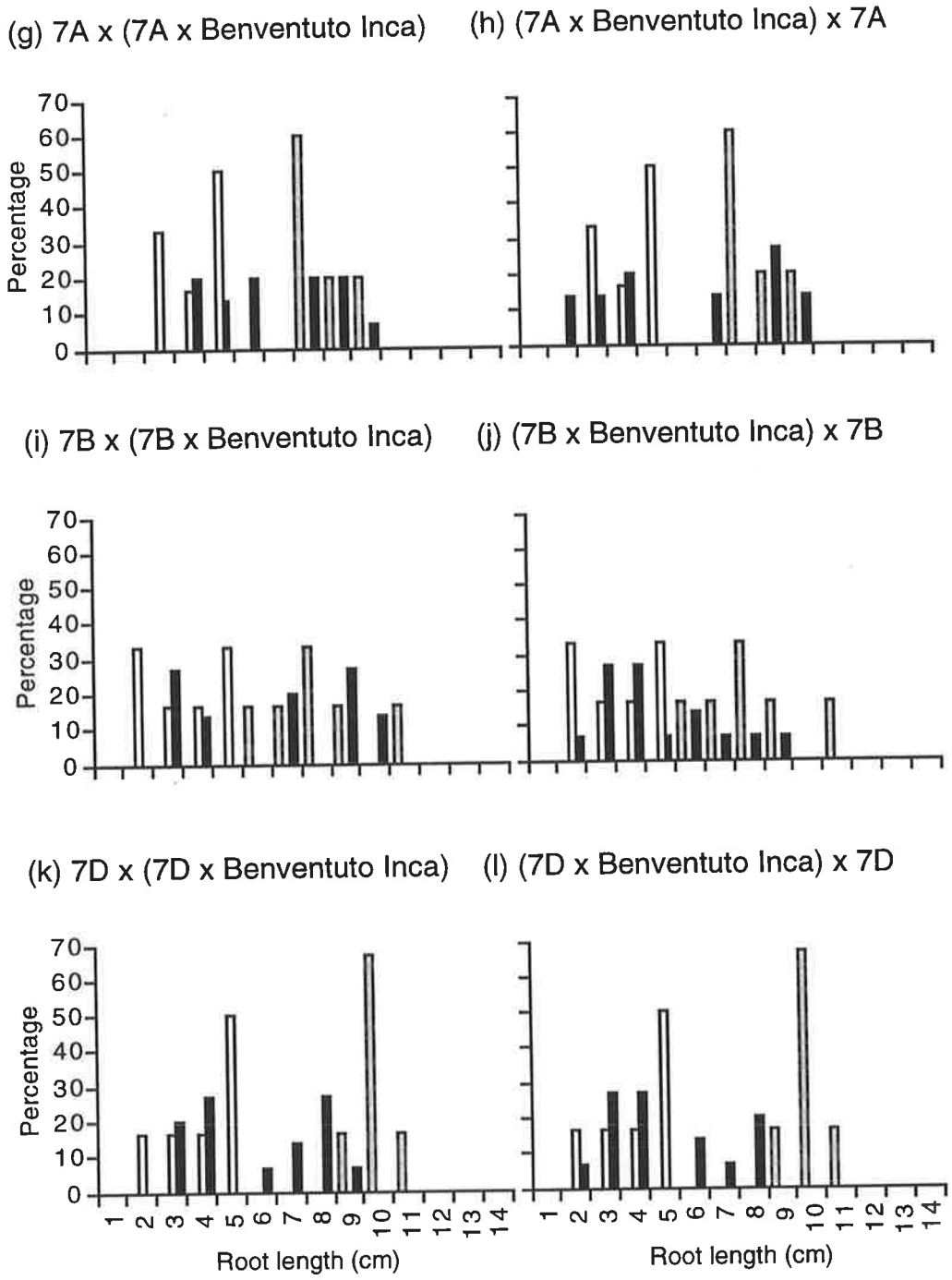


Table 7.11 Chi-square analysis of the observed and expected segregation ratio of 3 intermediate : 1 sensitive of F_1 backcross reciprocal families carrying non-critical chromosomes of Benventuto Inca and Condor, tested in filter paper at B100.

Family		Model	Frequency		
			Int[a]	Sens[b]	χ^2_1
Benventuto Inca 4B[c]	Obs[e]		6	9	
	Exp[f]	3 : 1	11.25	3.75	9.80**
Condor 4B[d]	Obs		10	5	
	Exp	3 : 1	11.25	3.75	0.55
Benventuto Inca 4D	Obs		5	5	
	Exp	3 : 1	7.5	2.5	3.33
Condor 4D	Obs		6	13	
	Exp	3 : 1	14.25	4.75	19.10**
Benventuto Inca 7A	Obs		10	5	
	Exp	3 : 1	11.25	3.75	0.55
Condor 7A	Obs		8	7	
	Exp	3 : 1	11.25	3.75	3.75
Benventuto Inca 7B	Obs		9	6	
	Exp	3 : 1	11.25	3.75	1.80
Condor 7B	Obs		5	10	
	Exp	3 : 1	11.25	3.75	13.89**
Benventuto Inca 7D	Obs		8	7	
	Exp	3 : 1	11.25	3.75	3.75
Condor 7D	Obs		6	9	
	Exp	3 : 1	11.25	3.75	9.80**
Total frequency	Obs		73	76	
	Exp	3 : 1	111.75	37.25	53.75**

[a] Intermediate, [b] Sensitive,

[c] Family derived from crossing between the F_1 monosomic of (Condor monosomic 4B x Benventuto Inca) as male and Condor monosomic 4B as female,

[d] Family derived from crossing between the F_1 monosomic of (Condor monosomic 4B x Benventuto Inca) as female and Condor monosomic 4B as male,

[e] Observed value, [f] Expected value,

** Significant difference at $P < 0.01$.

between the intermediate and sensitive plants from all families of G61450 (Tables 7.8 and 7.9), Benventuto Inca (Tables 7.10 and 7.11) and Condor was also consistent with the ratio of 1 intermediate : 1 sensitve but not 3 intermediate : 1 sensitve. This indicated that tolerance to boron of G61450 and Benventuto Inca relative to Condor was controlled by a single gene. The result of a single gene controlling boron tolerance in G61450 was consistent with Chapter 6.

Chi-square analysis indicated that segregation ratio of all of the non-critical families of India 126 and Condor was consistent with digenic ratio of 3 intermediate : 1 sensitive (Table 7.12, Figure 7.6), with the exception of chromosomes 7D (Table 7.12). The segregation ratios of the non-critical families for chromosomes 4B and 4D of India 126 were consistent with both monogenic (Table 7.13) and digenic ratios (Table 7.12). However, the chi-square values for chromosome 4B and 4D of India 126 at 1 : 1 ratio were 3.27 ($0.05 < P < 0.25$) (Table 7.13) in comparison to 0.02 ($0.75 < P < 0.95$) (Table 7.12) for 3 : 1 ratio, indicating that the segregation ratios of monosomic 4B and 4D of India 126 were closer to the digenic ratio than the monogenic ratio. The segregation of the total frequency between intermediate and sensitive plants of all families of India 126 and Condor was not consistent with the ratio of 3 : 1 (Table 7.12), a deviation mainly because of a low frequency of intermediate plants for chromosome 7D. This is possibly a result of poor germination of the seeds of these families. The segregation of the total frequency between intermediate and sensitive plants was consistent with the 3 : 1 ratio (Table 7.12) when the families for chromosomes 7D were not included. The consistency of the segregation of the non-critical families of India 126 and Condor at the digenic ratio of 3 intermediate : 1 sensitive demonstrated that tolerance to boron of India 126, in relative to Condor is probably controlled by two genes.

7.4 Discussion

Clearly, there is homologous chromosome variation with respect to boron tolerance between different families. The significant increase in root length resulting from the presence of three homologues of chromosome 4A relative to Condor indicates that

Table 7.12 Chi-square analysis of the observed and expected segregation ratio of 3 intermediate : 1 sensitive of F_1 backcross reciprocal families carrying non-critical chromosomes of India 126 and Condor, tested in filter paper at B100.

Family		Model	Frequency		
			Int[a]	Sens[b]	χ^2_1
India126 4B[c]	Obs[e]		11	4	
	Exp[f]	3 : 1	11.25	3.75	0.02
Condor 4B[d]	Obs		9	6	
	Exp	3 : 1	11.25	3.75	1.80
India126 4D	Obs		11	4	
	Exp	3 : 1	11.25	3.75	0.02
Condor 4D	Obs		9	6	
	Exp	3 : 1	11.25	3.75	1.80
India126 7A	Obs		10	2	
	Exp	3 : 1	9	3	0.44
Condor 7A	Obs		8	6	
	Exp	3 : 1	10.5	3.5	2.39
India126 7B	Obs		12	3	
	Exp	3 : 1	11.25	3.75	0.20
Condor 7B	Obs		9	6	
	Exp	3 : 1	11.25	3.75	1.80
India126 7D	Obs		6	9	
	Exp	3 : 1	11.25	3.75	9.80**
Condor 7D	Obs		7	8	
	Exp	3 : 1	11.25	3.75	6.42*
Total frequency	Obs		92	54	
	Exp	3 : 1	109.5	36.5	11.19**
Total frequency[g]	Obs		79	37	
	Exp	3 : 1	87	29	2.95

[a] Intermediate, [b] Sensitive,

[c] Family derived from crossing between the F_1 monosomic of (Condor monosomic 4B x India 126) as male and Condor monosomic 4B as female,

[d] Family derived from crossing between the F_1 monosomic of (Condor monosomic 4B x India 126) as female and Condor monosomic 4B as male,

[e] Observed value, [f] Expected value,

[g] Total frequency excluded families derived from chromosome 7D,

*, ** Significant difference at $0.01 < P < 0.05$ and $P < 0.01$, respectively.

Figure 7.6 The distributions of root lengths of individual plants within the backcross reciprocal families derived from the reciprocal crosses between the F_1 monosomic of (Condor monosomic x India 126) and Condor monosomic, in comparison with the disomic parents.

(a) 4A x (4A x India 126) = family derived from crossing between the F_1 monosomic of (Condor monosomic 4A x India 126) as male and Condor monosomic 4A as female.

(b) (4A x India 126) x 4A = family derived from crossing between the F_1 monosomic of (Condor monosomic 4A x India 126) as female and Condor monosomic 4A as male. This system also applied to the other crosses.

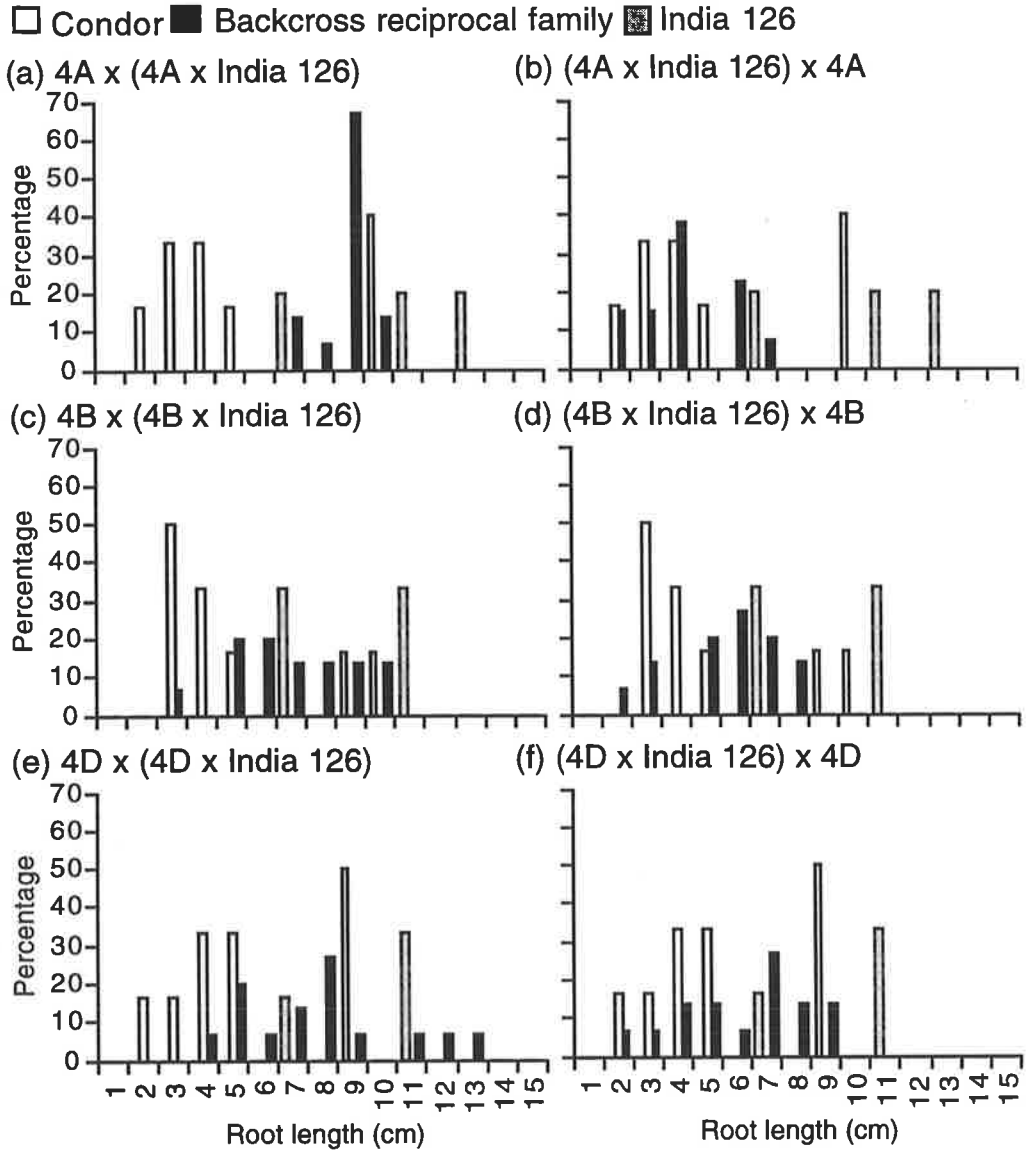


Figure 7.6 (continued).

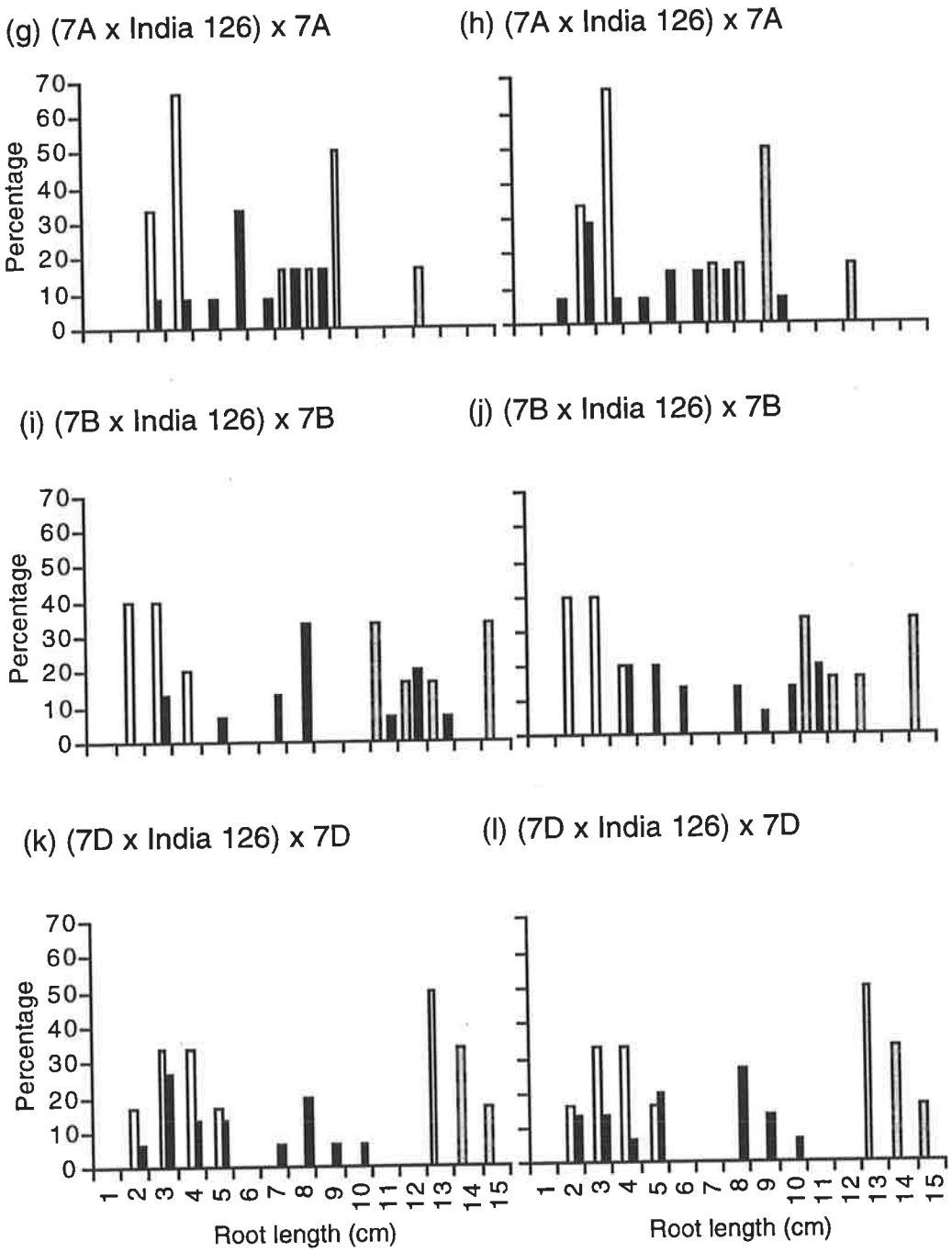


Table 7.13 Chi-square analysis of the observed and expected segregation ratio of 1 intermediate : 1 sensitive of F_1 backcross reciprocal families carrying non-critical chromosomes of India 126 and Condor, tested in filter paper at B100.

Family		Model	Frequency		
			Int[a]	Sens[b]	χ^2_1
India126 4B[c]	Obs[e]		11	4	
	Exp[f]	1 : 1	7.5	7.5	3.27
Condor 4B[d]	Obs		9	6	
	Exp	1 : 1	7.5	7.5	0.60
India126 4D	Obs		11	4	
	Exp	1 : 1	7.5	7.5	3.27
Condor 4D	Obs		9	6	
	Exp	1 : 1	7.5	7.5	0.60
India126 7A	Obs		10	2	
	Exp	1 : 1	6	6	5.33*
Condor 7A	Obs		8	6	
	Exp	1 : 1	7	7	0.29
India126 7B	Obs		12	3	
	Exp	1 : 1	7.5	7.5	5.40*
Condor 7B	Obs		9	6	
	Exp	1 : 1	7.5	7.5	0.60
India126 7D	Obs		6	9	
	Exp	1 : 1	7.5	7.5	0.60
Condor 7D	Obs		7	8	
	Exp	1 : 1	7.5	7.5	0.04
Total frequency	Obs		92	54	
	Exp	1 : 1	73	73	9.90**

[a] Intermediate, [b] Sensitive,

[c] Family derived from crossing between the F_1 monosomic of (Condor monosomic 4B x India 126) as male and Condor monosomic 4B as female,

[d] Family derived from crossing between the F_1 monosomic of (Condor monosomic 4B x India 126) as female and Condor monosomic 4B as male,

[e] Observed value, [f] Expected value,

*, ** Significant difference at $0.01 < P < 0.05$ and $P < 0.01$, respectively.

chromosome 4A of Benventuto Inca, G61450 and India 126 carry alleles responsible for boron tolerance. Chromosome 7B is responsible for boron tolerance of Halberd.

Chi-square analysis indicated that the segregation of root lengths of the plants in the non-critical families of G61450, Benventuto Inca and their reciprocal families was consistent with the monogenic ratio of 1 intermediate : 1 sensitive, indicating a single gene controlling tolerance to boron of the two varieties in relative to Condor and that only the chromosomes identified above have substantial effects on boron tolerance. The segregation of the non-critical families of India 126 and their reciprocal was consistent with the digenic ratio of 3 intermediate : 1 sensitive, suggesting that two genes are involved in controlling tolerance to boron of India 126 relative to Condor, one of which is located on chromosome 4A.

The segregation of root lengths of almost all of the non-critical families of Halberd/Condor monosomic crosses was consistent with a monogenic ratio. However, all families had a deficiency of intermediate plants (Table 7.6). It is probable that the low frequency of intermediate plants was a consequence of the closeness in response of Halberd and Condor (Figure 7.3) resulting in poor discrimination between the various classes and a high level of Type I and Type II errors.

The segregation of some of the non-critical families of G61450, Benventuto Inca, India 126 and their reciprocal families was consistent with both of the monogenic and digenic ratio. For example, the segregation of non-critical families for chromosomes 4B and 4D of India 126 was consistent with both monogenic (Table 7.13) and digenic ratio (Table 7.12). This is probably because the number of seeds (fifteen seeds per family) used for testing tolerance to boron was not sufficient to differentiate between the two ratios. The lower frequency of intermediate and higher frequency of sensitive plants than expected in some families is possibly as a result of misclassification of the intermediate as sensitive due to the poor germination (Table 7.12). For example, the frequency of the intermediate plants

of the non-critical family for chromosome 4B of G61450 and 4D of Condor was 3 in comparison to 12 of the expected frequency (Table 7.8).

It has been shown that chromosome dosage has no effect on root length of all the Condor monosomic families (Chapter 6). Thus, the differences in root length observed between each pair of the reciprocal families may be attributed to differences in response to boron of the tested chromosomes rather than chromosome dosage effects. Almost all of the families for the critical chromosomes from the tolerant varieties had, on average, shorter roots than the corresponding disomic parent, whereas the critical families for the chromosomes from Condor had a mean root length greater than the disomic Condor (Table 7.1, 7.2). For example, the mean root lengths of the critical family for chromosome 4A of Benventuto (8.39 cm) was shorter than that of Benventuto Inca (9.2 cm) (Table 7.1) while the mean root lengths of the family carrying chromosome 4A from Condor (4.25 cm) was longer than that of the disomic Condor (3.5 cm) (Table 7.1). This phenomenon may be explained by the frequency of tolerant-intermediate plants of the critical families for the chromosome of the tolerant variety (96%) and the corresponding families of the sensitive variety (25%) (Figure 7.1).

As the frequency of 72%, 24% and 4% for tolerant, intermediate and sensitive, respectively, was expected for the progenies of the family for the critical chromosome from the tolerant variety (Figure 7.2a) while the frequency of 1%, 24% and 75% was expected for the tolerant, intermediate and sensitive progenies of the family for the critical chromosome from Condor (Figure 7.2b), an expected mean can be calculated.

The expected mean of the family for the critical chromosome from tolerant variety

$$= ((72 \times m_1) + (4 \times m_2) + (24 \times m_3))/100$$

The expected mean of the family for the critical chromosome from sensitive variety

$$= ((1 \times m_1) + (75 \times m_2) + (24 \times m_3))/100$$

where m_1 = mean root length of the tolerant parent, m_2 = mean root length of the sensitive parent, $m_3 = (m_1 + m_2)/2$, the mean root length of the heterozygote.

The observed differences between the means root lengths of the families for the critical chromosomes from Halberd, G61450 and Benventuto Inca and those of the corresponding families for the critical chromosomes from Condor were similar to those expected (Table 7.14). This indicated that all the response of tolerance to boron of Halberd, G61450 and Benventuto Inca, relative to Condor, was attributable to chromosomes 7B or 4A. The difference between the observed mean of the critical family for India 126 and that of the family for Condor was 2.15 cm in comparison to an expected 4.76 cm indicating that there was probably more than one chromosomes responsible for tolerance to boron in India 126.

For the family derived from the reciprocal crosses between the non-critical monosomic of the tolerant and sensitive varieties, there is no monosomic plant with the gene for boron tolerance in the hemizygous condition. Approximately 50% of the plants of this family are heterozygous for the gene controlling boron tolerance and the other 50% of the plants are the sensitive. Thus on average, these families were more tolerant than Condor but less tolerant than the tolerant varieties and show an increased variation. For example, the average root lengths over all the non-critical families for G61450 and Condor were 5.87 and 5.59 cm, in comparison to the over all average 9.56 and 4.06 cm for the parents G61450 and Condor (Table 7.2).

The variance of the critical families for chromosomes from the tolerant varieties were in general lower than those of the corresponding critical families for chromosome of Condor which in turn were lower than those of the corresponding non-critical families. For example, the distribution of the seedling root lengths of the critical family for chromosome 4A from Benventuto Inca (Figure 7.5a) was substancially different from that for chromosome 4A from Condor (Figure 7.5b) and the non-critical family for chromosome 4B from Benventuto Inca (Figure 7.5c) and Condor (Figure 7.5d). There were only the tolerant-intermediate seedlings observed in the critical family for chromosome 4A of

Table 7.14 The expected and observed means of the F_1 backcross reciprocal families for the critical chromosomes of Halberd, G61450, India 126, Benventuto Inca and Condor, tested in filter papers at B100.

Family	Mean			
	Expected	Difference[c]	Observed	Difference
Halberd 7B[a]	5.90	2.13	6.42	2.13
Condor 7B[b]	3.77		4.29	
G61450 4A	9.03	3.90	8.38	3.80
Condor 4A	5.13		4.58	
India 126 4A	9.14	4.76	8.78	2.15
Condor 4A	4.38		6.63	
B. Inca 4A	8.30	4.05	8.39	4.14
Condor 4A	4.25		4.25	

[a] Family for chromosome 7B of Halberd, [b] Family for chromosome 7B of Condor,

[c] Difference between the BCR families.

Benventuto Inca (Table 7.5) and the variance of this family was 0.5 (Table 7.4). The root length of the plants for chromosome 4A of Condor, with the variance of 6.1 (Table 7.4), segregated at the ratio of 1 tolerant-intermediate : 3 sensitive (Table 7.5). The non-critical families for chromosomes 4B of both Benventuto Inca and Condor, with the variances of 11.3 and 8.9 (Table 7.4), respectively, segregated at the ratio of 1 intermediate : 1 sensitive (Table 7.10).

The results of the chromosome 4A and 7B of G61450 and Halberd, respectively, were consistent with the monosomic analysis experiment (Chapter 6). For Lin Calel and AUS 4041, there was no significant effect of chromosome 4B and 4D or chromosomes of group 7 in response to boron. This indicates a possibility of chromosome 4A or, alternatively, chromosomes of different homoeologous groups being responsible for boron tolerance in the two varieties. If chromosome 4A is responsible for boron tolerance in Lin Calel and Aus 4014, transgressive segregation should not be expected when the two varieties are crossed to G61450 and Benventuto Inca.

For India 126, the segregation of the non-critical families for India 126 and Condor was consistent with the digenic ratio suggesting two genes involved in controlling tolerance to boron of India 126. In contrast, backcross reciprocal monosomic analysis indicated that only one chromosome (4A) is responsible for tolerance to boron in India 126. However, there were large differences between the mean root lengths of the families for chromosomes 4D, 7A and 7B from India 126 and those of the families from Condor (1.48, 1.18 and 1.28 cm, respectively) (Tables 7.1 and 7.2), indicating that these chromosomes may be responsible for tolerance to boron in India 126. The non-significant effects of these chromosomes in response to boron relative to Condor could be because the effects of the alleles on each of these chromosomes are small in comparison to chromosome 4A. Alternatively, it is possible that a homoeologous groups other than group 4 and 7, were responsible for the tolerance to boron of India 126.

Chapter 8

Relationship between the South Australian boron tolerant variety BT-Schomburgk and exotic germplasms

8.1 Introduction

In the regions of southern Australia where high levels of boron predominate, wheat varieties that have been widely cultivated belong to a single family of varieties descended from Federation (Wrigley and Rathjen, 1981), which is moderately tolerant to boron (Paull et al., 1986). Most of the varieties in this family, including Halberd, are also moderately tolerant to boron (Paull, 1990). This suggests that the high concentrations of boron in the soil have had a significant selection pressure on the breeding and distribution of the wheat varieties grown in southern Australia.

More than 1500 accessions of wheat from the Australian Winter Cereals Collections, Tamworth, have been screened for tolerance to boron. Approximately 7% (107) of the lines, most of which were exotic, were more tolerant than Halberd (Moody et al., 1988). Most Australian varieties were classified as moderately sensitive. Lines from Afghanistan and Japan were predominantly tolerant whereas lines from semi-arid regions or along the earth's major fault lines including China, Turkey, India and South America, had a great diversity, probably associated with localized zones of depletion and accumulation of boron (Moody et al., 1988). These tolerant lines could be used as donor parents in a backcrossing program to increase the levels of tolerance in southern Australian wheats.

The introduction of CIMMYT wheats to Australia in the 1960's produced the most important new source of genetic variability this century. Although the CIMMYT derived wheats are generally moderately sensitive to boron (Moody et al., 1988), many are resistant to stripe rust (*Puccinia striiformis*) and as a result of the impact of this disease since 1979, farmers, particularly in Victoria, have continued to grow these varieties despite their moderately sensitive reaction to boron. The most tolerant of the current Australian varieties are Halberd, Spear, Dagger, BT-Schomburgk, Barunga, Trident and Frame. BT-

Schomburgk was developed by deliberately transferring the boron tolerant gene *Bo1* from Halberd, a moderately tolerant Australian variety, to the high yielding and well adapted variety Schomburgk by backcrossing. This same approach could be adopted to transfer the high level of tolerance identified in exotic accessions to the moderately tolerant Australian varieties.

To enable an efficient crossing and selection strategy to be devised it is necessary to elucidate the genetic relationship between the exotic sources of boron tolerance and the Australian varieties carrying the *Bo1* gene. If it can be demonstrated that the difference in response between a tolerant exotic and a moderately tolerant Australian variety is under simple genetic control, the backcrossing procedure could be utilized. On the other hand, where transgressive segregation occurs, as was observed among the F_2 progeny of the cross between the tolerant line G61450, introduced from Greece, and Halberd (Chapter 5) (Paull et al., 1991b), the potential exists to select very tolerant genotypes which have a combination of genes from the two varieties.

The experiment described in this Chapter was conducted to establish the genetic relationship, with respect to tolerance to boron, between BT-Schomburgk and four exotic lines derived from geographically diverse locations.

8.2 Materials and methods

Genetic material

The pedigree, boron response, Australian Winter Cereals Collection accession number and the country of origin for all lines, including the tolerant selections India 126, AUS 4903, Turkey 1473 and Klein Granador, used in this experiment are presented in Table 4.1. These lines were chosen on the basis of their responses to boron (Moody et al., 1988), and their geographical origins and genetic backgrounds which are diverse from Australian wheat varieties. The F_1 hybrid seeds from the crosses between BT-Schomburgk and the four exotic lines were kindly provided by Mr. D. B. Moody. These crosses were developed as part of his PhD project. He is now working with the Victoria Department of Agriculture (VIDA). The F_1 seeds were advanced to the F_2, F_3 and F_4 generations.

Generation of the tested populations and number of seeds

Approximately 100 random F_2 seeds were obtained from F_1 plants and advanced to F_3 and F_4 generations using a single seed descent method. Approximately 10 random F_1 seeds (including reciprocals) of each cross were sown and the F_2 seeds within each cross were bulked. In the second season, approximately 100 random F_2 seeds were sown and one random F_3 seed was separately harvested from each of the 100 F_2 plants and sown. The F_4 seeds which were harvested from individual F_3 plants are described as F_3 derived families.

These families were tested for tolerance to boron using the filter paper method (Chapter 4) with the concentration of boron of 100 mg l^{-1}. Twelve to fifteen seeds of each F_3 derived family and their parental lines were placed separately in filter papers.

Methods used for estimation of the number of genes responsible for boron tolerance

The methods (Chapter 3) used for estimation the number of genes responsible for tolerance to boron in four F_3 derived populations are described below.

(a) The F_3 derived families were classified into three categories (homozygous tolerant, segregating and homozygous sensitive) using the comparison between means of each F_3 derived family and their parents. The confidence intervals of the means of the two parents were calculated as described in Chapter 3. The LSD of the parental variances were also calculated (Chapter 3), however as these LSD are very subject to Type II errors (D. Pederson, pers. comm.), it can be observed that when the variance of one, or both parents, was high (see Table 8.4), the variances of virtually all families were below the LSD of the parental variances, despite overwhelming evidence of segregation within some of the families. In some instances, where the LSD of the parental variances was not comparatively high, families having variances less than or equal to the LSD of the parental variances and with means within the confidence interval of the mean of either the sensitive or tolerant parent could be classified as homozygous sensitive or tolerant, respectively. Families with variances higher than the LSD of the parental variances were classified as segregating. However, when the LSD of the parental variances was high, an arbitrary level of a little above the parental variances, at the upper level of those families obviously in the

homozygous categories, was taken for differentiation between the segregating and homozygous families. For the monogenic model, it was not immediately possible to classify a family into any of these three categories when its variance was similar to that of the parents but its mean was between the two parents. In this case, the individual families were investigated and the classification was based on the basis of the performance of the individual segregants. For example, if the root lengths of the plants within a family overlapped with the two parents, the family was classified as heterozygous. Whereas a family was classified as homozygous tolerant or sensitive when the root length of all plants within the family fell within the range of the tolerant or sensitive parent.

Chi-square analysis was used for testing the goodness of fit of the observed segregation ratios to frequencies expected for monogenic and digenic segregation. Since the single seed descent method was used for advancing the four populations to the F_4 generation, the expected monogenic and digenic segregation ratios of the F_3 families were 5 tolerant-segregating : 3 sensitive and 3 tolerant : 2 segregating : 3 sensitive or 55 tolerant-intermediate : 9 sensitive and 9 tolerant : 46 intermediate : 9 sensitive (Table 8.1), respectively.

Table 8.1 The expected frequencies for monogenic and digenic segregation ratios (these ratios apply to all additive genetic situations) of progenies from F_2, F_3 and F_4 populations derived from F_1 hybrids using the single seed descent method.

Generation	Genotype					
	Monogenic			Digenic		
	AA	Aa	aa	AABB	Int[a]	aabb
F_2	1	2	1	1	14	1
F_3	3	2	3	9	46	9
F_4	7	2	7	49	158	49

[a] Intermediate genotypes are AAbb, aaBB, AABb, AaBb, aaBb, AaBB and Aabb.

(b) The variance observed from the individual populations was compared with the expected variances for alternative genetic models. The observed and expected variances were not regarded as being significantly different when the expected variance fell within the confidence interval of the observed variance (Chapter 3). The expected variances were calculated from the following equations as described in Chapter 3.

For 1 gene; $V_{F4} = 7/8D + E$

For 2 genes, $d_a = d_b = d/2$; $V_{F4} = 7/8D + E$,

where d is the departure from the mid-point (m) of each homozygous genotype, and E is the environmental variance ($E = 1/4\ V_{P1} + 1/4\ V_{P2} + 1/2\ V_{F1}$; V_{P1} and V_{P2} are variances of the parents, V_{F1} is the variance of the F_1 hybrid between P_1 and P_2). Since the F_1 plants were not tested in this experiment, the variance of the F_1 hybrid was estimated to be equal to the average of the variance of the two parents ($V_{F1} = (V_{P1} + V_{P2})/2$) (see Chapter 3).

8.3 Results

The distributions of the mean root length of the F_3 derived families under high boron condition were observed to be bimodal or trimodal for all crosses (Figure 8.1). However, there was no obvious cut-off point between the sensitive and tolerant-segregating families in all crosses. Thus, the F_3 derived families of the four crosses were classified into two (tolerant-segregating and sensitive) and three (tolerant, segregating and sensitive) categories according to the means and variances of each family in comparison with the confidence interval of the means and the LSD of the variances of the parents (Chapter 3).

(BT-Schomburgk x India 126)

The LSD of the parental variances was much higher than the variances of virtually all families (Figure 8.2) and thus it was not used to differentiate among the families. An arbitrary level of a little above the variance of the parents, at the upper level of those families obviously in the homozygous categories, was taken for differentiation between the segregating and homozygous families.

Figure 8.1 Seedling root length of F_3 derived families (mean of 10-12 F_4 seedlings for each families) and parents (mean of 20-24 seedlings for each parent), tested in filter papers at B100.

(a) (BT-Schomburgk x India 126)

(b) (BT-Schomburgk x AUS 4903)

(c) (BT-Schomburgk x Turkey 1473)

(d) (BT-Schomburgk x Klein Granador)

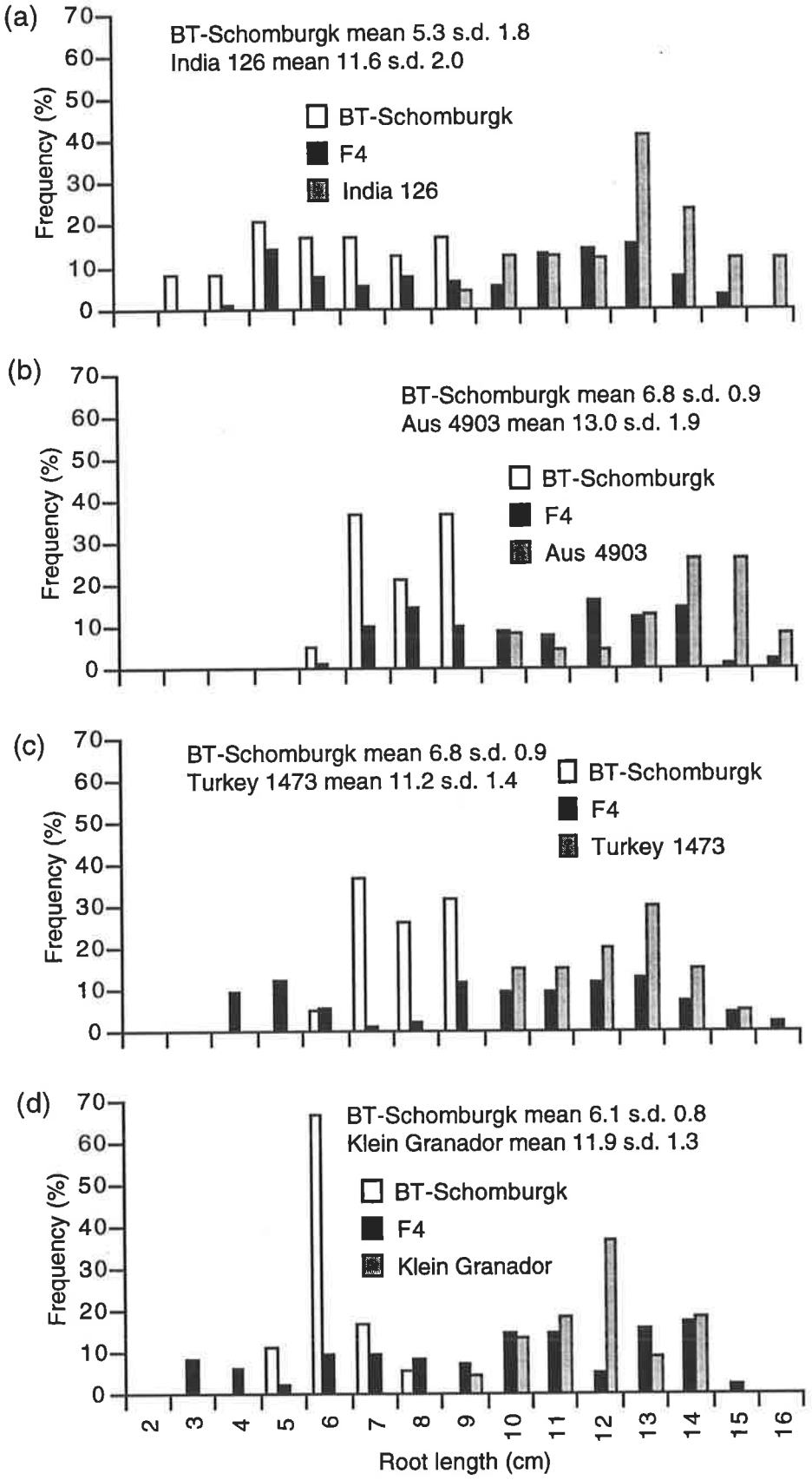
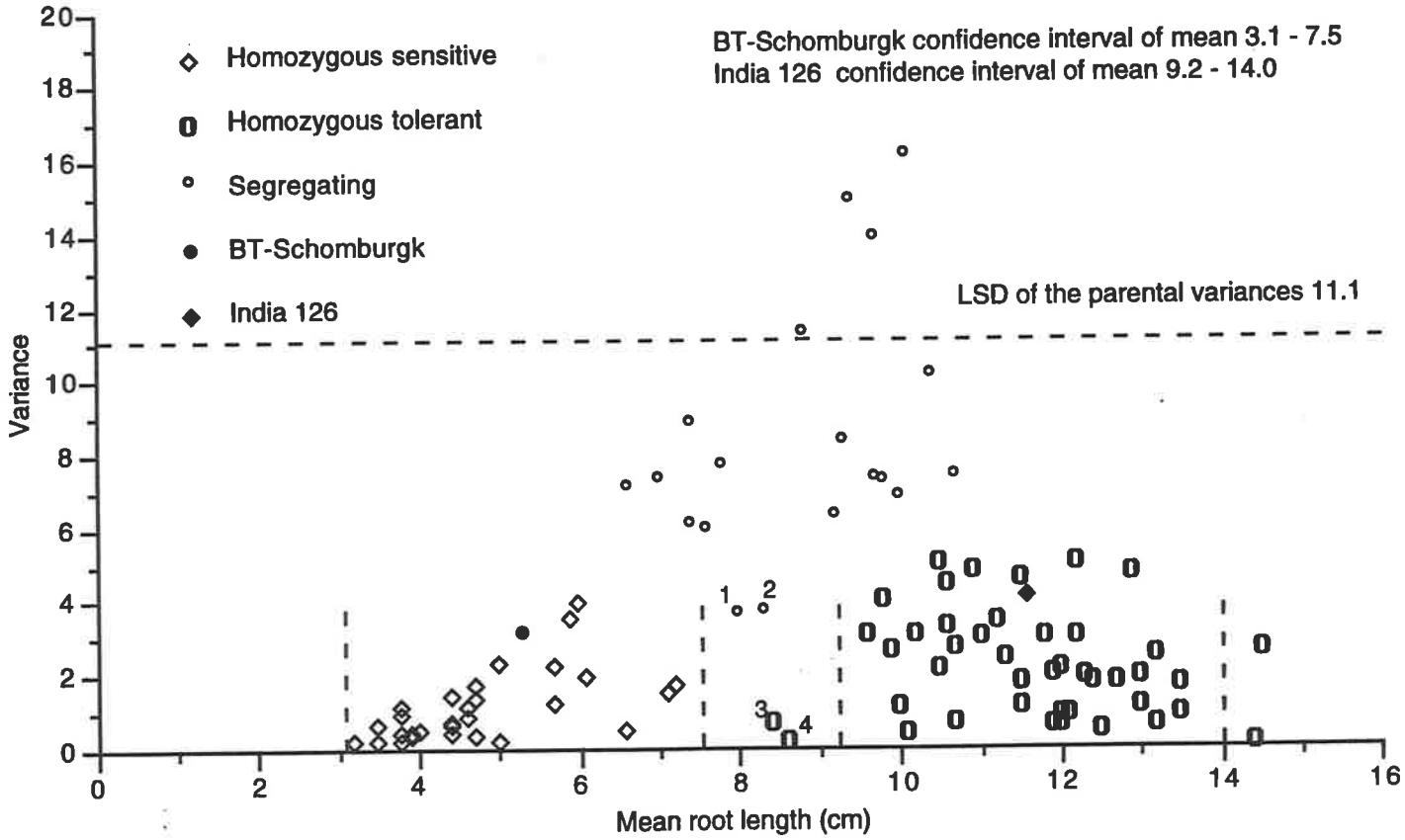

(a)
BT-Schomburgk mean 5.3 s.d. 1.8
India 126 mean 11.6 s.d. 2.0
BT-Schomburgk
F4
India 126
Frequency (%)
(b)
BT-Schomburgk mean 6.8 s.d. 0.9
Aus 4903 mean 13.0 s.d. 1.9
BT-Schomburgk
F4
Aus 4903
Frequency (%)
(c)
BT-Schomburgk mean 6.8 s.d. 0.9
Turkey 1473 mean 11.2 s.d. 1.4
BT-Schomburgk
F4
Turkey 1473
Frequency (%)
(d)
BT-Schomburgk mean 6.1 s.d. 0.8
Klein Granador mean 11.9 s.d. 1.3
BT-Schomburgk
F4
Klein Granador
Frequency (%)
0 10 20 30 40 50 60 70
2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Root length (cm)

Figure 8.2 Mean and variance of root length of 93 F_3 derived families and their parents for the cross (BT-Schomburgk x India 126), tested in filter papers at B100.

Note: The horizontal line is the LSD of the parental variances.

The vertical lines are the confidence intervals of means of the two parents.

The numbers on the diagram refer to the families which are depicted in Figures 8.3.

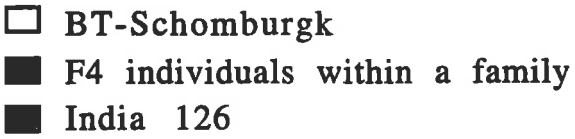

Homozygous sensitive
Homozygous tolerant
Segregating
BT-Schomburgk
India 126
BT-Schomburgk confidence interval of mean 3.1 - 7.5
India 126 confidence interval of mean 9.2 - 14.0
LSD of the parental variances 11.1
Variance
Mean root length (cm)
0
2
4
6
8
10
12
14
16
18
20
1
2
3
4

The F_3 families were classified into two categories (tolerant-segregating and homozygous sensitive). Families with a mean within the confidence interval of the mean of the sensitive parent and variance close to that of the sensitive parent were classified as homozygous sensitive. Families with a mean of greater than the confidence interval of the mean of the sensitive parent were classified as tolerant-segregating (Chapter 3). The F_3 families were also classified into three categories (homozygous tolerant, segregating and homozygous sensitive). Families with a mean within the confidence interval of the mean of the tolerant parent and variances close to that of the tolerant parent were classified as homozygous tolerant. Families with a mean outside the confidence interval of the means of the parents and variances much greater than those of the parents were classified as segregating. There were four families with variances that were little different from those of the sensitive and tolerant parents, but with a mean root length between the two parents (Figure 8.2). These individual families were inspected and classified on the basis of the performance of individual plants in comparison with the parents (Figure 8.3). The ranges of the root lengths of the sensitive and tolerant parents were 2-8 cm and 8-15 cm, respectively (Figure 8.3). Since there was overlap between the root lengths of plants within each of Families 1 and 2 and those of the two parents (Figure 8.3), these families were classified as heterozygous or segregating. The variation in root lengths of Families 3 and 4 was low and as almost all of the plants fell within the range of the tolerant parent (Figure 8.3), Families 3 and 4 were classified as homozygous tolerant.

Chi-square analysis indicated that the segregation of the F_3 derived families of (BT-Schomburgk x India 126) was consistent with the monogenic ratio of 5 tolerant-segregating : 3 sensitive and 3 tolerant : 2 segregating : 3 sensitive (Table 8.2) but not the digenic ratio of 55 tolerant-segregating : 9 sensitive (Table 8.3). Examples of the homozygous tolerant, homozygous sensitive and segregating F_3 families of (BT-Schomburgk x India 126) in comparison with the two parents are presented in Plate 8.1a.

As the expected variances for both one and two gene models of the F_3 derived population of (BT-Schomburgk x India 126) were in the range of the confidence interval of

Figure 8.3 Response of twelve plants within four F_3 derived F_4 families of (BT-Schomburgk x India 126) at B100 in comparison with the two parents.



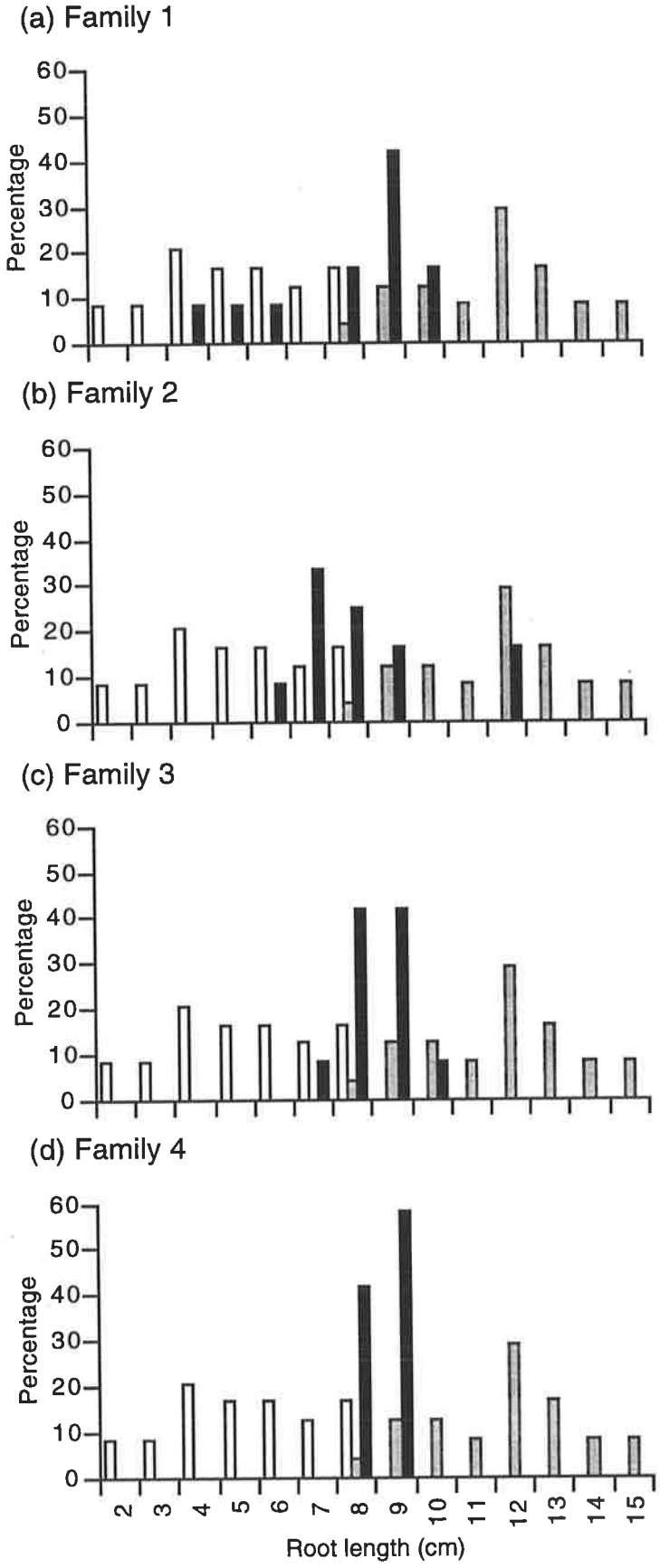

(a) Family 1
Percentage
60
50
40
30
20
10
0
(b) Family 2
Percentage
60
50
40
30
20
10
0
(c) Family 3
Percentage
60
50
40
30
20
10
0
(d) Family 4
Percentage
60
50
40
30
20
10
0
2
3
4
5
6
7
8
9
10
11
12
13
14
15
Root length (cm)

Table 8.2 Chi-square analysis of the observed and expected segregation ratios for a one gene model of F_3 derived F_4 families for four populations, tested in filter papers at B100.

Population (F_3 derived F_4)		Model	Frequency			
			Tol[a]+Seg[b]	Sens[c]		χ^2_1
(BT-SchomburgkxIndia126)	Obs[d]		64	29		
	Exp[e]	5 : 3	58.125	34.875		1.58
(BT-SchomburgkxAUS4903)	Obs		58	28		
	Exp	5 : 3	53.75	32.25		0.90
(BT-SchomburgkxTurkey1473)	Obs		57	27		
	Exp	5 : 3	52.5	31.5		1.03
(BT-SchomburgkxKleinGranador)	Obs		58	24		
	Exp	5 : 3	51.25	30.75		2.36
			Tol	Seg	Sens	χ^2_2
(BT-SchomburgkxIndia126)	Obs		44	20	29	
	Exp	3 : 2 :3	34.875	23.25	34.875	3.83
(BT-SchomburgkxAUS4903)	Obs		36	22	28	
	Exp	3 : 2 :3	32.25	21.5	32.25	1.01
(BT-SchomburgkxTurkey1473)	Obs		32	25	27	
	Exp	3 : 2 : 3	31.5	21	31.5	1.41
(BT-SchomburgkxKleinGranador)	Obs		25	33	24	
	Exp	3 : 2 : 3	30.75	20.5	30.75	10.18

Probability (P) of chi-square at 1 and 2 degrees of freedom.

P	0.50	0.25	0.05	0.01
χ^2_1	0.45	1.32	3.84	6.63
χ^2_2	1.39	2.77	5.99	9.21

[a] Tolerant, [b] Segregating, [c] Sensitive, [d] Observed value, [e] Expected value

Table 8.3 Chi-square analysis of the observed and expected segregation ratios for a two gene model of F_3 derived F_4 families for four populations, tested in filter papers at B100.

Population (F_3 derived F_4)		Model	Frequency		
			Tol[a]+int[b]	Sens[c]	χ^2_1
(BT-SchomburgkxIndia126)	Obs[d]		64	29	
	Exp[e]	55 : 9	79.92	13.08	22.55
(BT-SchomburgkxAUS4903)	Obs		58	28	
	Exp	55 : 9	73.91	12.09	24.36
(BT-SchomburgkxTurkey1473)	Obs		60	23	
	Exp	55 : 9	72.19	11.81	12.65
(BT-SchomburgkxKleinGranador)	Obs		70	12	
	Exp	55 : 9	70.47	11.53	0.02
			Tol	Int	Sens	χ^2_2
(BT-SchomburgkxKleinGranador)	Obs		5	65	12	
	Exp	9 : 46 : 9	11.53	58.94	11.53	4.34

Probability (P) of chi-square at 1 and 2 degrees of freedom.

P	0.50	0.25	0.05	0.01
χ^2_1	0.45	1.32	3.84	6.63
χ^2_2	1.39	2.77	5.99	9.21

[a] Tolerant, [b] Intermediate, [c] Sensitive, [d] Observed value, [e] Expected value

Plate 8.1 Response of homozygous tolerant, segregating and homozygous sensitive F_3 derived families of two crosses to the B100 treatment and comparison with the two parents.

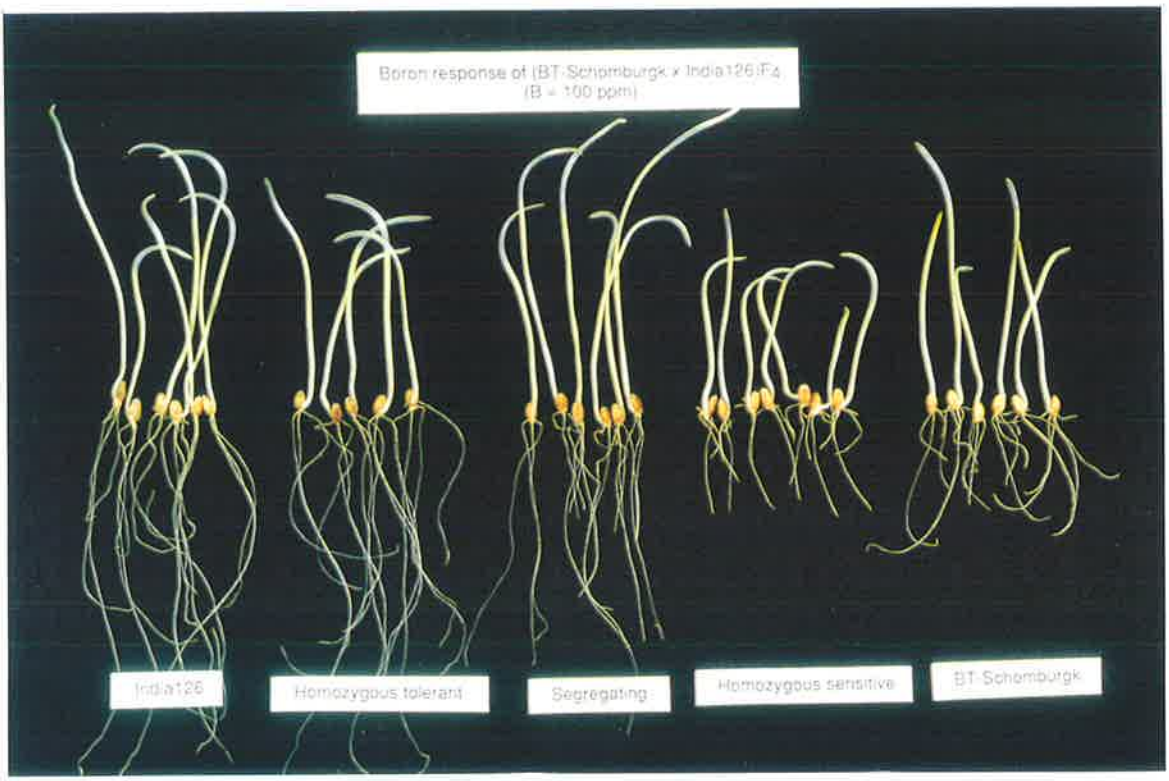
(a) (BT-Schomburgk x India 126)

From left to right: India 126, Homozygous tolerant, Segregating, Homozygous sensitive and BT-Schomburgk

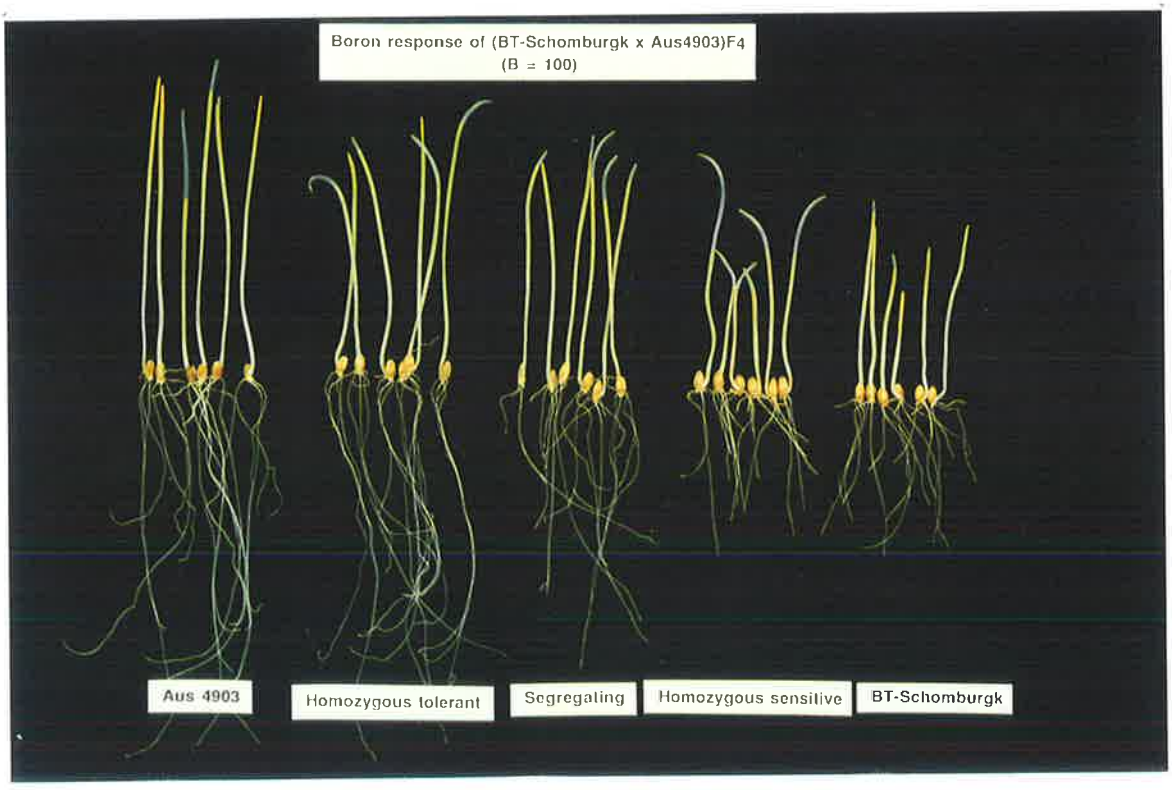
(b) (BT-Schomburgk x AUS 4903)

From left to right: AUS 4903, Homozygous tolerant, Segregating, Homozygous sensitive and BT-Schomburgk

(a)


Boron response of (BT-Schomburgk x India126)F4
(B = 100 ppm)
India126
Homozygous tolerant
Segregating
Homozygous sensitive
BT-Schomburgk

(b)


Boron response of (BT-Schomburgk x Aus4903)F4
(B = 100)
Aus 4903
Homozygous tolerant
Segregating
Homozygous sensitive
BT-Schomburgk

the observed variance (Table 8.4), this failed to differentiate between the one or two genes models for tolerance to boron for this cross.

(BT-Schomburgk x AUS 4903)

The LSD of the parental variances (6.9) for this cross was also higher than most families (Figure 8.4) and thus was not used to differentiate between the families. Families were classified into two (tolerant-segregating and homozygous sensitive) and then into three (homozygous tolerant, segregating and homozygous sensitive) categories. There were nine families which could not be obviously classified because their means were intermediate between the two parents but with variances not much greater than those of the two parents (Figure 8.4), so the performance of the individual plants of these families was inspected. The ranges of the root lengths of the sensitive and tolerant parents were 5-8 cm and 9-16 cm, respectively (Figure 8.5). Families with root lengths overlapping the two parents were classified as segregating (Families 2-8). The root lengths of Family 1 also overlapped the two parents, however it was classified as homozygous sensitive because the variation in root lengths of this family was lower than those of the other families. Family 9 with root lengths within the range of the tolerant parent was classified as homozygous tolerant (Figure 8.5). Chi-square analysis indicated that the segregation ratio of the F_3 derived families of this cross was consistent with the monogenic ratios of 5 tolerant-segregating : 3 sensitive and 3 tolerant : 2 segregating : 3 sensitive (Table 8.2) but not the digenic ratio of 55 tolerant-intermediate : 9 sensitive (Table 8.3). Examples of the homozygous tolerant, homozygous sensitive and segregating F_3 derived families of (BT-Schomburgk x AUS 4903) in comparison with the two parents are presented in Plate 8.1b.

In contrast to the chi-square analysis, the comparison between the observed and expected variances (Table 8.4) indicated segregation at two genes for (BT-Schomburgk x AUS 4903).

Table 8.4 The comparison between expected and observed variances of four F_3 derived F_4 populations for monogenic and digenic models.

Parents and F_4	d [a]	E [b]	Expected variance[c]		Observed	Confidence
			1 gene	2 genes	variance	interval[d]
BT-Schomburgk					3.2	
F_4	3.2	3.6	12.6	8.1	10.4	14.2 - 7.9
India 126					4.0	
BT-Schomburgk					0.8	
F_4	3.1	2.2	10.6	6.4	6.4	8.8 - 4.9
AUS 4903					3.6	
BT-Schomburgk					0.8	
F_4	2.2	1.4	5.6	3.5	11.6	16.1 - 8.7
Turkey 1473					2.0	
BT-Schomburgk					0.6	
F_4	2.9	1.2	8.6	4.9	10.3	14.4 - 7.7
Klein Granador					1.7	

[a] d = the departure of one of a pair of corresponding homozygotes from their mid-point,

[b] E = environmental variance = 1/2 P_1 + 1/2 P_2 (Chapter 3),

[c] the expected variances were calculated on the assumption of no dominance, no linkage and no epistacy,

[d] confidence interval of observed variance at $P = 0.95$.

Figure 8.4 Mean and variance of root length of 86 F_3 derived families and their parents for the cross (BT-Schomburgk x AUS 4903), tested in filter papers at B100.

Note: The horizontal line is the LSD of the parental variances.

The vertical lines are the confidence intervals of means of the two parents.

The numbers on the diagram refer to the families which are depicted in Figures 8.5.

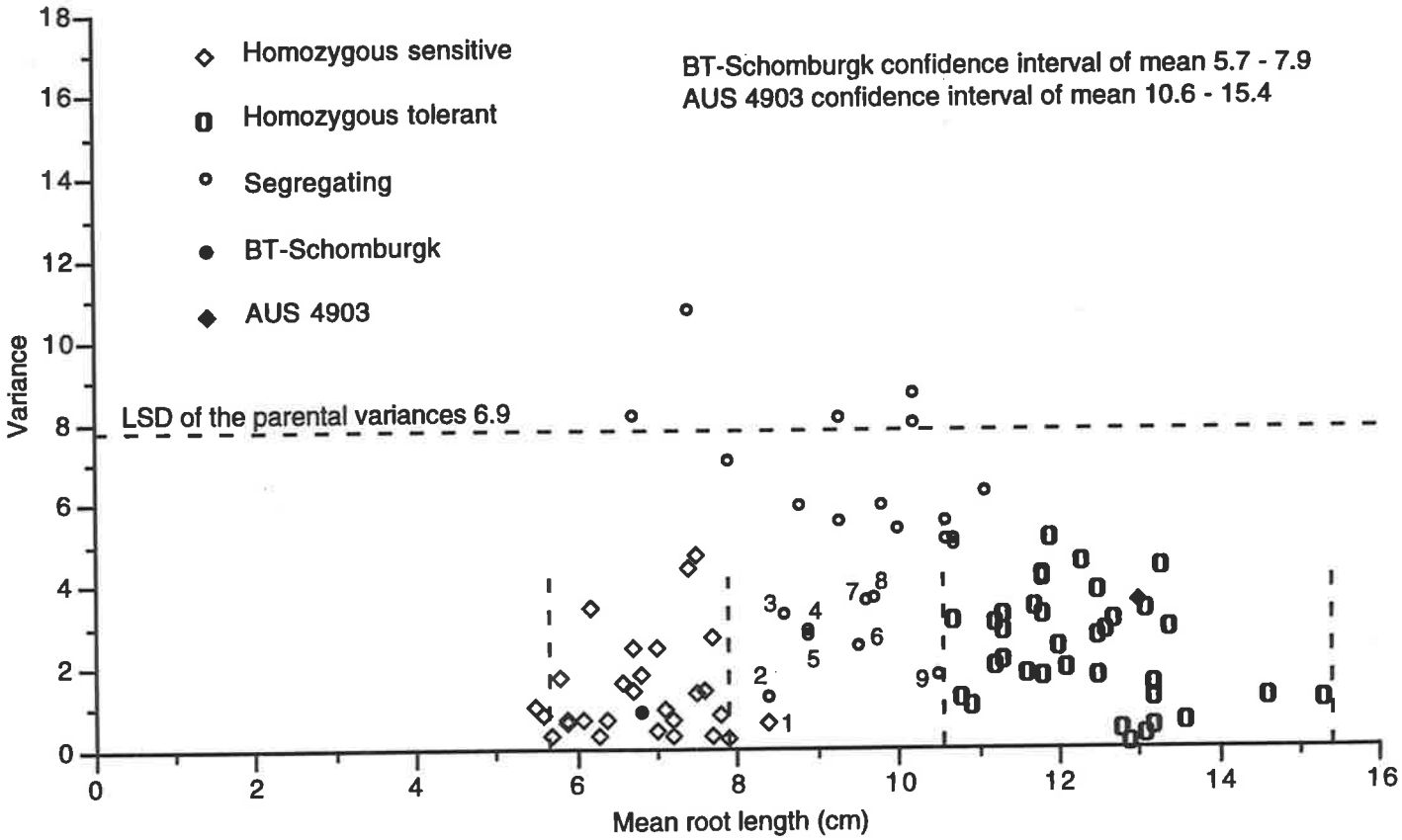
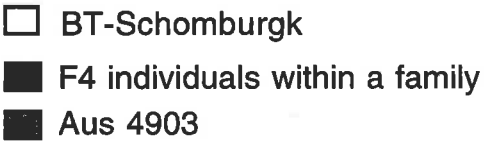
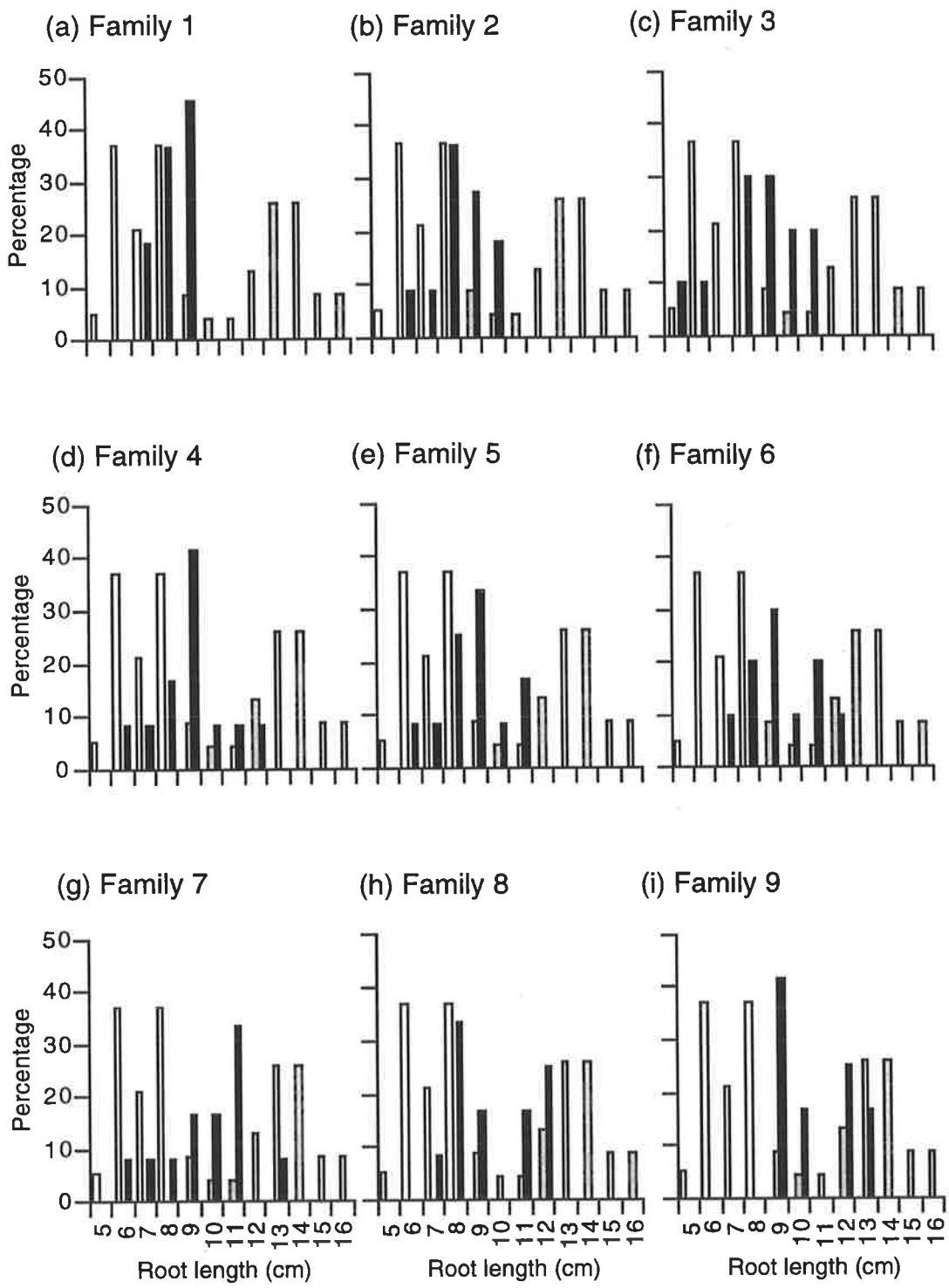

Homozygous sensitive
Homozygous tolerant
Segregating
BT-Schomburgk
AUS 4903
BT-Schomburgk confidence interval of mean 5.7 - 7.9
AUS 4903 confidence interval of mean 10.6 - 15.4
LSD of the parental variances 6.9
Variance
Mean root length (cm)
0
2
4
6
8
10
12
14
16
18

Figure 8.5 Response of twelve plants within nine F_3 derived F_4 families of (BT-Schomburgk x AUS 4903) at B100 in comparison with the two parents.




(a) Family 1
(b) Family 2
(c) Family 3
(d) Family 4
(e) Family 5
(f) Family 6
(g) Family 7
(h) Family 8
(i) Family 9
Percentage
0
10
20
30
40
50
5 6 7 8 9 10 11 12 13 14 15 16
Root length (cm)

(BT-Schomburgk x Turkey 1473)

The LSD of the parental variances (4.4) was used to differentiated between homozygous and heterozygous families (Figure 8.6). Families with variances of less than or equal to 4.4 were classified as homozygous. Families were classified into two and three categories using the comparison of the mean of each family with the confidence interval of the means of the two parents. There were many families with a mean root length below the confidence interval of the sensitive parent, BT-Schomburgk, and several above the confidence range of Turkey 1473 (Figure 8.6). This suggested transgressive segregation among the progeny of this cross.

There were thirteen families with mean root lengths intermediate to the confidence intervals of the parents, but with low variances. Six of these families were inspected for the performance of the individual plants in comparison with the sensitive and tolerant parents with ranges of the root lengths of 5-8 cm and 9-14 cm (Figure 8.7), respectively. Family 6 was classified as segregating (Figure 8.7). The root lengths of Families 1-5 also overlapped with the parents, however these families were classified as homozygous intermediate because the variation in root length was comparatively low in comparison to that of the segregating family (Figure 8.7). Chi-square analysis, classifying the apparently transgressive segregants into the homozygous categories and the low variance intermediates as segregating, indicated that the segregation of (BT-Schomburgk x Turkey 1473) was consistent with the monogenic ratio of 5 tolerant-segregating : 3 homozygous sensitive and 3 homozygous tolerant : 2 segregating : 3 homozygous sensitive (Table 8.2). However, the observation of the homozygous intermediate families (Families 1-5) again indicated the probability of two genes controlling tolerance to boron in this cross.

There were twenty three sensitive families with means below the range of the sensitive parent (5.7-7.9) and five tolerant families with means above the range of the tolerant parent (Figure 8.6). If this was the result of transgressive segregation and all the twenty three families were classified as homozygous sensitive and all the other families were classified as tolerant-intermediate, chi-square analysis indicated that the segregation of this cross was not consistent with the digenic ratio of 55 tolerant-intermediate : 9

Figure 8.6 Mean and variance of root length of 84 F_3 derived families and their parents for the cross (BT-Schomburgk x Turkey 1473), tested in filter papers at B100.

Note: The horizontal line is the LSD of the parental variances.

The vertical lines are the confidence intervals of means of the two parents.

The numbers on the diagram refer to the families which are depicted in Figures 8.7.

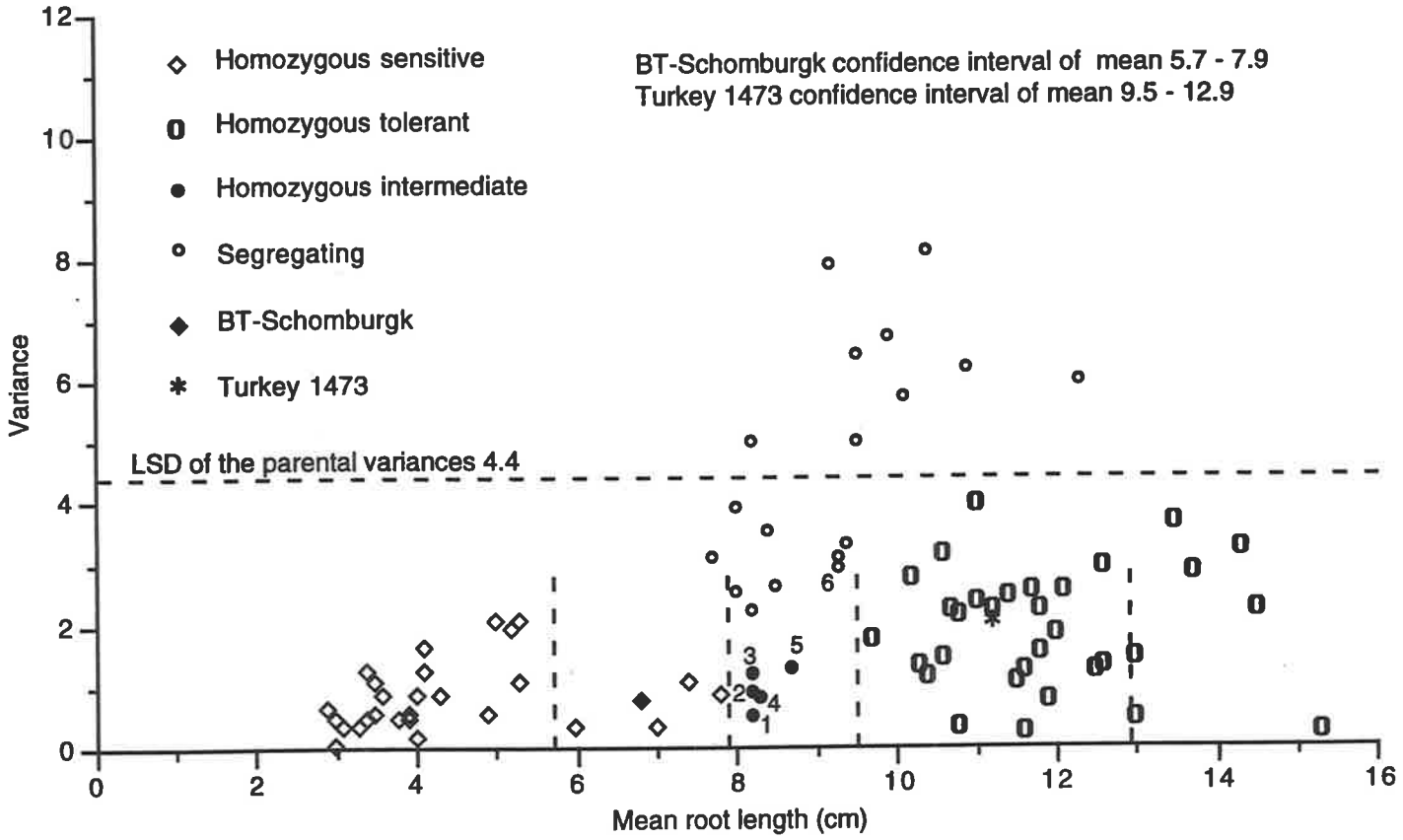
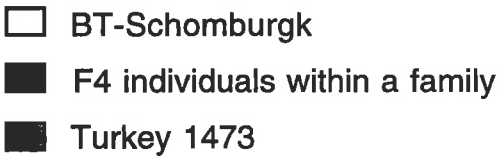
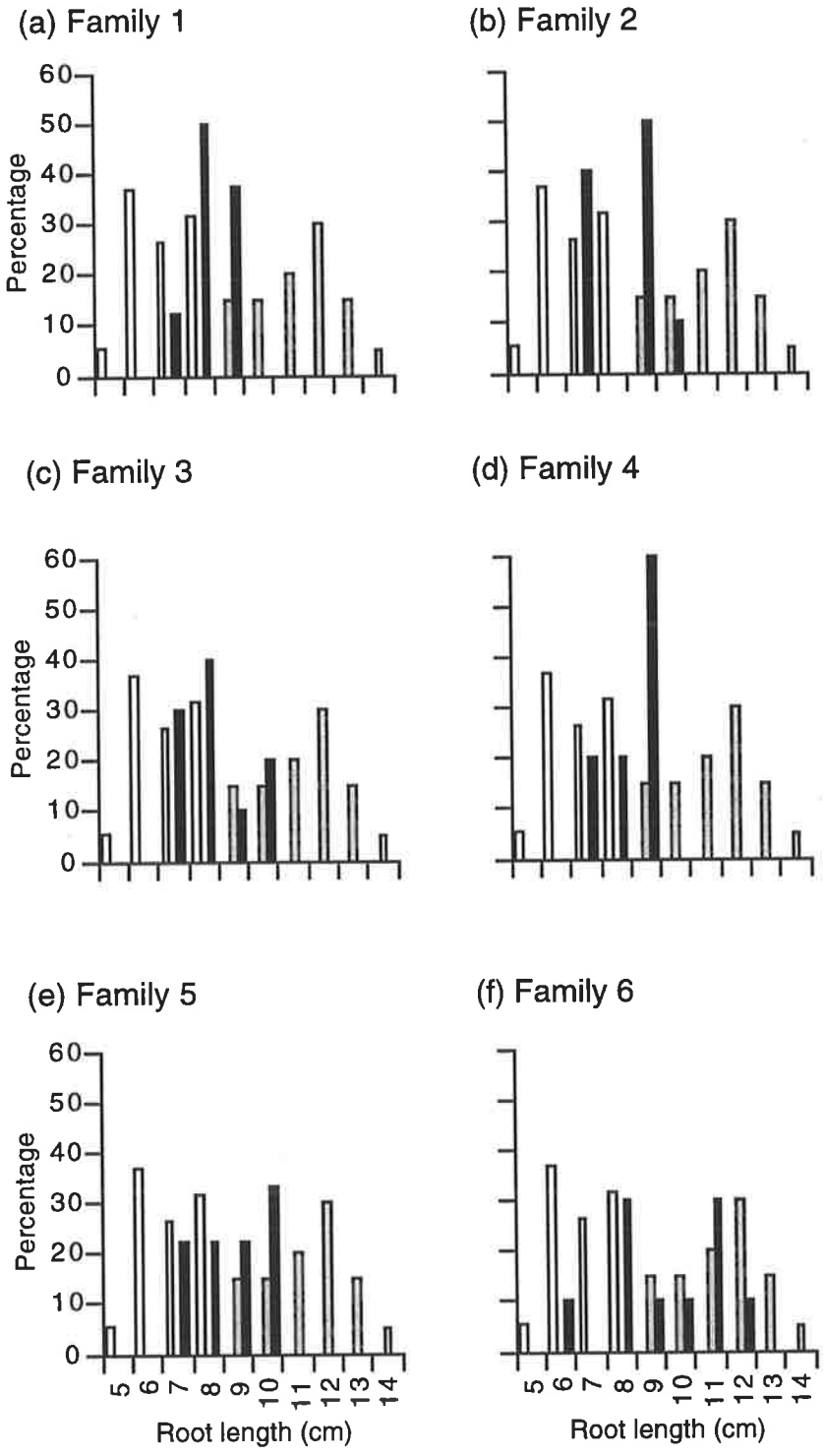

Homozygous sensitive
Homozygous tolerant
Homozygous intermediate
Segregating
BT-Schomburgk
Turkey 1473
BT-Schomburgk confidence interval of mean 5.7 - 7.9
Turkey 1473 confidence interval of mean 9.5 - 12.9
LSD of the parental variances 4.4
Variance
Mean root length (cm)
0
2
4
6
8
10
12
14
16

Figure 8.7 Response of twelve plants within six F_3 derived F_4 families of (BT-Schomburgk x Turkey 1473) at B100 in comparison with the two parents.




(a) Family 1
(b) Family 2
(c) Family 3
(d) Family 4
(e) Family 5
(f) Family 6
Percentage
0
10
20
30
40
50
60
5
6
7
8
9
10
11
12
13
14
Root length (cm)

homozygous sensitive (Table 8.3). The failure to observe digenic segregation in this cross is possibly because one of the two genes had a comparatively large effect compared to the other. If this was the case, transgressive segregation can be tested using the expected ratio of 49 tolerant-intermediate : 15 homozygous sensitive as outlined below.

Transgressive segregation would be expected from the F_3 population of the cross between variety X (AAbb) and variety Y (aaBB). For an additive genetic system, the families derived from genotypes aabb, Aabb and aaBb would, on average, be more sensitive than either of the parents. However, if 'A' is the allele of large effect, Aabb would be similar to aaBB and more tolerant than aaBb and aabb. Therefore only the genotypes aabb and aaBb would be more sensitive than the either parent. The heterozygous families of the genotype aaBb would also have relatively low variances as it would be segregating at only the minor locus. The expected frequencies of families derived from F_3 individuals of the genotypes aabb and aaBb are 9/64 and 6/64, respectively and thus, the frequency of the families being more sensitive than either parent would be expected as 15/64.

Therefore, for 84 families of the F_3 population derived from (BT-Schomburgk x Turkey 1473), approximately 19 families are expected to be more sensitive than BT-Schomburgk.

Families with means less than the confidence interval of the mean of the sensitive parent were classified as sensitive whereas families with means within or greater than the confidence interval of the two parents were classified as tolerant-intermediate. Chi-square analysis indicated that segregation was consistent with the transgressive segregation ratio expected for control by two genes (Table 8.5).

Table 8.5 Chi-square analysis of the observed and expected ratios for transgressive segregation of F_3 derived F_4 families for (BT-Schomburgk x Turkey 1473), tested in filter papers at B100.

Population (F_3 derived F_4)		Model	Frequency		
			Tol[a]+Int[b]	Sen[c]	χ^2_1
(BT-SchomburgkxTurkey1473)	Obs[d]		61	23	
	Exp[e]	49 : 15	64.31	19.69	0.73

Probability (P) of chi-square at 1 and 2 degrees of freedom.

P	0.50	0.25	0.05	0.01
χ^2_1	0.45	1.32	3.84	6.63

[a] Tolerant, [b] Intermediate, [c] Sensitive, [d] Observed value, [e] Expected value

The observed variance of (BT-Schomburgk x Turkey 1473) was significantly different from both of the expected variances for the one and two genes models and the observed variance of 11.6 was much greater than the expected variance of 5.6 for the one gene model (Table 8.4). An observed variance greater than that expected for a one gene model is consistent with transgressive segregation as a high proportion of the progeny fall at the extremes of the distribution. Two homozygous sensitive and two homozygous tolerant families of (BT-Schomburgk x Turkey 1473) are demonstrated in Plate 8.2.

(BT-Schomburgk x Klein Granador)

When the F_3 derived families of (BT-Schomburgk x Klein Granador) were divided into two and three categories using the comparison between means and variances of the families and those of the two parents (Figure 8.8), it is obvious that a considerable number of families had a mean root length less than BT-Schomburgk or greater than Klein Granador.

There were twelve sensitive families with means less than the range of the sensitive parent and five tolerant families with means greater than the range of the tolerant parent (Figure 8.8). If this was the result of transgressive segregation and the twelve families were

Plate 8.2 Response of F_3 derived families of (BT-Schomburgk x Turkey 1473) to the B100 treatment and comparison with the two parents.

Note: left to right; T1473 = Turkey 1473, 924647 and 24224 = homozygous tolerant families, 924671 and 24230 = homozygous sensitive families, BTSch = BT-Schomburgk.

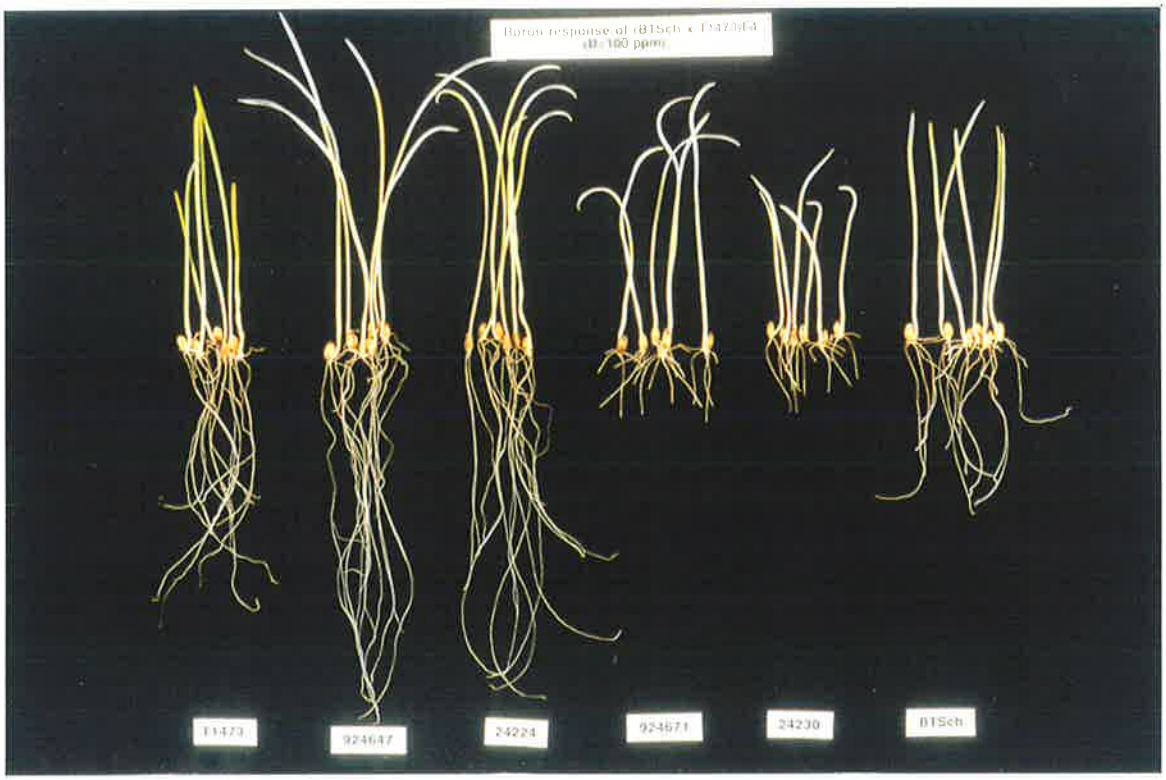
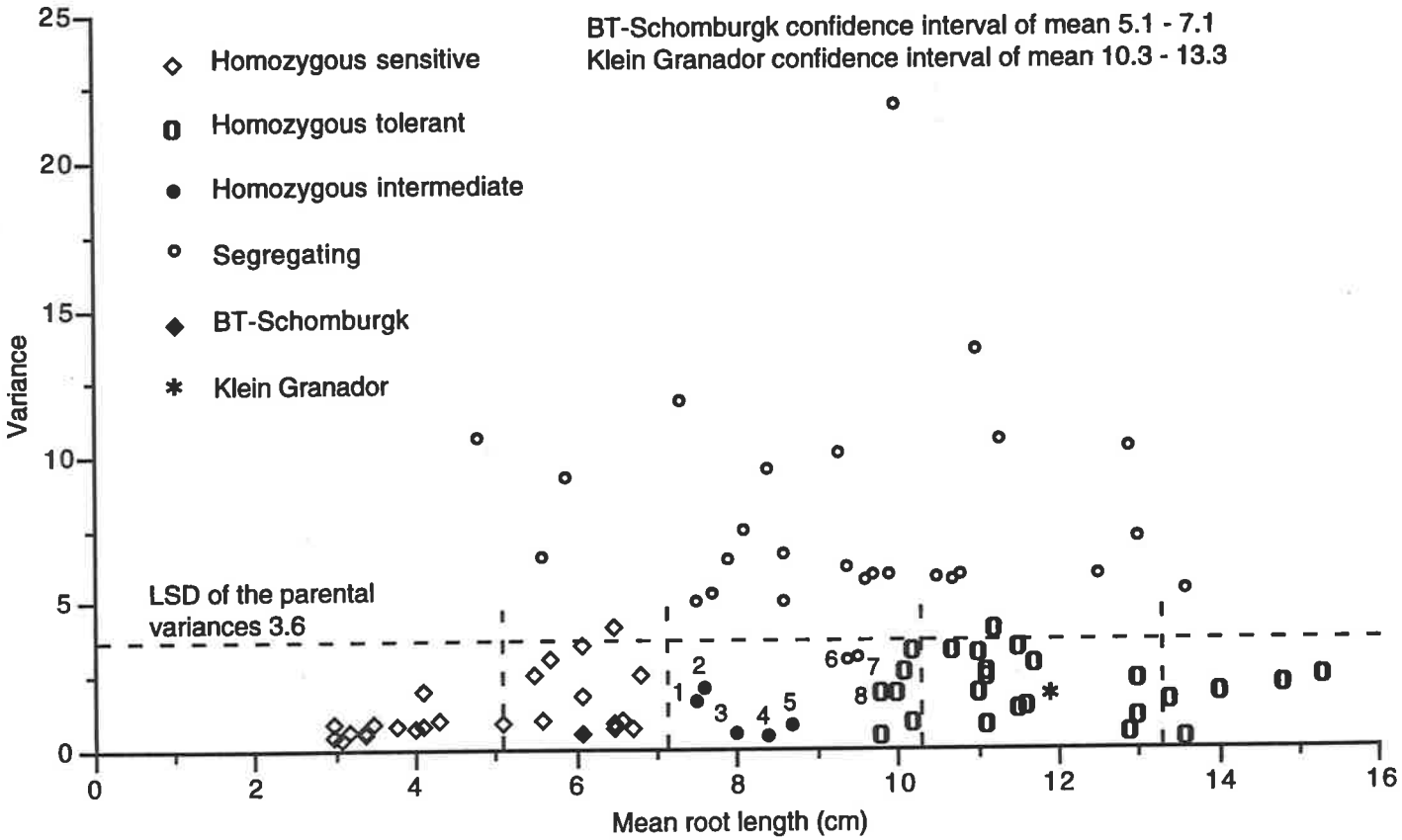

Boron response of (B1Sch x D472b54
dB=100 ppm)
D1473
924647
24224
924671
24230
B1Sch

Figure 8.8 Mean and variance of root length of 82 F_3 derived families and their parents for the cross (BT-Schomburgk x Klein Granador), tested in filter papers at B100.

Note: The horizontal line is the LSD of the parental variances.

The vertical lines are the confidence intervals of means of the two parents.

The numbers on the diagram refer to the families which are depicted in Figures 8.9.


BT-Schomburgk confidence interval of mean 5.1 - 7.1
Klein Granador confidence interval of mean 10.3 - 13.3
Homozygous sensitive
Homozygous tolerant
Homozygous intermediate
Segregating
BT-Schomburgk
Klein Granador
LSD of the parental variances 3.6
Variance
Mean root length (cm)
0
5
10
15
20
25
0
2
4
6
8
10
12
14
16

classified as homozygous sensitive and the other families were classified as tolerant-intermediate, chi-square analysis indicated that the segregation was consistent with both digenic ratios of 55 tolerant-intermediate : 9 sensitive and 9 homozygous tolerant : 46 intermediate : 9 homozygous sensitive (Table 8.3). When the segregation ratios were tested for transgressive segregation by the assumptions applied to (BT-Schomburgk x Turkey 1473) (see results of (BT-Schomburgk x Turkey 1473) for the assumptions in fitting the 49 : 15 model), chi-square analysis also indicated that the segregation was consistent with the transgressive segregation ratio expected for control by two genes (Table 8.6).

Table 8.6 Chi-square analysis of the observed and expected ratios for transgressive segregation of F_3 derived F_4 families for (BT-Schomburgk x Klein Granador), tested in filter papers at B100.

Population (F_3 derived F_4)		Model	Frequency		
			Tol[a]+Int[b]	Sen[c]	χ^2_1
(BT-SchomburgkxKleinGranador)	Obs[d]		70	12	
	Exp[e]	49 : 15	62.78	19.22	3.54

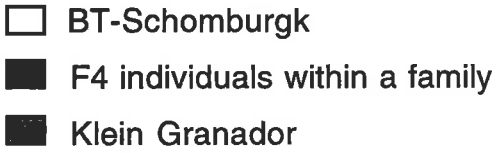
Probability (P) of chi-square at 1 and 2 degrees of freedom.

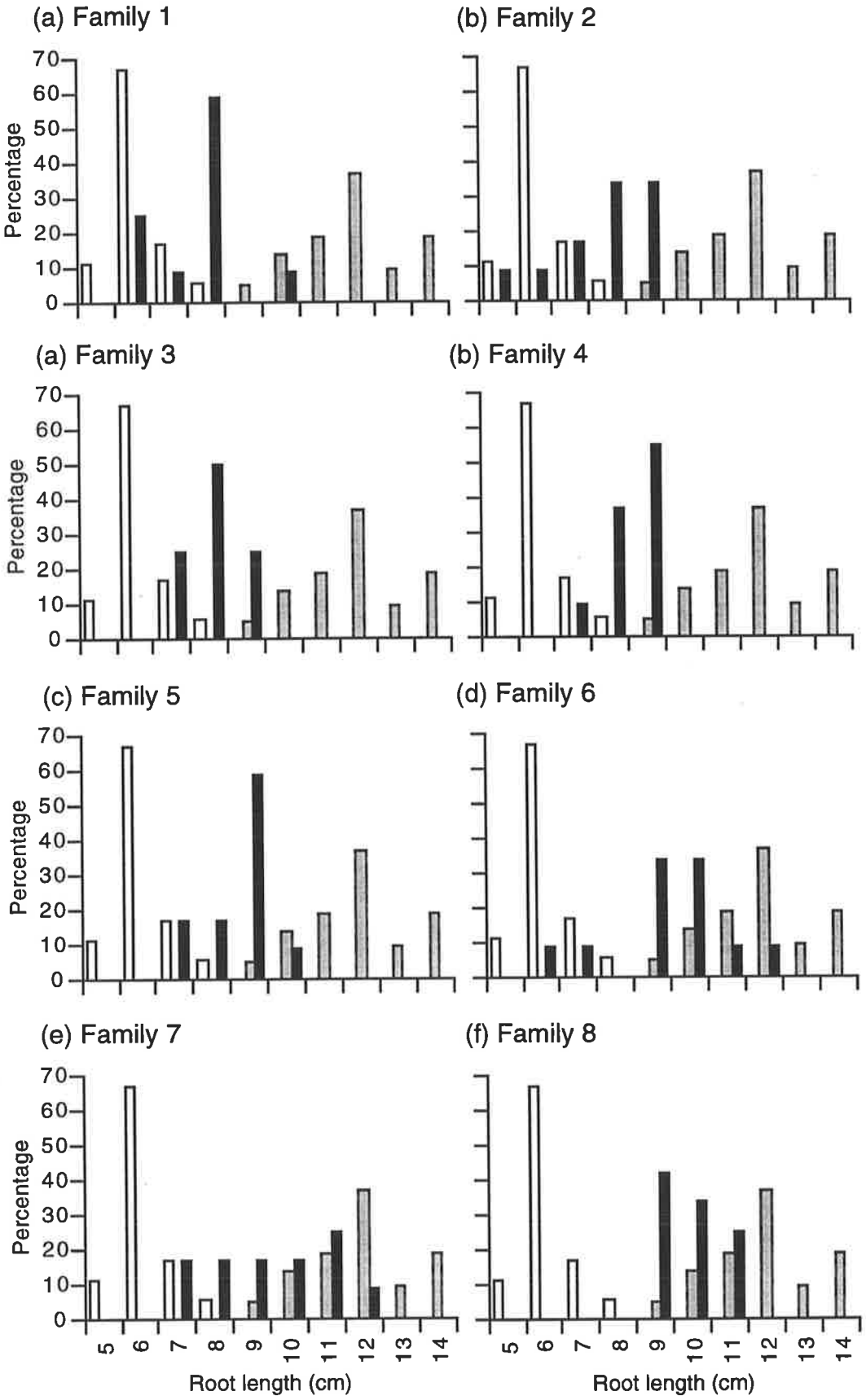
P	0.50	0.25	0.05	0.01
χ^2_1	0.45	1.32	3.84	6.63

[a] Tolerant, [b] Intermediate, [c] Sensitive, [d] Observed value, [e] Expected value.

Eight families (Figure 8.8) were inspected individually. All of the root lengths of the plants within Family 8 fell within the range of the tolerant parent, whereas for the other families, the root lengths of the plants overlapped the two parents (Figure 8.9). Therefore, Family 8 was classified as homozygous tolerant and Families 1-5 were classified as homozygous intermediate because the variation in root length of the plants of these families was comparatively low. Families 6 and 7 had a higher variation in root length than those of the homozygous families and thus were classified as segregating (Figure 8.8). The

Figure 8.9 Response of twelve plants within eight F_3 derived F_4 families of (BT-Schomburgk x Klein Granador) at B100 in comparison with the two parents.




(a) Family 1
(b) Family 2
(a) Family 3
(b) Family 4
(c) Family 5
(d) Family 6
(e) Family 7
(f) Family 8
Percentage
0
10
20
30
40
50
60
70
5
6
7
8
9
10
11
12
13
14
Root length (cm)

observation of the homozygous intermediate families (Families 1-5) also indicated that it was probable that two genes controlled tolerance to boron of this cross.

The observed variance of (BT-Schomburgk x Klein Granador) is above that expected on the monogenic model, although the monogenic but not the digenic models fall within its limits (Table 8.4).

8.4 Discussion

The result of this experiment indicate that control of boron tolerance of AUS 4903 differs from BT-Schomburgk at a single gene while transgressive segregation was observed between BT-Schomburgk and Turkey 1473 and Klein Granador. The relationship between BT-Schomburgk and India 126 is more complicated than those of the other crosses.

AUS 4903

The segregation ratio of the F_3 families of (BT-Schomburgk x AUS 4903) was consistent with both monogenic ratios of 5 tolerant-segregating : 3 homozygous sensitive and 3 homozygous tolerant : 2 segregating : 3 homozygous sensitive confirming an allelic difference at a single gene between AUS 4903 and BT-Schomburgk. The slightly low frequency of the homozygous sensitive families observed in this cross (Table 8.2) may be due to there being some misclassification of the homozygous sensitive families (Figure 8.4).

It is not possible to determine from the combinations tested whether AUS 4903 is of the same genotype or how it relates to G61450, the reference genotype for the *Bo4* locus. The genotype of the AUS 4903 is either:

Bo1Bo1Bo2Bo2Bo3Bo3Bo4Bo4

or

Bo1Bo1Bo2Bo2Bo3Bo3bo4bo4Bo5Bo5.

To test the two hypotheses, this variety should be crossed with G61450 and the progeny tested for segregation in response to boron. If AUS 4903 is of the former genotype, the progeny would segregate at a single gene, the *Bo1* locus, however, if of the latter genotype,

transgressive segregation would be observed among the progeny due to contrasting genotypes at the *Bo1*, *Bo4* and *Bo5* loci.

Turkey 1473 and Klein Granador

The distribution of the F_3 derived families of the crosses (BT-Schomburgk x Turkey 1473) and (BT-Schomburgk x Klein Granador) showed that the mean root lengths of some families exceeded the range of the parents indicating transgressive segregation among the progeny of these crosses. As two genes seem to be segregating, these varieties cannot be of the same genotype as AUS 4903. It is probable that the genotypes of Turkey 1473 and Klein Granador are different from that of G61450 (*bo1bo1Bo2Bo2Bo3Bo3Bo4Bo4*) because the level of tolerance to boron of these varieties, on the basis of root length in response to high boron was longer than that of G61450 (Table 4.6 of Chapter 4). At B150, the mean root length of Klein Granador (8.9 cm) and Turkey 1473 (7.4 cm) were significantly longer than that of G61450 (5.3 cm) and therefore it is unlikely that either of them has the same genotype as G61450 (*bo1bo1Bo2Bo2Bo3Bo3Bo4Bo4*). While these two varieties have a non-tolerant allele at one of the three loci with tolerant alleles in BT-Schomburgk, there is no evidence to discriminate between whether this occurs at the *Bo1, Bo2* or *Bo3* locus. Thus, there are five possible genotypes for the two varieties (Table 8.7) and transgressive segregation should be observed in the progenies from the cross between both of these varieties and G61450. Test crossing both of the two varieties with the homozygous tolerant line 418T-1 (*Bo1Bo1Bo2Bo2Bo3Bo3Bo4Bo4bo5bo5*) (Chapter 5) could result in monogenic segregation if the genotypes of the two varieties were *Bo1Bo1bo2bo2Bo3Bo3Bo4Bo4* or *Bo1Bo1Bo2Bo2bo3bo3Bo4Bo4* (Table 8.7) whereas transgressive segregation should be observed if Turkey 1473 or Klein Granador are one of the other three genotypes.

Table 8.7 The expected genotypes of Turkey 1473 and Klein Granador.

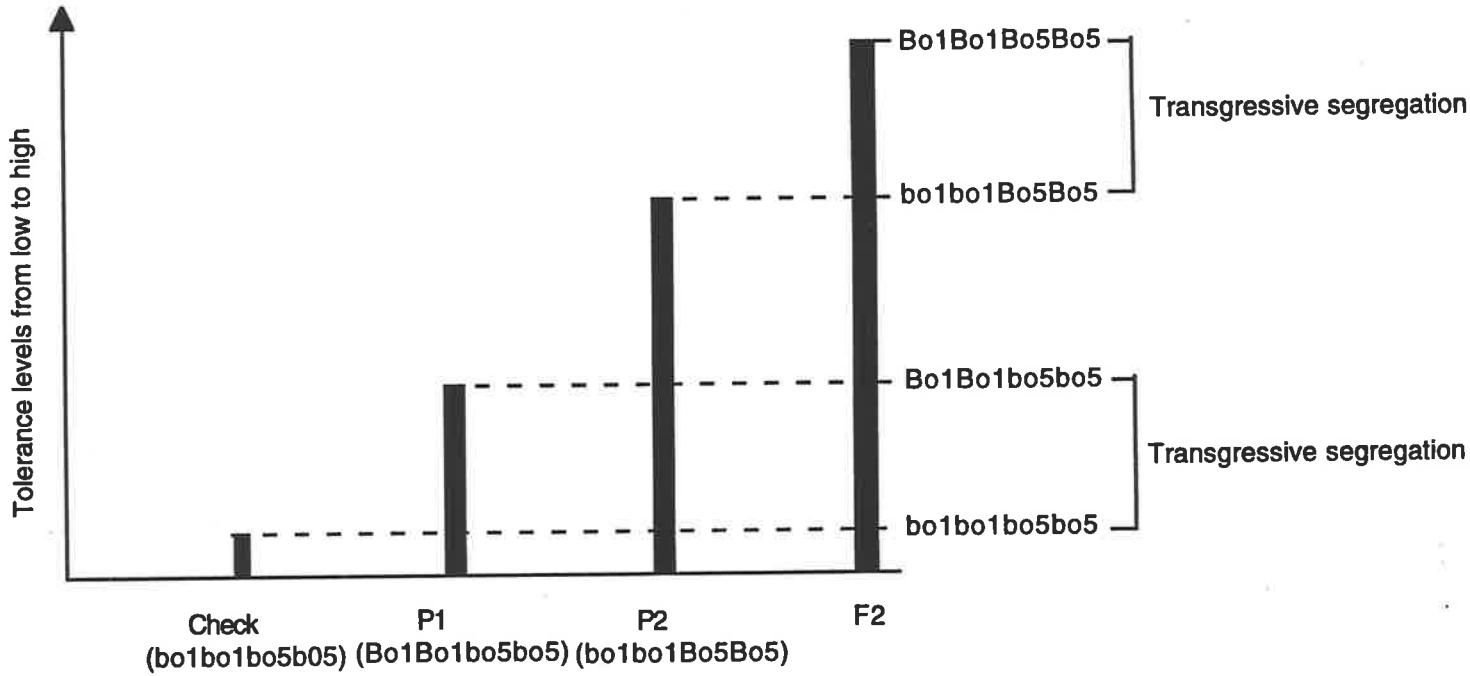
Expected genotypes	Conditions
Bo1Bo1bo2bo2Bo3Bo3Bo4Bo4bo5bo5	if *Bo2* has a small effect.
Bo1Bo1Bo2Bo2bo3bo3Bo4Bo4bo5bo5	if *Bo3* has a small effect
Bo1Bo1bo2bo2Bo3Bo3bo4bo4Bo5Bo5	if *Bo2* has a small effect.
Bo1Bo1Bo2Bo2bo3bo3bo4bo4Bo5Bo5	if *Bo3* has a small effect.
bo1bo1Bo2Bo2Bo3Bo3bo4bo4Bo5Bo5	if *Bo5* has a much greater effect than *Bo4*.

An example of transgressive segregation of a F_2 population derived from the cross between a moderately tolerant variety BT-Schomburgk or Halberd (*Bo1Bo1Bo2Bo2Bo3Bo3bo4bo4bo5bo5*) and a tolerant variety such as Turkey 1473 or Klein Granador with an expected genotype of *bo1bo1Bo2Bo2Bo3Bo3bo4bo4Bo5Bo5* is demonstrated in Figure 8.10. The F_2 plants with the genotype of (*Bo1Bo1Bo2Bo2Bo3Bo3bo4bo4Bo5Bo5*) would be more tolerant than the tolerant parent and the F_2 plants with the genotype of (*bo1bo1Bo2Bo2Bo3Bo3bo4bo4bo5bo5*) would be more sensitive than the sensitive parent (Figure 8.10) but the same as Condor (*bo1bo1Bo2Bo2Bo3Bo3bo4bo4bo5bo5*).

Chi-square analysis indicated that the segregation ratio of (BT-Schomburgk x Klein Granador) was consistent with both digenic ratios of 55 : 9 and 9 : 46 : 9 (Table 8.3). The segregation of the F_3 derived families of (BT-Schomburgk x Turkey 1473) and (BT-Schomburgk x Klein Granador) were consistent with the transgressive segregation ratio of 49 : 15 (Tables 8.5 and 8.6). The observed variances of 11.6 and 10.3 for (BT-Schomburgk x Turkey 1473) and (BT-Schomburgk x Klein Granador), respectively, were above the expected variances for a single gene model (Table 8.4), indicating transgressive segregation for the two crosses. The low frequency of families more tolerant than the upper confidence limit for the tolerant parent in the two crosses was not unexpected, due to the

Figure 8.10 Diagram of transgressive segregation in a F_2 population derived from crossing a moderately tolerant variety BT-Schomburgk or Halberd ($Bo_1Bo_1bo_5bo_5$) and a tolerant variety such as Turkey 1473 or Klein Granador ($bo_1bo_1Bo_5Bo_5$). The scheme is simplified to show only the two genes segregating between the two genotypes.

Note: P_1 = BT-Schomburgk or Halberd, P_2 = Turkey 1473 or Klein Granador, Check = Condor.


Tolerance levels from low to high
Bo1Bo1Bo5Bo5
Transgressive segregation
bo1bo1Bo5Bo5
Bo1Bo1bo5bo5
Transgressive segregation
bo1bo1bo5bo5
Check
(bo1bo1bo5b05)
P1
(Bo1Bo1bo5bo5)
P2
(bo1bo1Bo5Bo5)
F2

concentration of boron used in this experiment (100 mg l^{-1}) being insufficient to separate the homozygous very tolerant from the tolerant parental types.

India 126

The results of Chapter 7 indicated that chromosome 4A was responsible for tolerance to boron in India 126, while the *Bo1* gene from Halberd, responsible for boron tolerance in BT-Schomburgk, was located on chromosome 7B (Chapters 6 and 7). Those results suggest that there are at least two different genes controlling tolerance to boron between India 126 and BT-Schomburgk and that transgressive segregation would be expected from the cross between these two varieties. However, the results of this experiment superficially support only a single gene difference.

The reason for the apparent monogenic segregation but not digenic or transgressive segregation for the cross (BT-Schomburgk x India 126) may be because the BT-Schomburgk parent had a much larger variance (3.2) (Table 8.4), lower mean (5.3 cm) (Figure 8.1) and larger confidence interval (3.1-7.5 cm) (Figure 8.2) than it did (~1.0, ~6.5 cm and 5.5-7.5 cm, respectively) when tested with the three other crosses. This was due to a number of plants of BT-Schomburgk with short roots when screening its cross with India 126. This was possibly due to factors other than boron toxicity (Figure 8.1a) such as damaged seeds giving poor vigor seedlings. If the BT-Schomburgk had responded as it had with the other crosses (Figure 8.1b, 8.1c and 8.1d), the most sensitive families in the (BT-Schomburgk x India 126) cross (Figure 8.2) would have been classified as transgressive segregants. The variance of (BT-Schomburgk x India 126) (10.4) is similar to that of (BT-Schomburgk x Turkey 1473) and (BT-Schomburgk x Klein Granador) for which transgressive segregation was observed. The other possible reason is that the concentration of boron used in this experiment (100 mg l^{-1}) was not enough to separate the segregating or intermediate families from sensitive families and a higher concentration of boron (for example, 150 mg l^{-1}) may be required for differentiation between those genotypes.

Since chromosome 4A was responsible for tolerance to boron in G61450 (at the *Bo4* locus) (Chapters 6 and 7) and India 126 (Chapter 7), the boron tolerance genes on

chromosome 4A of these two varieties are possibly at the same locus (*Bo4*) but are different alleles. Therefore the possible genotype of India 126 is $bo1bo1Bo2Bo2Bo3Bo3Bo4_bBo4_b$ in comparison to $bo1bo1Bo2Bo2Bo3Bo3Bo4_aBo4_a$ of G61450. However, the result of Chapter 7 indicated that the effect of the 4A locus from India 126 was less than that for the 4A locus for G61450. In addition, India 126 is more tolerant than G61450. This more or less rules out this hypothesis.

Alternatively, the transgressive segregation is a result of different alleles at several loci. In Chapter 7, India 126 had several chromosomes with a small effect in response to boron. The lack of significance of these could be due to

(a) their comparatively small effect

and

(b) the low level of replication.

At this moment, the second hypothesis is the more likely one.

Variance

In contrast to chi-square analysis, the observed variance of (BT-Schomburgk x AUS 4903) was consistent with the expected variance for a two gene model (Table 8.4). This was possibly because the expected variance of (BT-Schomburgk x AUS 4903) was higher than those of (BT-Schomburgk x Turkey 1473) and (BT-Schomburgk x Klein Granador) due to high values of *d* and *E*, as described in Section 5.4 of Chapter 5.

The observed variance of (BT-Schomburgk x India 126) was similar to those of (BT-Schomburgk x Turkey 1473) and (BT-Schomburgk x Klein Granador) (Table 8.4) for which transgressive segregation was observed, indicating the possibility of two genes controlling for tolerance to boron of (BT-Schomburgk x India 126). However, the observed variance of (BT-Schomburgk x India 126) was not higher than the expected variance for a single gene model (Table 8.4). This is possibly because the expected variances of the one gene model for this cross were very high due to the high values of *d* and *E*, both of which would be increased if some of the seedlings of BT-Schomburgk were low in vigor.

The use of the LSD of the parental variances to differentiate between homozygous and segregating families is not appropriate where the variances of the two parents are high through experimental variation because this increases the LSD of the parental variances. For example, in (BT-Schomburgk x India 126), the variances of BT-Schomburgk and India 126 were 3.2 and 4.0, respectively, and the LSD of the parental variances was 11.1 (Figure 8.2), much higher than the 3.6 of (BT-Schomburgk x Klein Granador) where the variances of BT-Schomburgk and Klein Granador were 0.6 and 1.7 (Figure 8.8), respectively.

It should also be recognized that the use of the confidence interval of the root length of the two parents for classification of the families into homozygous and segregating categories was subject to errors due to the over (error Type II) and under (error Type I) estimation (Chapter 3).

Breeding

Since India 126, Turkey 1473 and Klein Granador showed more tolerance to boron than G61450 (Table 4.6 of Chapter 4), tolerant transgressive segregants from crosses between these varieties and BT-Schomburgk should produce lines more tolerant than the lines produced from (Halberd x G61450). These highly tolerant lines could be used as donor parents in a backcrossing program for the development of boron tolerant varieties.

Chapter 9

General discussion

The comparison of wheat genotypes with diverse responses to boron using the filter paper method (Chapter 4) indicate that the length of roots of the tested varieties were consistent in response to boron between the three boron treatments. As the correlations between the root length at B0 and the three boron treatments were non-significant, the differences in root length at the high boron treatments could not be attributed to inherent differences in root growth among the varieties (Chapter 4). Seedling root lengths at the three levels of boron in the filter paper technique were highly significantly correlated with the three characters determined for plants grown in soil containing high levels of boron, namely, the concentrations of boron in the shoots, plant dry weight and plant symptoms (Chapter 4). This indicates that root length could be used as a selection criterion in a genetic study or breeding program for boron tolerance. Shoot lengths and number of roots of different varieties under high boron conditions were not consistent with the levels of tolerance of the varieties when grown in high boron soil, suggesting that the two latter parameters are not appropriate for screening of boron tolerance.

Root lengths were observed to be affected by some unexplained factors. The variation of root lengths of some varieties were sometimes observed to be higher than expected (for example, the high variance of the BT-Schomburgk parent of the cross (BT-Schomburgk x India 126) (Table 8.4)) and this could have been a result of instability in the temperature control of 15°C in the room used for storing the treated seeds or due to unevenly germinated or poor growth from damaged seeds or contamination of the filter papers.

The number of genes controlling tolerance to boron between G61450 and Halberd was estimated (Chapter 5). The F_2 and F_2 derived populations of the crosses between a homozygous sensitive (442S-1) (a homozygous sensitive line derived from the combination between G61450 and Halberd) and a tolerant (418T-1) line from the same source, and

between both these lines and the varieties G61450, Halberd and Schomburgk and between Schomburgk and Condor were tested for segregation in response to boron. The results indicated that there were two alternative genes *Bo1* and *Bo4* controlling tolerance to boron of Halberd and G61450, respectively (Chapter 4). This was consistent with the transgressive segregation observed from the F_2 progenies of this combination (Paull et al., 1991b). The genes *Bo2* and *Bo3* are responsible for the boron tolerance of the moderately sensitive genotypes (*bobo1Bo2Bo2Bo3Bo3bo4bo4*) Schomburgk, Condor and 442S-1, whereas, these plus the genes *Bo1* and *Bo4* are responsible for boron tolerance of the homozygous tolerant line 418T-1 which showed greater tolerance to boron than G61450. Therefore varieties more tolerant than G61450 can be bred by transferring the two genes *Bo1* and *Bo4* to well adapted varieties using the backcrossing method. Backcrossing for transferring the *Bo4* gene from G61450 to a moderately tolerant variety BT-Schomburgk is now being undertaken as part of the wheat breeding program at the Waite Agricultural Research Institute.

The chromosomal location of genes for tolerance to boron was undertaken by F_2 monosomic analysis (Chapter 6) and backcross reciprocal monosomic analysis (Chapter 7). The results for the monosomic analysis using aneuploid stocks of a Condor selection demonstrated that chromosomes4A and 7B are responsible for tolerance to boron in G61450 and Halberd, respectively. This was consistent with the result of Paull (1990) which indicated that chromosome 7B is the location of a gene controlling boron tolerance in Federation, an ancestor of Halberd, relative to Chinese Spring. This difference in the critical chromosome between G61450 and Halberd (Chapter 6) was consistent with the result of Chapter 5 in showing that there were two alternative genes controlling response to high boron conditions between G61450 and Halberd.

Backcross reciprocal monosomic analysis indicated that there was chromosomal variation between the different varieties (Chapter 7). The significant increase in root length resulting from the presence of chromosome 7B and three homologues of chromosome 4A relative to Condor indicated that chromosome 7B of Halberd and 4A of Benventuto Inca, G61450 and India 126 were responsible for tolerance to boron. The significant difference

between the mean root length of the critical family for chromosome 4A from India 126 and that of the family for chromosome 4A from Condor was less than expected, indicating that there was probably another chromosome also responsible for tolerance to boron in India 126 (Chapter 7). The results for the chromosomes 4A and 7B of G61450 and Halberd (Chapter 7) were consistent with the monosomic analysis experiment (Chapter 6).

The finding that chromosomes 4A of Benventuto Inca, G61450 and India 126 and 7B of Halberd were the locations of genes controlling boron tolerance will facilitate further studies. The establishment of linkage maps between the genes controlling boron tolerance and molecular markers, which could be used for indirect selection for tolerance to boron in a breeding program, may not be necessary because the selection can be conducted rapidly and accurately using the filter paper technique. Actually isolating the genes for boron tolerance might be valuable as there will be a continuing need to select for adaptation to varying levels of soil boron. This may facilitate more direct methods of genetic manipulation (e.g. transformation if the techniques can be refined) of these genes. An interesting topic for further study would be to find out the location of the extra genes in India 126 and this could be attempted by F_2 monosomic analysis using the Condor selection aneuploid stocks.

The relationship between an Australian wheat variety BT-Schomburgk, with the *Bo1* gene from Halberd, and four tolerant exotic varieties was studied. The F_3 derived families from the crosses between BT-Schomburgk and the four varieties (Chapter 8) were tested for tolerance to boron using the filter paper technique at B100. The result indicated that there was a single gene difference between BT-Schomburgk and AUS 4903. Transgressive segregation was observed from the F_3 derived populations of (BT-Schomburgk x Turkey 1473) and (BT-Schomburgk x Klein Granador), indicating that at least two genes controlled tolerance to boron between BT-Schomburgk and Turkey 1473 and Klein Granador. The observed variance with India 126 was similar to those of (BT-Schomburgk x Turkey 1473) and (BT-Schomburgk x Klein Granador) in which the transgressive segregation was observed, again indicating the possibility of two genes being responsible for tolerance to boron between BT-Schomburgk and India 126.

The information in this thesis on the genetic control of boron tolerance in exotic lines, relative to the most tolerant of the Australian varieties, enables a strategy to be devised to introduce a greater level of tolerance into Australian varieties. Backcrossing will be required because the sources of tolerance are all poorly adapted to Australia. The ease of handling a single gene as compared to two genes in backcrossing programs needs to be considered.

To breed very tolerant lines by combining genes from BT-Schomburgk and India 126 needs at least two generations of selfing after backcrossing to produce the segregants from which the very tolerant genotypes can be selected. A larger number of BC_1F_2 plants would need to be screened to produce the homozygous tolerant genotypes because approximately only 1/64 of the BC_1F_2 plants will be homozygous tolerant when two genes are segregating, as opposed to 1/8 where only a single gene is segregating. The tolerant lines could then be recrossed in the backcrossing program. On the other hand, a line with only one gene controlling tolerance to boron can be used directly in a backcrossing program. Thus, at least two generations fewer are needed when a line with a single gene is used as a source of tolerance to boron.

Agronomic information is required to indicate whether the very high levels of tolerance are required in southern Australia or if the level of tolerance of a variety such as AUS 4903 would be adequate. At B100, AUS 4903 had the mean root length of 11.3 cm in comparison to 6.4 cm of BT-Schomburgk (Table 4.6). Yield reduction in the areas of high concentrations of boron would indicate the levels of boron toxicity in those areas and whether or not the tolerant varieties should be bred. Double haploid lines which have similar genetic background but differ in boron tolerance from the crosses between BT-Schomburgk and the other tolerant varieties such as G61450, India 126, Turkey 1473 and Klein Granador, would be suitable experimental materials for field trials conducted across the regions of high boron soil. These would indicate the yield advantage of the various levels of tolerance. The double haploid lines could also be used for physiological studies on mechanisms of tolerance to boron.

Chi-square analysis and the comparison between the observed variances of the individual populations and the variances expected for alternative genetic models were used to estimate the number of genes controlling tolerance to boron in segregating populations (Chapter 5 and Chapter 8). From the results it is obvious that the two methods should be used together for the estimation of the number of genes. When the estimated number of genes was the same between the two methods, it increased the confidence that the estimate was correct. However, when the two methods had conflicting answers, the data of the individual populations (such as individual plants within a family) needed to be investigated through the comparison of root lengths of individual plants within each families and those of the parents (Chapters 5 and 6).

In conclusion, the results of this thesis indicate that there are several major genes which act additively to control tolerance to boron between wheat varieties. Tolerant varieties can be bred using a backcrossing method or by selection of transgressive segregants from crosses between two parents with at least two different genes controlling boron tolerance. These results can be applied to the other agricultural crops such as barley, peas and medics where yields are also effected by the high level of boron in soil. Breeding for boron tolerance will improve the adaptation of many crops to southern Australia.

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