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

by

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TABLE OF CONTENTS

ABSTRACT

This thesis describes studies into the genetic control of tolerance of wheat to high concentrations of boron (B). Initially, experiments were conducted to determine selection criteria for distinguishing between tolerant and sensitive genotypes for both glasshouse and field grown wheat. Responses of plants to high concentrations of B, under glasshouse conditions, included reduced vigour, delayed development, expression of symptoms of toxicity and reduced grain and total dry matter yields. Significant differences between tolerant and sensitive genotypes resulted for all parameters, however the greatest discrimination for tolerance to B, between genotypes, resulted during vegetative growth. Tolerant genotypes accumulated less B than the more sensitive genotypes for both glasshouse and field experiments. The concentration of boron in shoots was a highly heritable character and B concentrations in shoots were significantly correlated between high boron conditions in a glasshouse and the field. The concentration of B in grain was highly correlated with the concentration in shoots for field grown wheat, but this relationship did not occur for wheat grown in pots and the anomalous result was related to the artifical growth conditions. Grain is an appropriate tissue for analysis to determine the B accumulation, and therefore tolerance, for field grown wheat.

The tolerance to B for wheat varieties of historical importance in Australia was investigated. Many of the historically dominant varieties are tolerant to B and all tolerant Australian varieties are interrelated. The initial tolerant varieties were Federation and Currawa and members of the derived family include Ghurka, Quadrat, Insignia, Heron, Olympic, Halberd, Spear and Dagger. The distribution of Insignia, Heron and Halberd followed a similar pattern in South Australia and the regions where these varieties were most widely cultivated corresponds to the regions where the highest concentrations of B have been measured in barley grain samples. Thus, there is correlative evidence that the high concentration of B occurring in the subsoils has been a major selective force in South Australian wheat production.

Tolerance to high concentrations of boron is inherited as an additive character, however expression of tolerance varies from being a dominant to a partially dominant

character depending upon the concentration of applied boron. Major gene control of tolerance to boron was identified from the segregation patterns of F_2 and F_3 generations derived from parents of contrasting tolerance to boron. The parents used represented five levels of tolerance to boron and the difference between successive levels of tolerance was under the control of single genes. Three independent single gene differences were identified. Transgressive segregation resulted between two tolerant lines, Halberd and G61450, and this suggests they have contrasting genes controlling the uptake of boron. A genetic model comprising four independent loci, designated *Bor1*, *Bor2*, *Bor3* and *Bor4* was proposed for the five lines. The five lines and their proposed genotypes were : Kenya Farmer (very sensitive) *bor1 bor2 bor3 bor4*, (W1*MMC) (sensitive) *bor1 bor2 Bor3 bor4*, Warigal (moderately sensitive) *bor1 Bor2 Bor3 bor4*, Halberd (moderately tolerant) *Bor1 Bor2 Bor3 bor4* and G61450 (very tolerant) *bor1 Bor2 Bor3 Bor4*. As tolerance to high concentrations of B is under the control of major genes, incorporation of tolerance into sensitive but otherwise adapted local varieties should be readily achieved through backcrossing.

The chromosomal location of genes controlling tolerance to B was undertaken by the use of intervarietal substitution lines, monosomic analysis and interspecific addition lines. Chromosome 4A of the Chinese Spring - Kenya Farmer substitution lines had a significant effect upon tolerance to boron and the 4A substitution line was more sensitive than Chinese Spring. Results for monosomic analysis were inconclusive, however chromosomes identified as the more probable locations of genes controlling tolerance to boron included 4A and 7D for analysis of the F_3's of (Chinese Spring monosomics * G61450) and chromosomes 7B, 3A and 2B for reciprocal monosomic analysis between Chinese Spring and Federation. The Chinese Spring x *Ag. elongatum* amphiploid was more tolerant than Chinese Spring and the chromosome 7E addition line was also more tolerant than Chinese Spring. The results of three separate comparisons therefore implicate the chromosomes of homoeologous group seven in the control of tolerance to boron.

Random F_4 and F_5 lines derived from the tolerant Halberd and sensitive (W1*MMC) were tested under naturally occurring high B conditions in the field.

Chemical analysis of shoots and grain by inductively coupled plasma spectrometry found uptake of B to be independent of nine other elements. The correlation between tolerance to B, as measured by B uptake, and yield among lines of this population was tested at six sites to identify conditions where tolerance to B resulted in a yield advantage. A significant correlation between tolerance to B and yield resulted only at sites where high boron concentrations of grain resulted. Genetic variation for concentrations of several other elements in shoots and grain also occurred within this population and significant correlations between the efficiency of nutrient uptake and grain yield resulted for Mn at Two Wells and Minnipa while genotypes with low Na accumulation produced significantly higher yields than genotypes with high Na uptake at Rudall. Genetic variation in response to soil elements, other than B, may explain the variable performance of varieties, between environments, and this is an area which warrants further investigation.

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(J.G.Paull)

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Chapter 1.

GENERAL INTRODUCTION

The efficiency and progress of breeding programs are enhanced by the recognition of constraints to yield, or quality, and the identification of genetic variation in response to the limiting factors. Constraints to yield include such factors as pathogens, nutritional imbalances and climatic conditions. Cartwright *et al.* (1984) reported a foliar symptom consisting of black spots and chlorotic lesions developing from the leaf tips for a barley crop at Gladstone in the mid-north of South Australia. The symptoms were not associated with the presence of a foliar pathogen. The symptoms developed in patches within the paddock and when soil data and concentrations of elements in shoots were compared, the greatest difference between healthy and affected plants was for boron (B) concentration. The same symptom of barley was described by Christensen (1934) and was also attributed to B toxicity. The average concentration of B in shoots of slightly affected plants was 13.8 mg kg^{-1} and for severly affected plants was 62.4 mg kg^{-1} and the reduction in grain yield for affected quadrats, compared with unaffected or slightly affected plants was 17%. This was the first report of naturally occurring B toxicity in non-irrigated areas of south eastern Australia and the first for cereal crops in Australia, although B toxicity of citrus and vines had been previously reported in the Sunraysia district of Victoria (Penman and McAlpin, 1949; Sauer, 1958, respectively).

The characteristic symptoms of B toxicity for barley were observed at 25 sites and high concentrations of B were measured in shoots of barley at 16 sites covering the full extent of the cereal belt of South Australia and one site in western Victoria (Cartwright *et al.*, 1986). A survey of the B concentration in grain of barley in South Australia, based on the delivery of grain to silos in 1983, revealed many regions where the B concentration was of the same order as those associated with toxicity at Gladstone (3 mg kg^{-1}) (Cartwright and Hirsch , 1986). These results indicated that B toxicity may be a major problem for cereal production in South Australia.

The concentration of B in the soil profile at Gladstone increased with depth (Cartwright *et al.*, 1984) and a similar distribution of B was measured for subsequent soil

profiles from many sites (e.g. Cartwright *et al.*, 1986, 1987). The soils with high concentrations of B were generally calcareous and more sodic than nearby soils where plant growth was apparently normal. As the occurrence of B at depth in the profile precludes soil amendment as a feasible means of overcoming the problem of toxicity, genetic variation in tolerance to high concentrations of B was sought. Many Australian wheat varieties and advanced breeding lines from the wheat breeding programs of the Waite Agricultural Research Institute and Roseworthy Agricultural College were evaluated under high B conditions at Two Wells in 1984. A greater than two-fold range for concentration of B in shoots and grain was measured among the lines and the concentration of B in the grain was lowest for the highest yielding lines (Cartwright *et al.*, 1987). Thus, there was evidence of genetic variation in tolerance to high concentratons of B.

The genetic variation in tolerance to B was investigated under controlled conditions and the concentration of B in the grain for field grown wheat was confirmed to be related to tolerance to B *per se* (Paull, 1985). The concentration of B in shoots of tolerant lines when grown in B amended soil in pots was also lower than for the more sensitive lines (Paull, 1985; Paull *et al.*, 1988). The variety Halberd was among the most tolerant lines, and the concentration of B in the grain of a number of the ancestors of Halberd were also low when grown at Two Wells in 1984 (unpublished). Collectively this family, which can be traced to Federation and Currawa, has dominated wheat production in south eastern Australia, in particular, and Australia in general, for most of the twentieth century. There was therefore a correlation between concentration of B in the grain and the extent and persistence of varieties in cultivation in Australia. This further suggests that high concentrations of B may be a significant factor influencing cereal production in regions of Australia.

A number of collaborative projects are being undertaken between the wheat and barley breeding programs of the Waite Agricultural Research Institute and the CSIRO Division of Soils to investigate the soil factors associated with, and the extent of, high concentrations of B and the physiological basis and genetic control of tolerance to B. The

ultimate objectives of these projects are to breed B tolerant varieties and to be able to provide recommendations where tolerant varieties should be grown.

The aim of the work presented in this thesis was to provide information on the genetic control of tolerance to high concentrations of B and so assist in the breeding of tolerant varieties. At the commencement of the project there was no information on the genetic control of tolerance to B and the only known report of variation between genotypes of wheat for tolerance to B (Mehrotra *et al.*, 1980) did not relate the results of a glasshouse experiment to the field.

The project reported here consisted of three areas, firstly the identification of selection parameters and evaluation of varieties, secondly the study of the genetic control of tolerance to B and thirdly the measurement of the grain yield and interaction between B uptake and other nutrients for a population comprised of related tolerant and sensitive lines under field conditions. The initial experiments examined the response for a limited number of genotypes of known, but contrasting, tolerance to B in order to identify parameters which may be applied for assessment of segregating generations in the later genetic studies (Chapter 4). The tolerance of past and current Australian varieties, and in particular the ancestors of Halberd, was examined to determine whether varieties more tolerant than Halberd either have been, or currently are, in cultivation in Australia and therefore a potential source of B tolerance (Chapter 5). A knowledge of the relative tolerances of related varieties may provide an indication of the inheritance of tolerance while information on the tolerance of the current varieties would allow objective recommendations for areas where high concentrations of B prevail.

The genetic control of tolerance to B was investigated for the F_1, F_2 and F_3 generations among five genotyes of distinct levels of tolerance ranging from very sensitive to tolerant (Chapter 6) as the mode of inheritance of tolerance determines the strategy employed when breeding for tolerance. If tolerance is under the control of major genes the backcrossing method can be used to transfer tolerance to sensitive but otherwise adapted varieties. Studies on the chromosomal location of genes controlling tolerance to B were undertaken by aneuploid techniques with monosomic, intervarietal substitution and interspecific addition lines (Chapter 7).

The success of the transfer of B tolerance to adapted sensitive varieties will be influenced by interactions between tolerance to B and other genetic and environmental factors. The interaction between B tolerance and other factors was studied with random lines developed from Halberd and a sensitive line of high yield potential. These lines were evaluated over multiple sites and seasons for both grain yield and concentrations of elements in shoots and grain (Chapter 8).

The results of individual chapters are discussed separately while the overall results and conclusions are presented in a general discussion (Chapter 9).

In the text there are a number of references to personal communications and these are, in the main, to other members of the project studying B toxicity of cereals. These investigators and areas of research are : Dr. A.J. Rathjen, wheat breeder, Waite Agricultural Research Institute; Dr. B. Cartwright, soil scientist, CSIRO Division of Soils; Dr. R.O. Nable, plant physiologist, CSIRO Division of Soils and Mr. D.B. Moody, research officer - wheat breeding, Waite Agricultural Research Institute.

Chapter 2.

REVIEW OF LITERATURE - RESPONSE OF PLANTS TO BORON

2.1 INTRODUCTION

Boron (B) is one of seven micronutrients required for normal plant growth (Keren and Bingham, 1985). The role of B as an essential micronutrient was demonstrated by Warington (1923) for the broad bean and for a greater number of species by Sommer and Lipman (1926) and Brenchley and Warington (1927). The phytotoxic nature of excessive concentrations of B, under laboratory conditions, was recognized at a similar time as its requirement for growth (e.g. Brenchley and Warington, 1927; Collings, 1927; Haas, 1929) as were naturally occurring toxic concentrations of B in irrigation water in California (Kelley and Brown, 1928).

The range between deficient and toxic concentrations of B in plant tissues is narrow (Eaton 1944) and consequently imbalances of B nutrition are widespread. B deficiency is the most widespread of known micronutrient deficiencies (Keren and Bingham, 1985). A number of regions and crops for which either B toxicity or deficiency have been reported are listed in Table 2.1 (world) and Table 2.2 (Australia).

Boron toxicity is most common in arid and semi-arid regions, while deficiency is most widespread in the cool humid regions. High concentrations of B may occur naturally in the soil (e.g. Cartwright *et al.*, 1984), or in ground water (e.g. Eaton 1935). The concentration of B in sea water ranges from 0.5 - 9.6 mg kg^{-1} and the average concentration is approximately 4.6 mg kg^{-1} (Morgan, 1980) and high concentrations of B are common in soils formed in parent materials of marine origin (Norrish, 1975). High concentrations of B may occur in fumarole condensates and all major B concentrations are associated with deep seated fault systems of the arid regions (Morgan, 1980). Toxic concentrations of B may be applied to crops through over-application of B fertilizer when correcting deficiency, or as a contaminant of sewerage sludge (Baker and Chesnin, 1975) and municipal and industrial waste, such as coal fly ash (Elseewi *et al.*, 1981).

Table 2.1 Regions of the world where boron toxicity and deficiency have been reported. (Where many reports have arisen from the one region only the initial ones are presented).

Country / Region		Crop	Reference
Toxicity			
U. S. A.	California	Fruit trees	Haas, 1929
	California	Fruit trees	Eaton, 1935
	Minnesota	Barley	Christensen, 1934
India	Agra	Wheat	Chauhan &Power, 1978
		Lentil, barley, oats	Chauhan & Asthana, 1981
Pakistan	Lahore	Wheat	Sheik & Khanum, 1976
Phillipines	IRRI	Rice	Cayton, 1985
Iraq		not specified	Khudairi, 1961
Peru		Cotton, alfalfa	Fox, 1968
Deficiency			
U. S. A.	43 states	B def reported for one or more crops	Sparr, 1970
Canada	Prince Edward Island	Barley	Gupta, 1972
India	North Bihar	Wheat	Singh *et al.*, 1976
	North Bengal	Wheat	Chatterjee *et al.*, 1980
		Wheat, tomato	Gulati *et al.*, 1980
		Rice	Garg *et al.*, 1979
Pakistan		Wheat	Khan *et al.*, 1979
South Africa		Sunflower	Blamey *et al.*, 1984
Madagascar		Wheat	Oliver *et al.*, 1974
New Zealand		Clover, lucerne	Sherrell, 1983
Sweden		Clover	Eriksson, 1979
England		Many	Wallace, 1951
China		not specified	Liu *et al.*, 1981

Table 2.2 Regions of Australia where B toxicity and deficiency have been reported and crops affected.

State / Region		Crop	Reference
Toxicity			
Vic.	Sunraysia	Citrus	Penman & McAlpin, 1949
Vic	Sunraysia	Grapes	Sauer, 1958
Vic.	Sunraysia	Grapes	Antcliff & Webster, 1962
S. A.	Mid north	Barley	Cartwright *et al.*, 1984
Deficiency			
N.S.W.	Southern Tablelands	Subclover, lucerne	Anderson, 1952
Vic.	Koetong	Pinus radiata	Hopmans & Flinn, 1984
Tas.	53 sites	Brassicas-various	Lamp, 1964
6 states	21 sites (most on slopes of Great Dividing Range)	Various	Jackson & Chapman,1975

Boron toxicity in Australia has been described for citrus (Penman and McAlpin,1949) and grapes (Sauer, 1958) in the Sunraysia district of Victoria, and more recently for barley and wheat in the cereal belt of South Australia (Cartwright *et al.*, 1984). The highest concentrations of B in the soils of South Australia occur in the subsoil and therefore it has not been possible to reduce the incidence of B toxicity through amelioration of the soil conditions. Genetic variation in the tolerance of South Australian wheat varieties and breeding lines to high concentrations of B has recently been identified (Paull, 1985). This variation could be utilized for the breeding of tolerant varieties for cultivation in regions where high concentrations of B in the soil prevail. An understanding of the genetic control of tolerance to high concentrations of boron would facilitate the breeding of tolerant varieties.

This review of the literature addresses factors associated with the interaction between boron and wheat specifically, and those flowering plants in general, which are significant to a study of the genetic control of boron tolerance for wheat. There is evidence that the response to low and high concentrations of B are interrelated (Eaton, 1944), therefore response to B deficiency will also be considered.

It is considered beyond the scope of this project to formally review the interaction between soil and boron and the origins of B in soil. The section on B in soil will be discussed from the point of view of the major soil properties influencing B availability to plants.

2.2 CHEMISTRY OF BORON

Boron is the first member of the third periodic group, with an atomic number of 5, atomic mass of 10 and a constant valence of 3+. In aqueous solution B occurs as boric acid, $B(OH)_3$, which is a weak acid. It acts as a Lewis acid and accepts a hydroxide ion to form $B(OH)_4^-$. For the first dissociation of boric acid,

$$B(OH)_3 + 2H_2O \leftrightarrow B(OH)_4^- + H_3O^+ \quad pKa = 9.25$$

thus at physiological pH values the undissociated acid predominates (Raven, 1980). Boric acid has a trigonal planar structure whereas the borate ion has a tetrahedral structure in aqueous solution (Keren and Bingham, 1985).

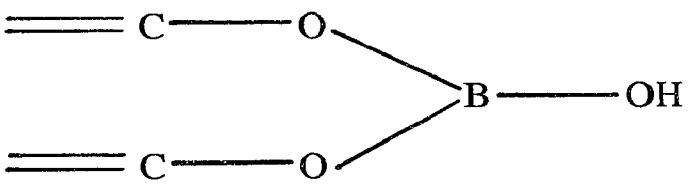
Boric acid is able to react with di-hydroxy organic compounds which have a cis-diol configuration (Fig 2.1). Compounds which have the correct configuration include a number of common sugars found both within the plant (Raven, 1980) and in the breakdown of organic matter in the soil (Parks and White, 1952). These compounds will affect the distribution and concentration gradients of B within plants (Raven, 1980) and the retention / release of B by organic matter (Parks and White, 1952).

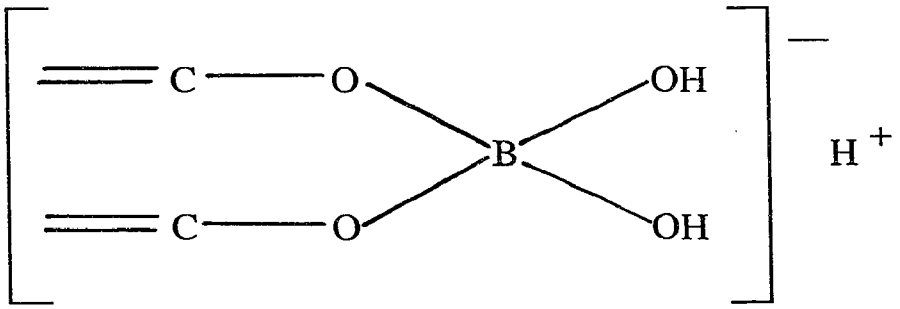
2.3 THE ROLE OF BORON IN PLANT METABOLISM

The requirement of B for completion of a plants growth cycle was demonstrated by Warington (1923) for broad bean and for a wider range of plants by Sommer and Lipman (1926) and Brenchley and Warington (1927). Since then the role of B in plant metabolism has been much studied, but a specific single role has not been elucidated (Dugger, 1983), nor is B considered to be a constitutive component of specific plant enzymes or cofactors (Jackson and Chapman, 1975; Dugger, 1983) and in this respect is unique among trace element nutrients.

An imbalance in B nutrition has been shown to affect many metabolic processes, however the distinction between the primary role of B and secondary effects arising from B imbalances is not always clear. The role of B in plant metabolism has been recently reviewed by Dugger (1983) and Pilbeam and Kirkby (1983). Dugger (1983) arranged the reported effects of B on plant growth and development in sequence and this diagram has been reproduced as Figure 2.2. Dugger (1983) proposed that the primary effect that B has in plants is to regulate enzymatic reactions through complexing with cis di-hydroxy compounds, and possibly also with certain trans 1,2-diol configurations. Thus, when B complexes with compounds which are reactants or products of enzymatic reactions it may stimulate or inhibit the course of specific metabolic pathways, which may in turn interact with other pathways and so influence plant growth.

Figure 2.1 Complexes formed between boric acid and compounds with a di-hydroxy configuration.





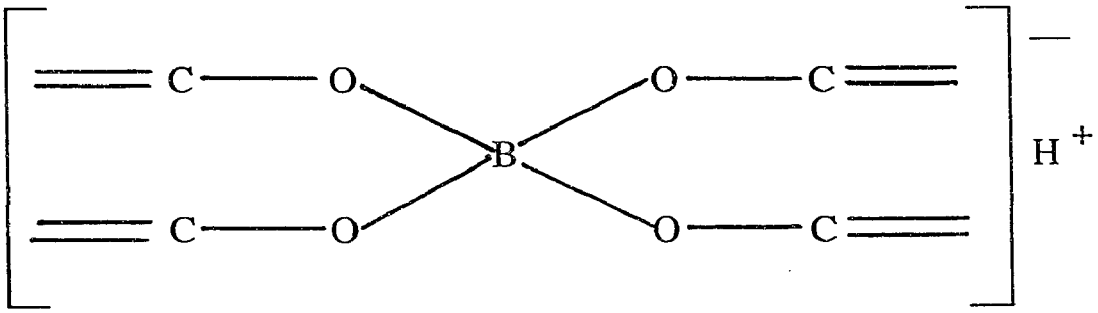
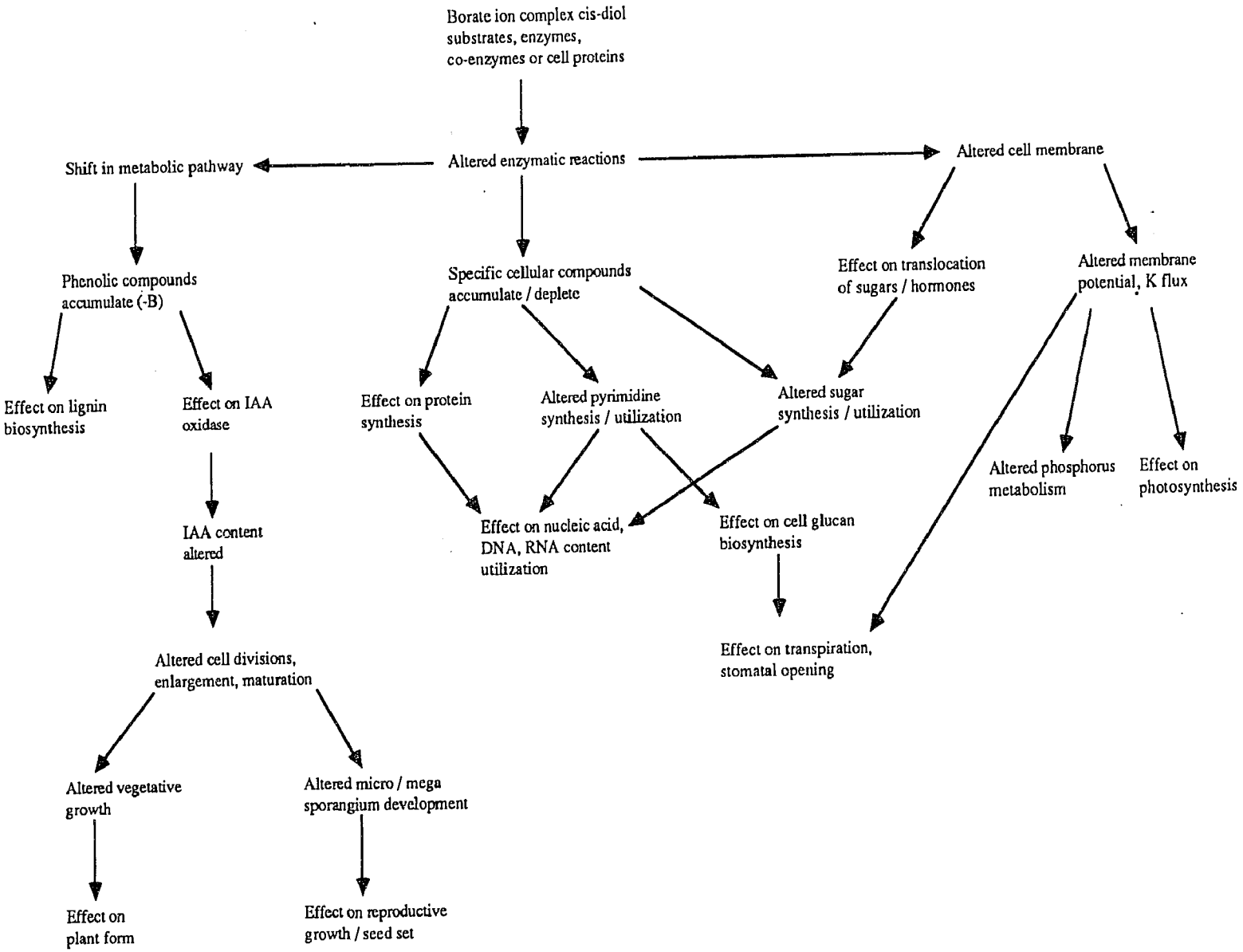


Figure 2.2 Proposed effects of B in plant metabolism. Reproduced from Dugger (1983).


Borate ion complex cis-diol substrates, enzymes, co-enzymes or cell proteins
Shift in metabolic pathway
Altered enzymatic reactions
Altered cell membrane
Phenolic compounds accumulate (-B)
Specific cellular compounds accumulate / deplete
Effect on translocation of sugars / hormones
Altered membrane potential, K flux
Effect on lignin biosynthesis
Effect on IAA oxidase
Effect on protein synthesis
Altered pyrimidine synthesis / utilization
Altered sugar synthesis / utilization
Altered phosphorus metabolism
Effect on photosynthesis
IAA content altered
Effect on nucleic acid, DNA, RNA content utilization
Effect on cell glucan biosynthesis
Effect on transpiration, stomatal opening
Altered cell divisions, enlargement, maturation
Altered vegetative growth
Altered micro / mega sporangium development
Effect on plant form
Effect on reproductive growth / seed set

2.3.1 Cell division and differentiation

Cell division and differentiation are both influenced by B supply. The essentiality of B for normal meristem development was recognized by Brenchly and Warington (1927). B deficiency has a very rapid effect on root growth and cessation of root elongation has been observed within 12 hours of transferring plants to a B deficient medium (Cohen and Lepper,1977; Hirsch and Torrey, 1980; Hirsch *et al.*, 1982). This effect may be attributed principally to inhibition of mitosis and disintegration of the meristematic region (Sommer and Sorokin, 1927; Albert and Wilson, 1961; Cohen and Albert, 1974; Cohen and Lepper,1977; Hirsch and Torrey, 1980; Hirsch *et al.*, 1982). The inhibitory effect of B deficiency on root elongation is reversible if B is supplied directly to the root system within 72 hours (Sommer and Sorokin, 1928; Neales, 1960).

Exogenous auxin may lead to rapid inhibition of root elongation (Coke and Whittington, 1967; Hirsch and Torrey, 1980) and B deficiency results in increased levels of endogenous auxin in roots (Hirsch *et al.*, 1982). As the inhibitory effect of B deficiency on mitosis occurred within six hours, but endogenous auxin levels did not increase until 24 hours of a nil B treatment, it was considered that the elevated auxin levels are a consequence, rather than a cause of symptoms of B deficiency, and B deficiency does not inhibit root elongation via an auxin effect (Hirsch *et al.*, 1982).

Other effects of B deficiency on cell morphology and differentiation include :

(1) thickened cell walls (Sommer and Sorokin, 1928; Albert and Wilson, 1961; Cohen and Lepper, 1977; Hirsch and Torrey, 1980);

(2) loss of dominance of main root tip and development of lateral roots close to the main root tip. The laterals that form on the portion of the root system under low B supply do not develop into normal secondary roots. The general appearance of roots is therefore stumpy (loss of dominance of the root tip) and bumpy (poorly developed laterals) (Sommer and Sorokin, 1928; Eltinge, 1936; Albert and Wilson, 1961);

(3) central vascular cylinder cells continue to elongate and progress into meristematic regions (therefore a continuous supply of B is not required for cell elongation, at least in the short term) (Cohen and Lepper, 1977);

(4) disintegration and lack of differentiation of tissues (Eltinge, 1936; Palser and McIlrath, 1956; Brown and Ambler, 1973).

The short term effect of B toxicity on cell division is not as well studied as the effect of B deficiency. Morris (1931) found elongation of roots of wheat seedlings to be severely inhibited during four days in solution at a concentration of 25 mg B l^{-1} and to be significantly reduced at a concentration of 4 mg B l^{-1}. Inhibition of root elongation and lateral root development of *Cucurbita pepo* occurred 72 hours after transfer of five day old plants to a high B medium, but it could not be determined whether the response was due to the direct effect of B on the roots, or a consequence of B accumulation in the leaves (Lovatt and Bates, 1984). Neither report indicated the relative contributions of inhibition of cell division and cell elongation to reduced root elongation under conditions of B toxicity.

2.3.2 Cellular membranes

Boron has been implicated in the control of membrane function. The activity of membrane functions, such as phosphate absorption, KCl-stimulated ATPase, and Cl^- and Rb^+ ion-uptake are reduced by B deficiency, but are rapidly restored following the application of B (Pollard *et al.*, 1977). The membranes of sunflower root tips lost integrity within six hours of transfer to a B deficient medium (Hirsch and Torrey, 1980), while the activity of membrane bound RNase was enhanced by B deficiency and was attributed to alteration or disruption of membrane permeability (Dave and Kannan, 1980). The loss of membrane integrity under conditions of B deficiency may account for the cessation of normal cell function, including cell division (Hirsch and Torrey, 1980), discussed earlier.

Tanada (1983) determined B in leaves of mung beans and found concentrations, expressed on a protein basis, to be : hypocotyl 1.0, protoplasts 1.8 and protoplast membranes 20.1 μg B mg^{-1} protein. Pollard *et al.* (1977) speculate that B may interact directly with the membrane by binding to polyhydroxyl compounds such as glycoproteins or glycolipids. This would be consistent with the high concentration of B in membranes.

2.3.3 DNA, RNA and proteins

The rapid effect of B deficiency on mitosis and cell division indicates an association between B deficiency and nucleic acid metabolism (Pilbeam and Kirkby, 1983). There is some discrepency in the effect of B deficiency upon DNA and RNA metabolism and this may result from differences in species and plant organs studied, and the degree of B deficiency. Cohen and Albert (1974) reported impaired DNA synthesis, as measured by incorporation of ^{3}H thymidine into nuclei of squash root tips after 20 hours of nil B treatment. On the other hand, Jackson and Chapman (1975) found no effect of 100 hours of nil B treatment on incorporation of ^{14}C thymidine in *Phaseolus aureus* root tips. High concentrations of B (20 and 40 mg B l^{-1}) inhibited synthesis of DNA in shoot apices of *Cucurbita pepo* by 50 and 70%, respectively after 24 hours of treatment, however DNA synthesis in root apices was not influenced by B treatments even after 72 hours, the time at which inhibition of root growth occurs. The reduced DNA synthesis in shoots was a consequence of the inability of plants to synthesize or salvage purine or pyrimidine nucleotides (Lovatt and Dugger, 1986).

There was less RNA in endosperm and embryo tissue for seeds of *Themeda triandra* imbibed in deionized water than in 0.05 mgB l^{-1} (Cresswell and Nelson, 1972). B deficiency was found to enhance RNase activity in primary and trifoliate leaves in bean plants (Dave and Kannan, 1980) and the authors concluded that the reduction in RNA level associated with B deficiency is due mainly to enhanced RNase activity and not due to reduced synthesis of RNA. Other findings in support of this hypothesis include : incorporation of ^{14}C-uridine by excised root tips of *P. aureus* was greater for root tips derived from B deficient than B adequate plants (Jackson and Chapman, 1975), and B deficiency had no effect upon the biosynthesis, salvage or catabolism of uridine monophosphate nucleotides for B deficient squash roots (Lovatt *et al.*, 1981).

The rate of protein synthesis is decreased under conditions of B deficiency and can be measured in squash roots within six hours of transferring to a B deficient medium (Cohen, 1979). The activity of many enzymes is also affected by B supply even though B is not considered a constitutive component of specific plant enzymes. Dugger (1983) refers to 15 enzymes whose activities are influenced by B supply. The effect of B

deficiency on activity varies between enzymes and may either be stimulatory or inhibitory, while excess levels of B are generally inhibitory. These results may arise from either a direct effect of B upon the enzyme or interaction with a substrate through formation of cis - hydroxy complexes.

Smith and Johnson (1976) demonstrated inhibition of yeast alcohol dehydrogenase by borate, $B(OH)4^-$, *in vitro*, and attributed the effect to competitive inhibition with both $B(OH)4^-$ and the enzyme competing for the substrate NAD^+. Phosphoglucomutase of pea seed, but not muscle, was inhibited by boric acid, $B(OH)3$, *in vitro*. As the mechanism of action of both forms of phosphoglucomutase is similar it was concluded that the inhibitory effect was not likely to be due to formation of complexes with a substrate but rather to a specific interaction between boric acid and the pea seed enzyme (Loughman, 1961).

Boron nutrition may affect the amino acid balance in plants. The concentrations of Asp, Pro, Ala and Gly were significantly increased in the sap of B deficient tomato plants (Hernando *et al.*, 1975) and for wheat the levels of essential amino acids in the grain were generally increased, and non-essential amino acids decreased, as B supply increased from deficient to sufficient (Iqtidar and Rehman, 1984). An increase in the total protein in wheat grain has been measured for increasing levels of applied B in the range from deficiency to sufficiency, without reductions in grain yield (Dani *et al.*, 1970; Iqtidar *et al.*, 1979; Iqtidar and Rehman, 1984). No effect of B nutrition on grain protein was found for corn (Jellum *et al.*, 1973) or soybean (Touchton and Boswell, 1975).

2.3.4 Phenolic compounds and lignin

The requirement for B is restricted to angiosperms, gymnosperms, pteridophytes and some marine diatoms, while it is not essential for bryophytes and most algae (Lewis, 1980; Pilbeam and Kirkby, 1983). Thus B requirement is associated with vascular plants and lignification. Indeed, Lewis (1980) proposed that the evolution of vascular plants was dependent upon the essential role of B in lignin biosynthesis.

The role of B in lignin and phenol metabolism is included in the recent reviews of Lewis (1980), Dugger (1983) and Pilbeam and Kirkby (1983). B complexes with 6-

phosphogluconic acid and might therefore regulate the pentose shunt catabolic pathway by inhibiting 6-phosphogluconate dehydrogenase and so prevent the accumulation of phenolics. Under conditions of B deficiency, phenolics accumulate and these may lead to necrosis of tissue. A consequence of the accumulation of phenolic compounds in B deficient tissues is that lignin also accumulates (Dugger, 1983). Excess B supply to tomatoes resulted in significantly reduced concentrations of the polyphenolic flavonoid glycosides (Carpena *et al.*, 1984). Weak stems of barley have been observed at high soil B treatments (Paull, 1985; Riley, 1987) and this would be consistent with impaired lignin synthesis.

2.3.5 Carbohydrate transport and metabolism

One of the first roles hypothesised for B in plants was in the movement of carbohydrates. Gauch and Dugger (1953) proposed that as B is able to complex with sugars it may facilitate the movement of sugars through membranes. The uptake and movement of ^{14}C, applied exogenously as either $^{14}CO_2$ or ^{14}C-labelled sugars, is greater for B adequate than for B deficient plants (Sisler *et al.*, 1956; Lee *et al.*, 1966). B deficiency of the apical bud was not alleviated by the regular application of sugars (Sisler *et al.*, 1956; Skok, 1957), therefore the symptoms of B deficiency did not appear to be the result of carbohydrate deficiency, or impeded carbohydrate translocation due to B deficiency. While B may not have a direct role in translocation of sugar in plants, B is consistently found to influence absorption and movement of sugars. This may be because of an indirect role, or through an effect on auxin biosynthesis (Dugger, 1973).

The balance between starch and reducing sugars is influenced by B supply. Supra-optimal B levels result in increased levels of reducing sugars and sucrose (Dugger and Humphreys, 1960; Scott, 1960) and a reduction in starch content (Scott, 1960; Lovatt and Dugger, 1986). The accumulation of sugars in leaves of sunflower, reported by Scott (1960), was not a result of impeded transport due to B imbalance as the concentrations of sugars were the same in the roots as in the shoots. Germination of seeds of *Themeda triandra* in the presence of B (0.05 mgB l^{-1}) resulted in increased α-amylase activity and greater levels of sucrose and reducing sugars in embryo tissue

compared to seeds germinated in the absence of B (Cresswell and Nelson, 1973). On the other hand, α-amylase activity of germinating seeds of barley was reduced by 50 mgB l^{-1} (Crabb, 1970) and 1000 and 2000 mgB l^{-1} (Jiminez-Lucena and Barea, 1979) in solution.

2.3.6 Pollen germination and pollen tube growth

Boron is required for pollen germination and pollen tube growth. Dugger (1983) lists the possible roles of B in pollen germination and tube growth as:

(1) facilitates sugar absorption,

(2) increases respiration and

(3) pollen tube tube wall biosynthesis.

In addition to B, *in vitro* germination of pollen requires a carbohydrate as a nutrient source (De Bruyn, 1966). Sucrose is the most suitable form of carbohydrate but the optimum concentration varies between species, and even between varieties within a species (Vasil, 1960). IAA and GA are not able to replace the stimulatory effect of B on pollen germination (De Bruyn, 1966).

The responses of pollen germination and growth of seven species representing five families of plants were compared at a range of B treatments. Pollen of all plants responded to increasing B at low levels but high concentrations of B proved toxic. Considerable variation in response for both germination and growth of pollen tubes occurred between species and between varieties of the one species (Vasil, 1960). As there is no further information concerning the response to B at the whole plant level for the varieties studied, the genetic variation in response to B for pollen cannot be related to variation in response between plants.

The poor seed set which has been reported for wheat (Ganguly, 1979) and subterranean clover (Dear and Lipsett, 1987), despite apparently healthy vegetative growth, and the response of grain yield to applied B, is probably the result of a higher requirement of B for seed set than vegetative growth. The poor seed set under conditions of B deficiency might be related to unviable pollen or insufficient B on the stigmatic surface for pollen germination. In cereals, atrophy of anthers occurs at a B supply which

has no effect upon the embryo sac and surrounding tissue. The sporogenous layer, in particular, is affected and inhibited nuclear division results (Vasil, 1964).

2.3.7 Conclusions on the role of B in plant metabolism

The role of B in plant metabolism is by no means certain, despite being required for normal growth and development. The major roles that have been proposed can be categorized as (1) maintenance of membrane integrity and functioning and (2) regulation of enzymatic reactions. The major characteristic of B upon which most conclusions are based is the ability of $B(OH)_3$, the dominant form of B in plants at physiological pH, to form cis di-hydroxy complexes (Figure 2.1). The distinction between primary and secondary effects of imbalances in B nutrition is not clear. B has been shown to influence enzymatic reactions, *in vitro*, but to extrapolate from this situation to *in vivo* assumes that B and the enzyme, or substrates, are associated within the plant. On the other hand the influence of high or low concentrations of B upon reactions determined *in vivo* may be due to either primary or secondary effects. There is no information on the comparative responses of genotypes of contrasting tolerance to B.

This section has not identified a biochemical pathway which would account for the genetic differences in tolerance to high concentrations of B for wheat identified in South Australia (Paull, 1985; Cartwright *et al.*, 1987; Nable, 1988; Paull *et al.*, 1988).

2.4 BORON AND SOILS

2.4.1 Geochemistry

B is not uniformly distributed in the earth's crust but the concentration varies with rock type and origin. The total B concentrations of rock types have been summarized recently by Evans and Sparks (1983) and Kabata-Pendias and Pendias (1984). The values vary between authors, but in general total B concentrations are greater in sedimentary than igneous rocks. The highest concentrations are in marine argillaceous sediments and shale, while for igneous rocks the B content increases with the acidity of the rock.

Common total B concentrations (mg kg^{-1}) include :

Igneous Rocks : Ultrafumic rocks 1-5, Mafic rocks 5-20, Intermediate rocks 9-25, Acid rocks 10-30;

Sedimentary Rocks : Marine Argillaceous sediments 120, Shales 130, Sandstones 30, Limestones and dolomites 20-30 (Kabata-Pendias and Pendias, 1984).

Boron is found in a number of minerals, the most common being -

Hydrous borates : borax, kernite, colemanite and ulexite ;

Anhydrous borates : ludwigite and kotoite;

Complex borosilicates : tourmaline and axenite (Evans and Sparks, 1983).

The original source of B in most soils is tourmaline (3-4% B), however B within minerals is not available to plants (Graham, 1957; Norrish 1975). Weathering of B containing rocks and minerals brings B into solution, predominantly as $B(OH)_3$. Once B is released it can be rapidly leached, often leading to B deficiency, especially in acid soils in high rainfall areas (Gupta, 1979) or accumulation in evaporite deposits in arid regions (Evans and Sparks, 1983).

2.4.2 Boron adsorption sites

Boron may be bound in the soil in one of three ways :

(1) Incorporation in silicate structures. The tetrahedral anion $B(OH)_4^-$ may substitute for Al^{3+} or Si^{4+} in silicate structures following adsorption onto the clay surface (Couch and Grim, 1968). The tourmaline group is the main example of this form of B (Davies, 1980).

(2) Adsorption by clay minerals, sesquioxides and iron and aluminium hydroxy compounds. This may involve one of two types of binding, either (a) exchange of borate for hydroxyl ions, or (b) the formation of a borate-diol complex (Sims and Bingham, 1967, 1968a,b). Illite is the most reactive clay and adsorbs more B than

kaolinite or montmorillonite per unit surface area (Hingston, 1964), while the amount of B fixed also varies with the type of illite (Couch and Grim, 1968). Much of the affinity of clay for B can be attributed to sesquioxide and iron and aluminium hydroxy compounds on the surface of clays. Hydroxy-aluminium materials adsorb more B than do hydroxy-iron compounds (Sims and Bingham, 1968). Adsorption of B by clays is pH dependent and retention increases with increasing pH. This has been attributed to either $B(OH)_4^-$ being the dominant form of B in reaction (Hingston, 1964; Bingham *et al.*, 1971), or $B(OH)_3$ being adsorbed but the number of adsorption sites on the clay increases with pH (Hingston, 1964).

(3) Boron contained in organic matter. The role of organic matter upon the availability of B has long been recognized. Berger and Troug (1945) and Martens (1968) observed soils with a high level of organic matter to also have high levels of available B, while Olson and Berger (1946) found the destruction of organic matter resulted in a reduced ability of soil to fix B. Parks and White (1952) postulated that cis di-hydroxy organic compounds, resulting from the breakdown of soil organic matter may form B-diol complexes and so hold the B in a stable form. Gradual release of B in a plant-available form may result from the action of microbial decomposition.

Hatcher *et al.* (1959), for beans, and Keren *et al.* (1985), for wheat, have reported that plants only respond to the portion of B in solution. Stinson (1953) and Wear and Patterson (1962) also found the concentration of B in alfalfa shoots to be directly correlated with the soluble B content of the soil. It is therefore more appropriate to describe the B status of soils, with respect to plant response, as a function of B in solution, rather than total or adsorbed soil B. As the concentration of B will vary according to the method of extraction, direct comparisons of reported B concentrations may not reflect true differences in plant available B. The most common method of determining an index of plant-available B in soil is by hot water extraction (Berger and Troug, 1939), however other extractants, such as $CaCl_2$-mannitol may also give a highly significant correlation between B extracted and plant uptake of B (Cartwright *et al.*, 1983). The absolute concentration of B extracted by the $CaCl_2$-mannitol method was less than by the hot water soluble (HWS) method at low B concentrations but greater at

potentially toxic concentrations (Cartwright *et al.*, 1983). The concentration of B from a soil saturation extract was less than the mannitol extractable B, but the two were highly correlated with each other and the concentration of B in barley shoots (Cartwright *et al.*, 1984).

2.4.3 Soil factors affecting plant uptake of boron

When B is released from soil minerals, mineralized from organic matter or added to soils by means of irrigation water or fertilizer, part of the B remains in solution and part is adsorbed (Gupta *et al.*, 1985). The concentration of B in solution is only a small fraction of the total soil B. For example, the total B concentrations for 108 soil samples from eastern Canada ranged from 45-124 mg kg^{-1}, but the HWS-B ranged from only 0.38-4.67 mg kg^{-1} (Gupta, 1968). Although plants respond to the B in solution, the concentration of B measured by the HWS method may not give a measure of the B supplying power of soil over a growing season. The amount of B taken up by plants was greater than the initial HWS-B content of the soil for several soil types (McClung and Dawson, 1950; Haddad and Kaldor, 1984). Either the HWS method does not measure the total B available to plants for all soil types, or alternatively there is a state of equilibrium between adsorbed B and B in solution. Hatcher and Bower (1958) and Hingston (1964) found adsorption of B to be easily reversible and Hatcher and Bower (1958) proposed an equilibrium is established between dissolved and adsorbed B.

Factors affecting the equilibrium between the adsorbed and available B fractions, and therefore plant uptake, include texture, pH, organic matter, moisture content, reaction time and concentration of B. The availability of B may be measured either through extraction from the soil, or response of plants. The latter is of prime importance to this thesis and discussion will be concentrated on this area.

Texture

Boron is adsorbed to clay (Hingston, 1964; Couch and Grim, 1968) and consequently the uptake of applied B, by plants, is inversely related to the clay content, or particle size (Jordan and Powers, 1946; Wear and Patterson, 1962; John *et al.*, 1977;

Blamey *et al.*, 1979; Keren *et al.*, 1985). Wear and Patterson (1962) reported the B concentration of sunflowers to be greater when a given amount of B was applied to coarse textured than medium or fine textured soils. The change in the concentration of B in the plants per unit increase in HWS-B was also larger for the coarse soil. On the other hand, Keren *et al.* (1985) reported that although the B concentration in wheat shoots was more for a given B application to coarse than fine soil, the concentration of B in the shoots for three soil/sand mixtures and five concentrations of applied B lay on the same line when plotted against the B concentration in the saturation pastes.

The distribution and movement of B through the soil profile has also been related to soil texture. Kubota *et al.* (1948) found the movement of B applied to soils to be primarily related to texture and most rapid in soils of light texture. The rate of movement of B in the subsoil was reduced and B tended to accumulate for some soil types. This could be attributed not only to the heavier texture of the subsoil but also to reduced water movement.

pH

The pH of the growth substrate influences the plant uptake of B via two mechanisms :

(1) adsorption of B to clay particles is pH dependent and so pH affects the concentration of B in soil solution;

(2) pH affects the balance of $B(OH)_3$ and $B(OH)_4^-$ in the soil solution.

Midgley and Dunklee (1939) demonstrated that increasing levels of lime applied to acid soils resulted in decreased levels of HWS-B and more severe symptoms of B deficiency for flax, while the toxic effect of borax on soybeans was prevented by the application of lime (Cook and Millar, 1939). The B uptake by plants was not reported in either instance.

The uptake of B has been reported to decrease as the pH increases for a number of plants including clover (Bishop and Cook, 1957), alfalfa (Wear and Patterson, 1962), cotton (Fox, 1968), barley (Gupta, 1972; Gupta and MacLeod, 1981), tomato, cucumber and sweet corn (Prasad and Byrne, 1975), peas (Gupta and Macleod, 1981) and alfalfa,

red clover and timothy (Gupta, 1983). There was no effect of pH on the concentration of B in the shoots of tall fescue for the pH range 4.7 - 6.3 but B concentrations were significantly reduced at pH 7.4 (Peterson and Newman, 1976), while the effect of lime on B concentrations in wheat and barley shoots was inconsistent for the pH range 5.4 - 6.6 (Gupta, 1977). Contrary to the above results for experiments in which pH was increased by soil amendments, the plant availability of B for 15 untreated soils was positively correlated with soil pH (Martens, 1968). This probably reflects a greater reserve of B held against leaching by adsorption to clay and organic matter and therefore capable of becoming available to plants in soils having higher pH levels (Kubota *et al.*, 1948). Similarly, the HWS-B concentration was positively correlated with pH for 31 virgin soils over the pH range 4.7 - 6.7, but the correlation was negative for soils having pH greater than 7.1 (Berger and Troug, 1945).

The effect of liming on response to, and uptake of B by plants has been shown to be principally due to changes in pH rather than an interaction betwen $B(OH)_4^-$ and the cation of the base (Cook and Millar, 1939; Midgely and Dunklee, 1939; Muhr, 1940; Kubota *et al.*, 1948; Gupta and MacLead, 1977, 1981). The results are consistent with the increase in adsorption to clay at higher pH (Hingston, 1964; Bingham and Page, 1971).

The balance of $B(OH)_3$ and $B(OH)_4^-$ changes in favour of $B(OH)_4^-$ as the pH increases in the alkaline range. Uptake of B by roots has been described as both passive and non-metabolic with undissociated $B(OH)_3$ being favoured (Bingham *et al.*, 1970; Oertli and Grgurevic, 1975) and as actively absorbed in the ionic form (see Gupta *et al.*, 1985). The review of Raven (1980) argues in favour of uptake of B in the undissociated form and highlights the fact that most reports of active uptake have not allowed for B in tissues as borate-diol complexes when calculating B fluxes.

The results of the effect of substrate pH on B uptake by plants would also be consistent with uptake of $B(OH)_3$. The reduced uptake of B at higher pH may therefore arise from the joint effects of adsorption of $B(OH)_4^-$ to soil and organic matter and a reduced fraction of $B(OH)_3$ in the soil solution.

Despite the increase in B adsorbed at pH greater than 7, Olson and Berger (1946) found not more than 40% of added B was fixed at pH 10. It is therefore possible for the content of available B to reach toxic levels for highly alkaline soils, as is the situation in South Australia.

Moisture

The availability of B to plants is influenced by soil moisture content and in general B deficiency is more severe when moisture is limiting (Hobbs and Bertramson, 1949; Dible and Berger, 1952; Stinson, 1953; Hopmans and Flinn, 1984). A low moisture content may lead to low microbial activity and so result in minimal breakdown of organic matter and release of B from B-diol complexes (Parks and White, 1952). Hatcher *et al.* (1962) found the concentration of B in soil solution to be relatively constant, despite a several fold change in the water content of the soil. The change in concentration was largest for the soil having the lowest B adsorption capacity. This would indicate that B deficiency associated with dry conditions is probably due to increased exploration of the deeper horizons, which are generally lower in available B, rather than a fixation process (Davies, 1980). In semiarid regions, where leaching is limited, the B concentration in the subsoil often exceeds that in the surface (Gupta *et al.*, 1985) and such is the situation in South Australia (Cartwright *et al.*, 1984). Uptake of B is related to the proportion of the root system exposed to B (Bingham and Garber, 1970) therefore moisture conditions which encourage root exploration of the subsoil may have the opposite effect on these latter soils and exacerbate B toxicity .

Hatcher *et al.* (1962) found that repeated cycles of wetting and drying of soil resulted in irreversible adsorption of B. Soil should therefore not be allowed to dry to low moisture content while plant growth experiments are being conducted.

Boron concentration

The adsorption of B applied to soil has been attempted to be quantified according to soil properties by methods such as the Langmuir and Freundlich equations and a phenomenological equation (Keren and Mezuman, 1981). Deviations from the Langmuir

equation have often occurred, particularly at high B concentrations (see Gupta, 1985). From the point of view of the plant this deviation results from a reduced adsorption, as a percentage of applied B, at higher B treatments and therefore a higher proportion of B in solution (e.g. Keren *et al.*, 1985). The B concentration of wheat shoots was linearly related to B in soil solution, rather than B applied (Keren *et al.*, 1985).

Reaction time

The adsorption of B applied to soil requires a significant time to reach equilibrium. Adsorption of B by 2% suspensions of calcium-saturated kaolinite and bentonite had not reached equilibrium after four weeks (Parks and White, 1952), while greater than 24 hours was required for B to reach equilibrium between the adsorbed and solution phases for B applied to four soil types (Bingham *et al.*, 1971). For a pot experiment growing peppers at eight concentrations of applied B, the concentration of B in soil solution was maintained over a 10 week growing period (Hatcher *et al.*, 1962). Thus, while the concentration of extractable B measured immediately upon applying B to soil may be greater than at equilibrium, the equilibrium concentration should be relatively constant for the duration of pot experiments.

2.5 UPTAKE OF BORON AND DISTRIBUTION WITHIN THE PLANT

2.5.1 Uptake of B by roots

Oliver and Barber (1966) calculated that of the three means of reaching the root, namely root interception, mass flow and diffusion, mass flow accounted for at least 50% of the total B uptake by soybean, however B uptake was not directly related to transpiration. Altering environmental factors, such as temperature, humidity and light intensity, in favour of increased transpiration increased the B uptake by sugarcane but again there was not a simple stoichiometric relationship between uptake of B and water (Bowen, 1972). These results indicate that while the uptake of B is closely related to the uptake of moisture, B uptake by roots is not entirely passive but is at least partially controlled (Raven, 1980).

2.5.2 Translocation of B from roots to shoots

Once within the roots of sugarcane the translocation of B to shoots was passive and directly related to transpiration (Bowen, 1972). A contrary result was found between two tomatoes, one efficient (Rutgers) and the other inefficient (T3238), for B uptake to shoots. The efficient and inefficient genotypes had similar concentrations of B in root sap exudate (3.1 and 3.2 μgB ml^{-1}, respectively), however the B concentration in sap expressed from the top leaves was far less for the inefficient than the efficient genotype (1.7 and 25.8 μgB ml^{-1}, respectively). The greater susceptibility of T3238 to B deficiency resulted from reduced translocation of B from roots to shoots, a finding which was confirmed by reciprocal grafts between the two genotypes (Brown and Jones, 1971).

The concentration of B in shoots is under the control of the rootstock for several other species. For reciprocal grafts between sunflower (moderately tolerant) and artichoke (sensitive to high concentrations of B), the concentration in shoots was significantly lower for both scions when grown on sunflower as compared to artichoke rootstocks (Eaton and Blair, 1935). Similarly, the B tolerance of citrus, or the concentration of B in the scions reflected the rootstocks (Swingle *et al.*, 1928: Eaton and Blair, 1935: Haas, 1945; Penman and McAlpin, 1949; Smith *et al.*, 1949). However, the data presented do not indicate whether the effect of rootstock for these species is a consequence of differences in uptake by the roots, or translocation to the shoots.

When comparing five barley and six wheat genotypes of contrasting tolerance to B, Nable (1988) reported a similar ranking of genotypes with respect to B concentrations in roots and shoots. The mechanism controlling accumulation of B in shoots of cereals is therefore a physical or biochemical barrier to entry to the vascular tissue of the roots rather than restriction of translocation from roots to shoots as is the situation for tomatoes.

2.5.3 Distribution of B in shoots

The distribution of B within the shoots is far from uniform, both between organs, and within an individual leaf. Chlorotic and necrotic symptoms of B toxicity developed

first on the older leaves of barley and peas while within a leaf symptoms developed from the tip for barley and from the margins for peas (Brenchley, 1914). B toxicity symptoms are described for many plant species by Haas (1929), Eaton (1944) and Oertli and Kohl (1961). The pattern of symptoms differs between species, however a feature common to all is the association between symptom development and pattern of venation.

The distribution of B within the leaf corresponds with the toxicity symptoms and the highest concentration is coincident with the initial site of symptom expression. The B concentrations for a lemon leaf displaying symptoms of B toxicity were : midvein and petiole 47 mg kg^{-1}, green portion 438 mg kg^{-1}, yellowed portion 1060 mg kg^{-1} and apex and margin 1722 mg kg^{-1} (Eaton, 1935). A gradient for B concentration from petiole to tips and margins of lemon leaves also occurred at "normal" B concentrations (Oertli, 1960), and similar distribution patterns of B have been measured for Easter lilies (Kohl and Oertli, 1961), sugar beet, cotton and soybean (Oertli and Roth, 1969) and corn (Jones, 1970).

The coincidence of high B concentrations and symptoms of toxicity suggests that the symptoms are a direct effect of the high concentrations of B in the tissue (Eaton, 1935; Oertli and Kohl, 1961). The pattern of distribution of B within the leaf is indicative of B moving in the transpiration stream and accumulating at the site of transpiration (Eaton, 1935; Oertli and Kohl, 1961).

The concentration of B in plants grown in sand culture was higher in leaves than shoots and roots for 57 of 58 varieties representing 50 species, with the exception being a stone fruit (Eaton, 1944). (The distribution of B in field grown stone fruits was also found to be contrary to other species and the highest concentration of B was found in the bark (Eaton, 1935)). When shoots are further sub-divided the concentrations of B are greatest in the older leaves followed by upper leaves, growing tips, stems and grain, respectively (Hodgkiss *et al.*, 1942; Miller and Smith, 1977; Salinas *et al.*, 1981). For barley the concentration in leaves ranged from 55 mg kg^{-1} for the youngest leaf to 308 mg kg^{-1} for the fourth youngest leaf (Kluge and Podlesak, 1985).

The concentration of B in the grain is much lower than in shoots for cereals grown in pots (Gupta, 1971) and in the field (Cartwright *et al.*, 1984). When wheat,

barley and oats were compared for B concentrations in shoots and grain, the ranking for grain was barley << wheat < oats, however the ranking for B concentration in boot stage tissue was wheat = oats < barley (Gupta, 1971). B concentration in grain for field grown wheat (Cartwright *et al.*, 1987) was higher although of the same order, relative to the concentration in shoots, as for wheat grown in pots (Gupta, 1971). For example, in a pot experiment a B concentration of 86 mg kg^{-1} in shoots was associated with grain concentrations of 2.46 mg kg^{-1} (Gupta, 1971), while for wheat grown in the field in South Australia, boot stage B concentrations in the range 48 - 126 mg kg^{-1} were associated with grain values between 3.5 - 8.2 mg kg^{-1} (Cartwright *et al.*, 1987). On the other hand, the concentration of B in grain of barley grown in pots was not affected by increasing B applications and despite a B concentration in boot stage shoots of greater than 300 mg kg^{-1} the concentration in grain was only 0.37 mg kg^{-1} (Gupta, 1971). Experiments conducted at the Waite Institute have found that the B concentration of barley grain increases in response to B treatments in a similar manner to wheat (Paull, unpublished). It is therefore probable that there was an analytical error for the result of B in grain of barley published by Gupta (1971).

Concentrations of B in the grain of cereals vary between countries and are generally betwen one and five mg kg^{-1}, although concentrations as high as 15 mg kg^{-1} have been reported for wheat in U.S.S.R. (Kabata-Pendias and Pendias, 1984). By these standards the concentrations of up to 10 mgB kg^{-1} grain for wheat grown in the field in South Australia (Cartwright *et al.*, 1987) would be considered extremely high.

Boron in shoots is not readily remobilized and symptoms of B deficiency of the meristem may occur under conditions of low B supply, despite apparently adequate levels of B in the whole shoot (Eaton, 1944). When B was excluded from the nutrient medium the B concentration in the apical portion of lucerne was 8 mg kg^{-1} while the concentration in the whole shoot was 23 mg kg^{-1} (Dible and Berger, 1952). Brenchley and Warington (1927) and Eltinge (1936) found that a constant supply of B is required to prevent B deficiency of broad beans and maize, respectively. The age of plants and previous treatments had no influence on response to a current deficiency. Similarly, the growth of a lemon tree, suffering from B toxicity was not affected subsequent to transference to a

"normal" B medium (Haas, 1929). B in the older leaves is not translocated to the new actively growing regions.

Experiments using the split-root technique with sunflower (Husa and McIlrath, 1965) and tomato (Cerda and Roorda van Eysinga, 1981) have shown restricted lateral movement of B from the high B to the low B side of the plant, especially for the lower leaves. Roots in a plus B treatment were not able to supply B to the roots in a nil B treatment and these roots developed symptoms of B deficiency for sunflower (Husa and McIlrath, 1965) and tomato (Albert and Wilson, 1961). B applied to epicotyls did not alleviate B deficiency symptoms for roots in a nil B medium (Neales, 1960). These results, together with the effect of B supply on cell division, indicate that B is not translocated to the extremities of the root system and therefore roots would require a constant supply of B in the immediate root environment.

Boron within leaves is in a soluble form (Eaton, 1944; Oertli and Kohl, 1961), and is subject to leaching from the leaf (Oertli, 1962; Husa and McIlrath, 1965) and may also be lost from the leaf by guttation (Oerlti, 1962). The microdistribution of B within a leaf was homogeneous and the vacuolar and extra-cellular concentrations were close to equilibrium (Oertli, 1969), and B may enter and be transported in phloem (Oertli and Richardson, 1970). The immobility of B within plants is therefore not a consequence of chemical fixation or a barrier to the phloem. In an attempt to explain the lack of translocation of B from leaves, Oertli and Richardson (1970) proposed a counter-current system whereby B is cycled between the xylem and phloem in response to concentration gradients within the leaf. On the other hand, Raven (1980) argued that the major force determining the distribution of B is the unidirectional flow of the transpiration stream. Further evidence that B may be translocated in the phloem, if there is no opposing movement of water in xylem, is provided by fact that B may be translocated from a peanut plant in a B adequate environment to kernels where the fruiting medium is B free. As there is no water flux into buried peanuts, B is presumably translocated in the phloem (Campbell *et al.*, 1975).

2.6 RESPONSE OF PLANTS TO BORON IMBALANCE

The previous sections have described soil factors affecting the availability of B to plants, the function of B in plants, the responses at the metabolic level to imbalances of B nutrition, and the uptake and movement of B within the plant. The following sections review the response to B as manifested at the whole plant level, with particular reference to cereals. While B deficiency is undoubtedly a major problem for many crops, responses of plants to low concentrations of B will only be discussed from the point of view of genetic variation.

2.6.1 Germination

The response of seeds to B in the germination medium at first appears inconsistent between authors, with a number reporting no effect of high concentrations of B on germination, while others have found either delayed or reduced germination at higher B levels. These discrepencies may be accounted for, to a large degree, by such factors as the range in concentration of B in the germination medium, nature of the medium and species or variety.

B has been reported to have no effect on germination for wheat, barley, green gram and sesame in petri dishes to a concentration of 200 mgB l^{-1} (Khudairi, 1961), for *Agropyron elongatum* in soil to a level of 80 mg kg^{-1} water soluble B (Schuman, 1969), for barley in solutions to a concentration of 100 mgB kg^{-1} (Crabb, 1970), for wheat in petri dishes over the range 0.45 - 3.00 mgB l^{-1} (Sheik and Khanum, 1976) and for oats and berseem in soil to a level of 40 mg kg^{-1} water soluble B (Bajwa and Singh, 1977). The germination and emergence of seedlings of soybean from sand irrigated with 250 mgB l^{-1} was completely inhibited (Collings, 1927) as was the germination of *Themeda triandra* by 37 mgB l^{-1} in petri dishes (Cresswell amd Nelson, 1972).

The germination and emergence of corn was reduced at 100 mg kg^{-1} applied B for only one of three soil types (John *et al.*, 1977), while B had no effect on germination and emergence of soybean when applied as borax at 250 mg kg^{-1} to soil, but when applied at 25 mg kg^{-1} to sand, germination was significantly reduced although not

completely suppressed (Collings, 1927). The nature of the medium, and therefore the availablity of B, will influence the effect of B upon germination. Genotype may also influence response to B at germination as demonstrated by Paliwal and Mehta (1973) for three rice varieties.

High concentrations of B may result in delayed emergence without a reduction in the final emergence percentage (Collings, 1927; Jiminez and Aguilar, 1982; Paull, 1985; Paull *et al.*, 1988). This effect may arise either from a delay in germination or reduced rate of development subsequent to germination. Growth of roots of barley (Crabb, 1970) and radicles and shoots of sesame seeds (Khudairi, 1961) were reduced at increasing concentrations of B, even though germination was not affected. Such a reduction in rate of growth would result in delayed emergence for plants growing in high B soil. Germination and emergence of cereals is more tolerant to high concentrations of B than later growth. For nine genotypes of wheat and barley grown in soil, 150 mg kg^{-1} applied B delayed emergence without affecting the final emergence percentage, but only two of the nine genotypes survived to maturity at this treatment (Paull, 1985; Paull *et al.*, 1988).

High concentrations of B are inhibitory to germination and emergence, however the B concentrations at which these effects occur are far in excess of the B concentrations typically found in surface soils, or irrigation water. B is therefore unlikely to affect emergence of field grown crops, however for pot experiments where the B is mixed uniformly through the soil, there is the possibility of an effect of B treatments on early plant development.

2.6.2 Symptoms of B toxicity

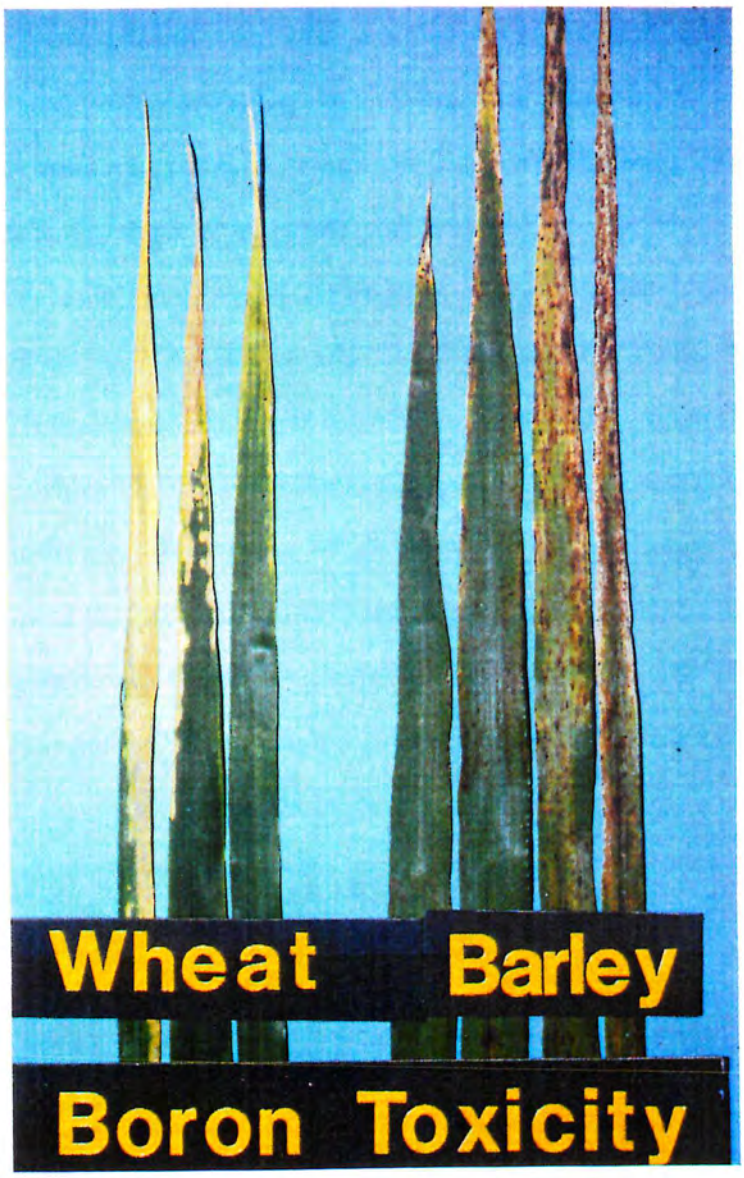
As described previously, the distribution of symptoms of B toxicity can be directly related to the passive movement of B in the transpiration stream and the symptoms coincide with the regions of accumulation of B. It is not proposed to describe the symptoms of B toxicity for all species (for comprehensive descriptions refer to Eaton (1944), Oertli and Kohl (1961) and Bradford (1973)), however the contrasting expression of symptoms among cereals is significant.

B toxicity symptoms of barley were described for plants grown in solution culture at a range of B treatments by Brenchley (1914) and consisted of yellow regions with black spots within them, developing on the tips of the oldest leaves first and progressing to the leaf base and to successively younger leaves. Naturally occurring symptoms of B toxicity for field grown barley were first described by Christensen (1934) in Minnesota, U.S.A. and were similar in appearance to symptoms of infection by *Helminthosporium sativum* . No causal organisms could be isolated and B was the only one of many applied nutrients which lead to similar symptoms under controlled conditions. The expression of symptoms, including such factors as size, shape, colour, number and distribution of spots varied among the 125 varieties evaluated. Eaton (1944) reported similar spotting of barley in response to high concentrations of B, and also indicated the difference in development of symptoms between B toxicity and *H. sativum*. Whereas *H. sativum* attacks at random over the entire leaf, symptoms of B toxicity are most severe at the leaf tip and for older leaves.

The contrasting symptoms of B toxicity for wheat, barley and oats were described by Gupta (1971) and also observed by Paull (1985). The symptoms for wheat and barley are shown in Plate 1. Barley develops brown spots within the necrotic regions, however toxicity symptoms for wheat appear as light browning developing from the leaf tips, and in particular for the older leaves. Gupta (1971) reported these symptoms to converge into light greenish blue spots, however this effect was not observed by Paull (1985). Toxicity symptoms of oats consisted of light yellow bleached leaf tips (Gupta, 1971). For rice, necrotic spots may or may not develop within the generally necrotic leaf tissue, depending upon variety (Cayton, 1985). Similar variation in development of necrotic spots has also been observed among genotypes of field pea (Materne, 1989), triticale and Medicago spp. in experiments conducted at the Waite Institute (unpublished).

Recognition of B toxicity in South Australia resulted from the initial observation of necrotic spots of barley (Cartwright *et al.*, 1984). As for Christensen (1934), no causal pathogen could be isolated and the only nutritional factor correlated with the appearance of symptoms was the concentration of B in the soil and shoots. The specific nature of the

Plate 1. Symptoms of boron toxicity for wheat and barley. The pattern of development is similar for the two species, progressing from the leaf tip, along the margin to the base, but only barley develops brown spots within the affected region.


Wheat Barley
Boron Toxicity

B toxicity symptoms for barley, as compared to wheat and oats, would enable the visual identification of crops affected by B toxicity.

2.6.3 Development and yield

High concentrations of B affect the developing seedling from an early stage and growth effects can be measured soon after emergence. Seedling height of wheat (Khudairi, 1961) and rice (Paliwal and Mehta, 1973) were reduced, and the rate of growth was also reduced, within seven days at a treatment of 20 mgB l^{-1}. There was no effect of B treatments upon the growth of coleoptiles of wheat (Khudairi, 1961) although no distinction was made between rate of growth and final length.

B supply affects tillering of wheat. At high concentrations, tillering was reduced relative to controls (Morris, 1931; Badenhorst and Burgers, 1973; Paull, 1985), while under conditions of B deficiency tillers continued to develop until plants reached the flowering stage (Brenchley, 1936). Phenological development is generally delayed by imbalances of B nutrition. Ear emergence of wheat (Morris, 1931; Gupta, 1971; Paull, 1985), flowering of peas (Salinas *et al.*, 1981) and maturity in soybeans (Touchton and Boswell, 1975) are delayed by increasing concentrations of B, while ear emergence of wheat (Morris, 1931) and flowering of peanuts (Harris and John, 1966) are delayed by B deficiency.

There are numerous reports of yield reductions due to B deficiency and toxicity. For cereals, and in particular for wheat, grain yield reductions due to B deficiency are the result of poor seed set resulting from pollen infertility. Consequently, yield responses to B applications are associated principally with an increase in grains per ear (Ganguly, 1979) and also an increase in 1000 grain weight (Iqtidar *et al.*, 1979). There is little information partitioning yield reductions due to B toxicity for cereals. High concentrations of B resulted in smaller heads for barley (Gupta,1971) and a decrease in spikelets, and hence grain number, for the main ear of several, but not all, wheat genotypes (Mehrotra *et al.*,1980).

It is axiomatic that for extreme B toxicity, whereby a plant dies before reaching the reproductive stage, grain production is more sensitive than vegetative growth to B

toxicity. This also appears to be the situation for moderate levels of toxicity. As the concentration of applied B increased, the grain and straw yields were 45% and 78% of the maximum yield, respectively, for barley, and 64% and 82%, respectively, for wheat (Gupta, 1971). Chauhan and Powar (1978) also reported a significant reduction in grain yield without a corresponding reduction in yield of straw, for wheat. Similar results have also been obtained for lentil (Chauhan and Asthana, 1981) and peas (Chauhan and Powar, 1978; Salinas *et al.*, 1981).

Boron toxicity may be identified either by expression of symptoms or yield reduction. It has long been recognized that symptoms of B toxicity may occur without yield reductions, for example for Faba beans (Brenchley and Warington, 1927) and for soybeans (Collings, 1927). Similar responses have since been reported for numerous crops including oats, bean, potato, tomato and cabbage (MacKay *et al.*, 1962), peas (Salinas *et al.*, 1981), linseed (Chauhan *et al.*, 1984), alfalfa, red clover and timothy (Gupta, 1984), tomato (Francois, 1984) and barley (Kluge and Podlesak, 1985). On the other hand, yield reductions, without symptom expression have been reported for oats and berseem (Bajwa and Singh, 1977), while 24 rice varieties suffered severe reductions in growth due to B toxicity but only 13 developed leaf symptoms (IRRI, 1979).

Eaton (1944) observed symptoms of B toxicity in 19 out of 72 plantings, representing 58 genotypes and 50 species, at or below B concentrations resulting in maximum growth. He attributed this to the distribution of B within the plant and noted that B may accumulate in the tips of lower leaves to such a concentration as to cause symptoms while the B supply to the actively growing tissue may be insufficient for maximum growth. This argument was expanded by Loneragan (1968) when comparing "critical nutrient concentrations", or the minimal concentration within the plant at maximal growth, and "functional nutrient requirement", or the minimal concentration of nutrient within the plant to sustain metabolic functions at rates which do not limit growth. The functional nutrient requirement is relatively constant and may be considerably lower than the critical nutrient concentration, especially for phloem immobile elements.

2.6.4 Critical concentrations of B in plants

Considerable discrepency exists within the literature for critical concentrations of B within plants, particularly for toxicity, but also for deficiency. While the following comments apply equally well to many other crops, discussion of critical concentrations is limited to toxicity of wheat and barley. Table 2.3 presents data on either defined critical levels of B in tissues, or yield and B values, as related to cultural conditions, tissue sampled and yield parameter. Reported critical values for B toxicity of wheat range from 0.6 mg kg^{-1} in whole plants at maturity (Sheik and Khanum, 1976) to more than 75 mg kg^{-1} in boot stage tissue (Gupta *et al.*, 1976) and more than 300mg kg^{-1} in grain (Chauhan and Powar, 1978), while for barley critical values range from 14 mg kg^{-1} in boot stage tissue (Gupta, 1972) to more than 195 mg kg^{-1} in six week old tissue (Manchanda and Yadav, 1978) and 768 mg kg^{-1} in the leaf below the flag leaf (Bingham *et al.*, 1985).

Possible reasons for the differences in critical concentrations of B for toxicity include:

(1) the phloem immobility of B which results in uneven distribution of B and in particular concentrating in "non-physiologically active regions" or older leaves and at the leaf tips;

(2) the strong influence of evapotranspiration on B uptake;

(3) differences in plant culture techniques;

(4) lack of uniformity of sampling procedures;

(5) loss of B from leaves due to leaching, either during growth for field grown plants or when rinsed prior to analysis;

(6) variation in analytical procedures and analytical error.

The first three factors interact strongly, for example differences in cultural techniques (glasshouse v field) will influence evapotranspiration which will affect B uptake.

There is little uniformity in sampling procedures for assessing the B status of plants, even among cereals. In their recent review, Keren and Bingham (1985) state "with small grains, the leaf below the flag-leaf at the time of early spike emergence is considered to be the appropriate leaf for chemical analysis of B." There is no reference to

experimental data to substantiate this point, and few other people have adopted this procedure, as evidenced by the array of tissues sampled listed in Table 2.3. Indeed, the procedure used by Bingham *et al.* (1987) for determining B status of wheat plants consisted of bulking the flag leaf and leaf below the flag leaf at early spike emergence. Kluge and Podlesak (1985) demonstrated different critical B concentrations, with respect to grain yield, between the youngest and older leaves of barley. For the whole shoot, a B concentration of 120-130 mg kg^{-1} was associated with a 10% grain yield reduction, however the concentration in individual leaves at this stage ranged from 55 mg kg^{-1} in the youngest expanded blade to 308 mg kg^{-1} in the fourth leaf.

The age of plants at sampling may influence derived critical values. The yield response to increasing applications of B varied for six week old plants as compared to plants at maturity (Manchanda and Yadav, 1978). A concentration of 275 mgB kg^{-1} in shoots at six weeks was associated with a yield reduction of plants at six weeks, but the yield of plants at the same treatment and grown to maturity was not reduced relative to the control plants. The contrasting response between sampling times for pot grown plants may result from the artificial cultural conditions with unlimited moisture and nutrients available to plants and redistribution of B within the soil due to leaching.

A considerable amount of research into B nutrition has been conducted by Gupta and co-workers at Prince Edward Island, Canada. Critical values for B toxicity and deficiency of cereals have been determined in both pots and the field using whole shoots at boot stage for B determinations. Critical values for toxicity of wheat and barley have generally been less than 20 mgB kg^{-1} in shoots (Gupta, 1971; Gupta, 1972; Gupta *et al.*, 1976; Gupta, 1977). There appears to be some reluctance in questioning the original critical value for toxicity, with respect to yield, of 16 mgB kg^{-1} for wheat and barley (Gupta, 1971). For example, Gupta *et al.* (1976) drew no attention to the fact that there was no reduction in grain yield associated with B concentrations in boot stage tissue in the range 9.4 - 35.6 mg kg^{-1} and 7.8 - 64.5 mg kg^{-1} for barley and wheat, respectively. Rather, they quote B toxicity values for symptom development of 14 and 16 mgB kg^{-1} for barley and wheat. In an even more extreme example there was no yield reduction of barley over the range of 56 - 219 mgB kg^{-1} in boot stage tissues (Gupta and MacLeod,

Table 2.3 Critical values or concentrations of B associated with a yield response for wheat and barley grown under different cultural conditions.

Culture	Tissue Sampled	Yield Determinant	[B] (mg kg^{-1}) v yield	Reference
Wheat				
Pot	boot stage shoot	grain	specified cv = 16	Gupta, 1971
			10% yield redn. 35	
		straw	10% yield redn. 35 - 86	
Pot	mature plants	whole plant	specified cv = 0.6	Sheik & Khanum, 1976
Pot	boot stage shoot	grain	no yield effect	Gupta *et al.*, 1976
			12.5 - 75.8	
Field	boot stage shoot	grain	Site 1 no yield effect 10.9	Gupta, 1977
			Site 2 no yield effect 14.9	
			Site 2 reduced yield 25.9	
			Site 3 reduced yield 12.4	
Pot	grain	total DM	no yield effect 33.6	Singh & Singh, 1976
Pot	3 m.o. shoots	straw	cv > 74	Chauhan & Powar, 1978
		grain	10% yield redn. 64 - 70	
	grain	grain	10% yield redn. 311 - 335	
Pot	leaf below flag	grain	specified cv = 55	Bingham *et al.*, 1985
Solution	35 d.o. shoots	shoot	not specified >200	Nable, 1988
Barley				
Pot	boot stage shoot	grain	specified cv = 16	Gupta, 1971
Pot	leaf below flag	grain	specified cv = 768	Bingham *et al.*, 1985
Pot	shoot Feekes 7-8	grain	cv = 120 - 130	Kluge & Podlesak, 1985
Pot	shoot 5 leaf	whole shoot	specified cv = 80	Davis *et al.*, 1978
Pot	boot stage shoots	grain	no yield redn. 9.4 - 35.6	Gupta *et al.*, 1976
Field	1 m.o shoots	straw, grain	10% yield redn. 50	Chauhan & Asthana, 1981
	3 m.o. shoots	straw, grain	10% yield redn. 89	
Field	shoots anthesis	grain	17% yield redn. 62.4	Cartwright *et al.*, 1984
Pot	6 w.o. shoots	6 w.o. shoots	no yield redn. 30 - 248	Gupta & MacLeod, 1981
Pot	boot stage shoots	grain	specified cv 50-70	Riley, 1987

d.o. = days old, w.o. = weeks old, m.o. = months old, DM = dry matter yield, cv = critical value

1981). In this paper there is no mention of critical values, either for symptom development or yield reductions. Moreover, the reviews of Gupta (1979) and Gupta *et al.* (1985) do not refer to the above two examples of "anomalous" results.

The contrasting "critical" values of B in plants associated with toxicity are probably a consequence of the immobility of B in the phloem which results in an uneven distribution of B, the intimate association between evapotranspiration and B uptake and the lack of uniformity of sampling procedures, alternative definitions of toxicity, different analytical procedures and possibly analytical error. In general it appears that critical values for B toxicity of wheat and barley derived in the higher transpiration environment of the glasshouse are greater than for field grown plants. A similar situation was reported for white clover by Parker and Gardner (1982), who questioned the use of critical values for B toxicity derived in the glasshouse for determining B toxicity in the field, while de Vries (1980) questioned the extrapolation of all glasshouse results to the field. It is most probable that if critical values for B toxicity can be derived in the field they will also differ between environments. Calibration data will therefore be required before the extent of B toxicity in southern Australia can be mapped.

2.7 INTERSPECIFIC VARIATION IN RESPONSE TO BORON.

A large range in B requirement and in the tolerance to high concentrations of B in the growth medium has been identified between plant species. Differences in B requirement were first recognized by Brenchley and Warington (1927) when assessing the essential role of B for a number of species. B proved essential to complete the life cycle for soybean, scarlet runner bean, several clovers, melon, broad bean and lucerne, whereas under the same conditions peas, barley and candytuft were able to develop to maturity. The difference in essentiality of B, between species, was probably a consequence of the degree of deficiency achieved in the experiments rather than some species not requiring B.

Extensive studies of the tolerance to high concentrations of B have been conducted for fruit trees (Haas, 1929), for more than 50 species representing most

classes of commercially grown plants (Eaton, 1935; Eaton, 1944) for 28 species of vegetables and cereals (Purvis and Hanna, 1938) and for 25 ornamentals (Francois and Clark, 1979). In general each species was represented by a single variety and on the basis of the response of the chosen variety, species have been assigned to arbitrary categories such as sensitive, semitolerant and tolerant to high concentrations of B. In view of the intraspecific variation in response to B which has been recognized (see Section 2.8) the generalisation of the species on the basis of response of a single variety is invalid and is undoubtedly one of the reasons for some conflict in the literature as to the tolerance of particular species.

There is little association between the concentration of B in leaves and the B tolerance rating over a diverse set of species (Eaton, 1944; Francois and Clark, 1979), therefore the more tolerant species do not necessarily have the lowest concentrations of B in tissues. For more closely related species, however, there is evidence that tolerance to high concentrations of B is associated with lower concentrations of B in leaves or shoots. Jerusalem artichoke, (*Helianthus tuberosus*) is considerably more sensitive to B, and has higher concentrations of B in leaves, than sunflower (*Helianthus annuus*), while the lemon (*Citrus limonin*) is more sensitive to B, and accumulates more B in leaves than the Chinese box orange (*Severinia buxifolia*). For both comparisons the response to B is related to B accumulation in shoots, which is under the control of the rootstock, and may be modified by reciprocal grafting (Eaton and Blair, 1935) (Section 2.5). Similarly, the cultivated tomato (*Lycopersicon esculentum*) is more sensitive and accumulates more B in shoots than the wild relative *L.cheesmanii* (Toledo and Spurr, 1984).

Oertli and Kohl (1961) analysed green, chlorotic and necrotic leaf tissue for 29 species grown in sand culture at a high concentration of B. The concentration of B in leaf tissue associated with chlorosis and necrosis was of the same order for all species, with a concentration in chlorotic tissue of approximately 1000 mgB kg^{-1} and in necrotic tissue of greater than 1500 mgB kg^{-1}. These values are similar to those reported by Eaton (1935) to be associated with chlorosis and necrosis of lemon leaves. The differences between species in the development of toxicity symptoms were attributed to differences in the rate of uptake or local accumulation of B, rather than the tolerance of tissues to B.

There is a general trend for species more tolerant to high concentrations of B to also have a higher B requirement, or be more prone to B deficiency. In a series of experiments assessing the response to B for more than 50 species, all plants classified as sensitive, and most classified as semi-tolerant produced maximum yields at either the trace or one mgB kg^{-1} treatment in sand culture. Plants classified as tolerant, by virtue of their ability to grow at the 15 and 25 mgB kg^{-1} treatments, produced maximum yields at either the 5, 10 or 15 mgB kg^{-1} treatments and had significantly reduced yield at the lowest B treatments (Eaton, 1944). Similar relationships for a smaller number of species include : tobacco most tolerant of five crops to B toxicity but also had the highest B requirement (Gandhi and Mehta, 1959), sugar beet more tolerant than cotton and soybean to high B, however yields reversed at low B supply (Oertli and Roth, 1969), and response of three *Eucalyptus* species reversed between low and high B supply (Malavolta *et al.*, 1978).

In contrast to the above results, Davies (1980) stated that plants with a high B requirement do not necessarily have high tolerance. The examples of lucerne and cabbage having a high B requirement but being classified as only semi-tolerant by Bradford (1966) were among others presented in support of this argument. On the other hand, lucerne was classified as tolerant to B by Eaton (1935) and cabbage as tolerant by both Eaton (1935) and Purvis and Hanna (1938). The variation in classification between Bradford (1966) and Eaton (1935) and Purvis and Hanna (1938) may have arisen from comparisons of different varieties within the species.

There have been no experiments conducted to specifically test for physiological and genetic independence of responses to low and high B supply. In the absence of such evidence it should be realised that when breeding wheat varieties for soils with high B concentrations there might be the possibility that the most tolerant varieties are not well adapted to soils with low B supply.

Wheat and barley were classified as semi-tolerant to B by Eaton (1935), however Bingham *et al.* (1985) classified wheat as sensitive and barley moderately tolerant on the basis of grain yield in relation to B concentration in nutrient solution. The conflicting classification may result from either different varieties chosen to represent the

two species, or a large variation in yield results of wheat at the low B treatments (Bingham *et al.*, 1985) which would have had a significant effect on determining the threshold level for toxicity. While barley had a greater apparent threshold for B toxicity, than wheat, the reduction in yield per unit increase of B in nutrient solution was greater for barley (Bingham *et al.*, 1985).

2.8 INTRASPECIFIC VARIATION AND GENETIC CONTROL OF RESPONSE TO BORON

Intraspecific variation in response to B nutrition has been described for a number of species, including *Triticum aestivum*, and the genetic control of response has also been determined in several cases.

2.8.1 Intraspecific variation

The response to applied B differed between wheat varieties in the low B regions of North Bihar and West Bengal, India. The local (unclassified) variety and Sonalika outyielded other varieties in the absence of applied B, while the other varieties showed a much greater response with respect to grain yield than the local variety and Sonalika (Singh *et al.*, 1976; Chatterjee *et al.*, 1979; Ganguly, 1979). No visible manifestation of B deficiency was observed during the vegetative stage, and the difference in yield between Sonalika and Janak (high B requirement) could be attributed to differences in seed set (Ganguly, 1979). The local variety and Sonalika were therefore adapted to soils with low B status and less sensitive to B deficiency. The poor seed set was probably the result of atrophy of anthers or unviable pollen, as discussed in Section 2.3. The most probable explanation for the difference in response, between varieties, would be that they differ in efficiency of uptake of B from soil; alternatively there may be genetic differences in the tolerance of anthers to low concentrations of B.

High concentrations of B have been reported in the irrigation water in regions of India, e.g. Punjab (Singh and Kanwar, 1963) and Rajasthan (Mather *et al.*, 1964). Due to the potential of B toxicity, Mehrotra *et al.* (1980) studied the genetic variation of Indian

wheats to high concentrations of B. Genotypes varied in response to increasing concentrations of applied B with respect to height, tiller number, ears, spikelets on the main ear, grains on the main ear, grain weight on the main ear, 1000 grain weight, grain yield per plant and B content in leaves. An index of growth and yield was determined by expressing the yield at the highest B treatment as a percentage of the maximum yield for each parameter. The indices for each genotype were averaged to give an overall measure of tolerance to B. The number of grains on the main ear was identified as the most appropriate selection criterion for breeding B tolerant wheat varieties. B concentrations in leaves (leaves analysed not specified) increased with the B treatments, however no comment was made upon the differences in B concentrations; between genotypes. From the data presented it is possible to calculate that the correlations between the mean tolerance indices and the B concentration in leaves for the two, four and six mgB l^{-1} treatments were $r = -0.71$, $r = -0.71$ and $r = -0.76$, respectively. Thus, the B concentration in leaves was lowest for the most tolerant genotypes and this difference was manifested at all treatments.

Genetic variation in response to high concentrations of B has also been described for barley (Christensen, 1934), strawberries (Blatt, 1976) and rice (Ponnamperuma *et al.*, 1979; Cayton, 1985). The severity of symptom expression for barley differed between genotypes (Christensen, 1934), but as yield measurements in the presence and absence of B were not determined it is not possible to conclude with certainty that the genotypes differed in tolerance to B.

High concentrations of B occur in the ground water used for irrigation at the International Rice Research Institute, Phillipines. The resulting B toxicity has prompted studies on genetic variation in tolerance to B (Ponnamperuma *et al.*, 1979; Cayton, 1985). The typical symptoms of B toxicity for rice consist of necrotic spots developing from the leaf tips, however some varieties did not develop spots but rather they developed chlorotic and necrotic leaf tips and margins. The number and size of spots, or severity of foliar symptoms, was not a good index of susceptibility to B toxicity, as measured by yield response (Cayton, 1985).

Three strawberry varieties were compared for response to increasing concentrations of B and P. Even though the P treatments had a significant interaction with B treatments, the relative responses of the three varieties to increasing B were the same for all levels of P. The variety "Midway" was the most sensitive to high concentrations of B and had the highest concentrations of B in tissues at all B treatments. The tolerant variety "Redcoat" had the lowest B concentrations in shoots and was the most susceptible to B deficiency (Blatt, 1976). This is another example of a B tolerant plant also being more susceptible to B deficiency, as discussed for interspecific variation in response to B. Again the mechanism of tolerance is reduced B accumulation in shoots.

2.8.2 Inheritance of response to B

Heritability studies for concentration of elements, including B, in plant tissues have been conducted for corn (Gorsline *et al.*, 1964, 1968), alfalfa (Hill and Jung, 1975) and sunflower (Blamey *et al.*, 1984). For sunflower, six genotypes known to differ in response to low concentrations of B (Blamey *et al.*, 1980) were studied in a half diallel analysis. The concentration of B in the uppermost leaf was highest for varieties and offspring least susceptible to B deficiency. General combining ability effects were highly significant for the concentration of B in the uppermost leaf, both in the presence and absence of B, for deficiency symptoms in the absence of B, and for grain yield. Specific combining ability effects were non-significant for B concentration in leaves, B deficiency symptoms and grain yield in the presence of applied B. The three characters were highly heritable and additive or additive epistatic gene action predominated. Responses of the offspring to low B conditions could therefore be predicted from the performance of the parents (Blamey *et al.*, 1984).

The inheritance studies for corn (Gorsline *et al.*, 1964, 1968) and lucerne (Hill and Jung, 1975) involved multi-element analysis, including B, but the parents were not chosen to necessarily differ in concentration of B in tissues. Nevertheless, significant differences in the concentration of B in tissues occurred between parents, and between offspring and parents. The concentration of B in shoots of lucerne and in the ear leaf of corn were under the control of additive gene action. B concentration in the grain of corn

was also determined but was not correlated to B concentration in the ear leaf (Gorsline *et al.*, 1964). Correlations between concentrations of all elements in lucerne shoots were calculated and there were no significant genotypic correlations between B and other elements (Hill and Jung, 1975).

Response to B deficiency is under monogenic control for celery (Pope and Munger, 1953), tomato (Wall and Andrus, 1962) and red beet (Tehrani *et al.*, 1971). B deficiency of celery results in a characteristic cracked stem for susceptible varieties. The F_1 derived from a cross between normal and susceptible parents was normal while a ratio of 3 normal : 1 susceptible plants was obtained for the F_2 generation when grown under low B conditions. Susceptibility to B deficiency was therefore under the control of a single recessive gene (Pope and Munger, 1953). No information is provided as to whether the susceptible parent had a lower rate of uptake of B, or a higher B requirement within the shoots.

Considerable variation in the tolerance of red beet (*Beta vulgaris*) to B deficiency was described by Kelly and Gabelman (1960). On the basis of the apparently continuous array of tolerance they concluded that the genetic system controlling the response was probably complex. However, Tehrani *et al.* (1971) identified a single gene controlling response to low B between the sensitive line Czerma and the tolerant W163-8. All F_1's were susceptible, the F_2 segregated 3 susceptible : 1 tolerant and the BC_1 to the tolerant parent segregated 1 susceptible : 1 tolerant. Susceptibility to B deficiency is therefore under the control of a single dominant gene for this particular cross. The B concentrations in leaves of tolerant plants were greater than for susceptible plants. The large variation described by Kelly and Gableman (1960) may have arisen from either multiple alleles with different effect at the one locus, or multiple loci.

The brittle stem condition of the mutant tomato line T3238 has been described in Section 2.5 on B uptake and distribution. Genetic studies on the expression of brittle stem, with the two genotypes T3238 (B inefficient) and Rutgers (B efficient) demonstrated the inefficient condition is under the control of a single recessive gene (Wall and Andrus, 1962). A second sublethal mutant resulting in inefficient transport of Fe from shoots to roots has also been identified in T3238 (Brown *et al.*, 1971). The

mechanisms controlling the inefficient transport of Fe and B were found to be genetically independent (Wann and Hills, 1973).

The genetic studies described have shown that B uptake, or concentration in shoots, is a highly heritable character, and for tomato (Wall and Andrus, 1962) and beet (Tehrani *et al.*, 1971) monogenic inheritance has been identified. There have been no genetic studies conducted on the tolerance of plants to high concentrations of B, but in view of the apparent relationship between responses to low and high concentrations of B and the fact that among related species tolerance to high concentrations of B is generally associated with lower concentrations of B in shoots, it is probable that genetic control of tolerance to high concentrations of B is also a highly heritable character.

Chapter 3.

MATERIALS AND METHODS - GENERAL PROCEDURES

3.1 FIELD EXPERIMENTS

3.1.1 Experimental sites

The principal location for field experiments was Two Wells (Lat. 34°35'S, Lon. 138°30'E) approximately 40 km N of Adelaide, on the property of Messrs. N and J. Sharpe. The soil at this site was classified by Dr. B. Cartwright of the CSIRO Division of Soils, Adelaide, as a Typic Natrixeralf, clayey, mixed (calcareous), thermic (Soil Survey Staff, 1975). Naturally occurring high concentrations of B are found in the subsoil and typical profiles are presented in Table 3.1.

Experiments were conducted at Two Wells in all years for the period 1985 - 1989. Further experiments were conducted at Rudall (farm of Mr. J. Norris), Minnipa (a research station of the South Australian Department of Agriculture), Urrbrae (Waite Agricultural Research Institute), Windsor (farm of Mr. R. Earle) and Walpeup (a research station of the Victorian Department of Agricultural and Rural Affairs). The distributions of B in representative soil profiles for all sites except Walpeup, kindly provided by Mr. D. Moody, are also included in Table 3.1. The location of all experimental sites is indicated in Figure 3.1, while monthly rainfall data for all relevant seasons is presented in Appendix A.

The Two Wells site was chosen specifically for the high boron concentrations in the subsoil, while the other sites, with the exception of the Waite Institute, were chosen as they represent physical and biological conditions typical of large areas in South Australia and western Victoria and are used as selection sites by the Waite Agricultural Research Institute wheat breeding program. Private farms are used extensively as selection sites because yield results obtained from such sites are, in general, a better predictor of the regional performance of varieties than results from research stations (Rathjen and Pederson, 1986).

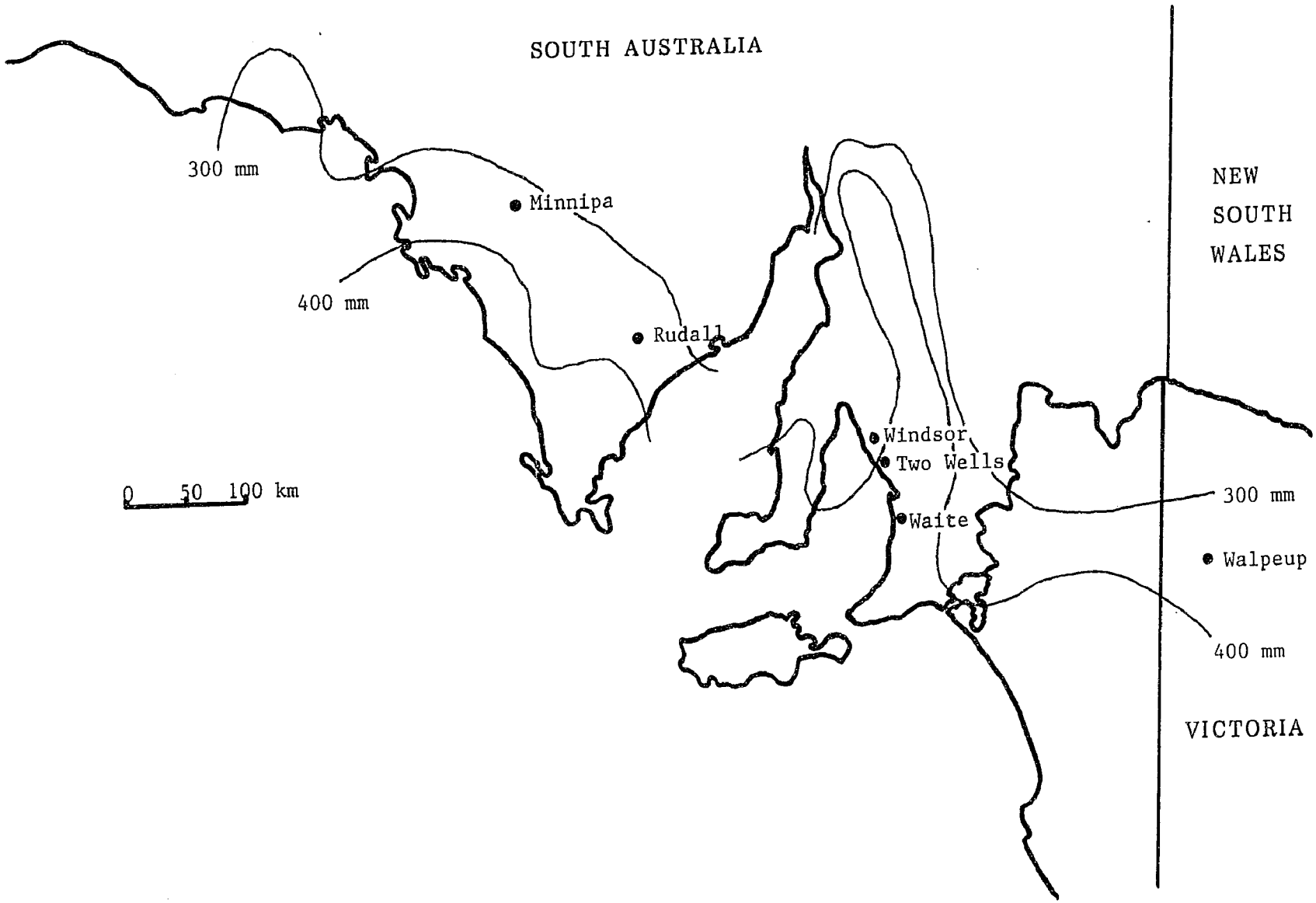
Table 3.1 Examples of the concentration of extractable B in the soil profile for the field trial sites. Data for Two Wells and Waite 1983 from Cartwright *et al.* (1987) and other results provided by D.B. Moody. All B extractions by the Mannitol-$CaCl_2$ method (Cartwright *et al.*, 1983).

Depth (cm)	Extractable B (mg kg-1)				
	Two Wells				
	1983	1987 P-1[a]	1987 P-2	1987 P-3	1988
0-10	3.6	1.8	2.5	2.3	12.3
10-20	52.8	7.2	10.7	13.7	40.0
20-30	104.4	48.1	34.6	39.5	62.4
30-40	101.1	70.6	41.9	43.8	63.1
40-50	82.5	50.4	34.9	36.0	49.0
50-60	67.4	48.1	35.4	47.7	57.8
60-70	57.3	56.6	47.7	45.9	71.6
70-80	51.2	54.2	44.7	38.8	63.8
80-90	45.3	46.4	35.7	31.2	39.3
90-100	42.9	34.7	23.9	27.6	27.3

	Rudall			Minnipa		Windsor		Waite	
	1985	1986 P-1	1986 P-2	1988 P-1	1988 P-2	1987 P-1	1987 P-2	1983	1987
0-10	1.2	2.7	2.0	2.5	1.7	1.3	1.0	1.1	0.8
10-20	2.1	3.6	3.5	2.0	1.6	1.1	1.3	-	0.9
20-30	3.0	3.7	6.8	1.9	1.8	1.0	1.5	1.3	-
30-40	-	4.5	16.0	4.4	1.6	0.9	1.7	1.2	1.0
40-50	4.0	7.7	-	15.2	2.7	0.8	1.9	1.3	1.7
50-60	-	15.3	30.1	29.9	7.4	0.8	2.9	1.2	-
60-70	20.8	24.0	30.3	35.5	15.9	0.8	9.5	1.2	1.9
70-80	17.7	29.0	28.4	36.1	29.2	0.7	17.7	1.2	2.4
80-90	19.2	-	26.7	34.5	33.5	0.7	20.7	-	-
90-100	17.5	-	26.0	27.4	34.6	0.9	22.1	1.3	-

[a] P-1 = profile 1

Figure 3.1 Location of field experimental sites. The 300 and 400 mm isohyets are indicated.


SOUTH AUSTRALIA
300 mm
Minnipa
400 mm
Rudall
0 50 100 km
NEW
SOUTH
WALES
Windsor
Two Wells
300 mm
Waite
Walpeup
400 mm
VICTORIA

3.1.2 Experimental design

Field experiments were conducted in conjunction with experiments of the Waite Agricultural Research Institute wheat and barley breeding programs and therefore were sown to the same layout and at the same time.

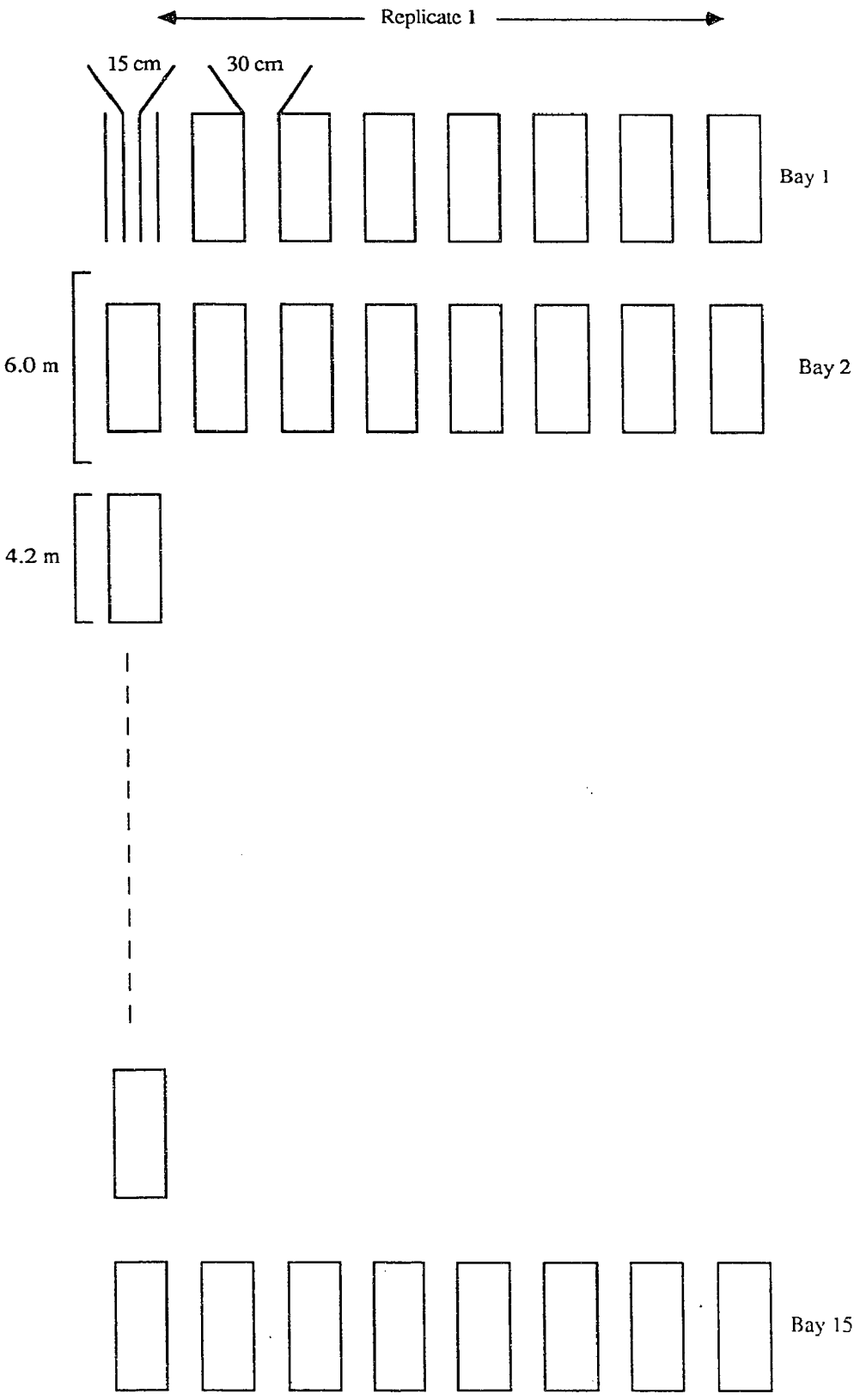
The standard field arrangement (Fig 3.2) consisted of 15 bays, each 6m from the midpoint of adjacent pathways so that the experimental site was 90m wide and entries in experiments were arranged by multiples of 15. Pathways of 1.8m were cut between bays to allow for spraying operations and the automatic cleaning of harvesters between plots to prevent contamination of samples. Individual plots were arranged within bays and consisted of four drill rows 15 cm apart. Plots were separated by one missing row, or 30cm. The total length of each plot was 4.2m and the sown area was 4.2m x 60cm, or 2.52m^2. Plots were sown at the rate of 30 g plot $^{-1}$ (sown over the 6m length) or approximately 60 kg ha^{-1}. The plots were sown by a modified 14 row drill and three plots were sown simultaneously.

An alternative planting arrangement was also utilized and consisted of short plots, each 1.2m long with 0.3m between bays. These plots were also four drill rows wide with 15 cm between rows. Short plots were used for early generation experiments when insufficient seed was available to allow full yield plots to be sown. Short plots were generally sown at a rate of 8 g $plot^{-1}$ but varied according to seed supply.

The management of the field experiments, including cultivation methods, was in accordance to the local district practices and the previous crops for the paddocks in which experiments were conducted varied according to the rotations for the individual farms and research stations. Topfos® double strength superphosphate was applied to all experiments at the rate of 100 kg ha^{-1} at seeding. Herbicides applied for weed control included Hoegrass® (diclofop-methyl 375g ai l^{-1}) at the rate of 1.0 l ha^{-1} for rye grass and 1.5 l ha^{-1} for wild oats, Buctril M® (bromoxynil 200g ai l^{-1}) at the rate of 1.0 - 1.4 l ha^{-1}, Ally® (metsulfuron-methyl 600g ai kg^{-1}) at 7 g ha^{-1} and Ally + MCPA at 6 g Ally + 100 ml MCPA ha^{-1} for control of broad leaf weeds.

Grain of individual plots was harvested at maturity, using harvesters designed and built at the Waite Agricultural Research Institute.

Figure 3.2 Arrangement and dimensions of plots used for field experiments.


Replicate 1
15 cm
30 cm
Bay 1
6.0 m
Bay 2
4.2 m
Bay 15

3.2 GLASSHOUSE EXPERIMENTS

3.2.1 Soil

The soil used for glasshouse experiments was a bulk surface sample (0 - 15 cm) obtained from paddocks W6 or W7 at the Glenthorne Research Farm, O'Halloran Hill, South Australia. The hot $CaCl_2$ extractable B concentration (Aitken *et al.*, 1987) of this soil was 2.3 mg kg^{-1} and as such was not likely to contribute significantly to the effect of applied B. The soil was a Typic Haploxeralf (Soil Survey Staff, 1975) and the chemical and physical properties for samples collected in 1985 and 1986 are presented in Table 3.2.

As outlined in the review of literature, when B is applied to soil, a portion is adsorbed and therefore unavailable to plants. The amount adsorbed varies with soil properties such as texture, pH, organic matter and clay type. The soil used for the initial experiments was of the same origin (paddock W7) as that used by Paull (1985). During the course of the project it became necessary to obtain soil from an alternative paddock at Glenthorne. Prior to collecting a bulk sample from the new source (paddock W6), the surface soil was sampled and the B adsorption characteristics were compared with the original soil (paddock W7) and the soil used by Rathjen (1987) (obtained from paddock C3) by the method of Elrashidi and O'Connor (1982). The soil samples are referred to as Glenthorne I (GI) for the original soil of paddock W7, GII for paddock C3 and GIII for the alternative source from paddock W6.

Twenty cm^3 of 0.01M $CaCl_2$ containing B, as $B(OH)_3$, at concentrations as indicated in Table 3.3, were added to 50 cm^3 centrifuge tubes containing 20 g of air dry soil. The tubes were capped, shaken end over end for 23 hours and then centrifuged at 3000 revs min^{-1} and 20°C for five min . The extract was filtered through Whatman No 42 filter paper into 15 cm^{-1} polycarbonate tubes. The concentration of B in the filtrate was determined by inductively coupled plasma (ICP) - spectrometry. The concentration of B adsorbed by the soil was equal to the difference between the B concentration of the standard solution and the concentration of the filtrate. Each treatment was duplicated. The B adsorption characteristics of the new source (paddock W6) were identical to the original

Table 3.2 Chemical and physical properties of two samples of Glenthorne I soil obtained from paddock W6 in 1985 and 1986. Analyses were conducted by CSIRO Division of Soils Routine Analysis Service.

Property	Extraction/Reference	1985	1986
pH	1 : 5 soil : H_2O	7.4	7.6
E.C. dS / m	1 : 5 soil : H_2O	0.25	0.21
Cl mg / kg	1 : 5 soil : H_2O	67.0	180.0
C (total) %	(Merry and Spouncer, 1988)	4.5	2.8
N (total) %	(McLeod, 1982)	0.27	0.23
P ($NaHCO_3$ extr) mg / kg	(McLeod,1982)	144	60
Exchangeable cations pH 8.5	(Tucker, 1974; McLeod and Zarcinas, 1976)		
Ca (mmol (+) / kg)		199	164
Mg		25.1	16.7
Na		4.7	2.6
K		28.6	20.8
Total		257	204
C.E.C. (NH_4)		281	243
Texture (%)	(Hutton, 1955)		
Clay		34	35
Silt		24	23
Sand - fine		37	6
Sand - coarse		5	32

soil (paddock W7) (Table 3.3). It was therefore considered a suitable soil and the range of B treatments originally adopted still suitable. The Glenthorne II soil adsorbed much less B than the other two samples and this is consistent with the results of Rathjen (1987) and Moody (unpublished). The percentage of applied B which was adsorbed by the soils decreased as the applied concentration increased. Paull (1985) and Paull *et al* (1988) reported little decrease in the concentration of extractable B for B amended Glenthorne I soil when stored in polythene bags for six months.

The Glenthorne I soil was used for experiments of Chapters 4 and 5 and the Glenthorne III soil for experiments of Chapters 6 and 7.

3.2.2 Application of B to soil

The bulk soil sample was sieved to pass a five mm screen. A weighed amount of air dry soil was placed in a soil tumbler with a capacity of approximately 500kg. The soil was continuously mixed as the B solution, as $B(OH)_3$ was slowly poured over it. The uniformity of mixing by this method was determined by taking five random samples from mixtures of both 50 and 100 mg kg^{-1} applied B. The B concentrations of the samples were determined by mannitol $CaCl_2$ extraction (Cartwright *et al.* 1983) and were;

50 mg kg^{-1} applied B : mean 22.04, s.d. 1.42, min 19.93, max 23.51 mgB kg^{-1}

100 mg kg^{-1} applied B : mean 64.32, s.d. 1.76, min 61.25, max 65.49 mgB kg^{-1}.

Boron treatments are designated B0, B25, B50, B100 etc. where these treatments are nil, 25, 50 and 100 mg kg^{-1} applied B.

3.2.3 Culture of plants

Experiments consisted of two types (1) comparing a limited number of genotypes at a range of B treatments (i.e. factorial experiments) and (2) mass screening of plants at a single level of B. The cultural conditions varied according to the types of experiments and the former were grown in pots while the latter were grown in large boxes or trays.

Table 3.3 B adsorption characteristics of the Glenthorne soils used for pot experiments. The concentration of B in the filtrate is expressed with respect to the soil and is an approximate measure of the relative amount that would be readily available to plants. Glenthorne I and III were used for experiments described in this thesis while Glenthorne II was used by Rathjen (1987). Values are the means of two samples.

Applied B ($mg\ kg^{-1}$)	B conc in filtrate ($mg\ kg^{-1}$)		
	Glenthorne I Paddock W7	Glenthorne II Paddock C3	Glenthorne III Paddock W6
0	0.16	0.13	0.07
12.5	3.01	8.05	3.00
25	7.69	17.59	7.79
50	20.58	39.34	20.95
75	34.84	60.48	33.74
100	52.25	84.63	50.25
125	67.03	108.70	67.53
150	87.25	131.28	87.33
200	123.50	179.83	123.00

Pot experiments

B amended soil was weighed into plastic pots lined with polythene bags to prevent leaching. The pot size varied according to the number of plants to be grown and the duration of the experiment. The pots were watered to weight with distilled water. The soil was maintained between 50and70% of field capacity (46% for G I and 35% for G III).

Prior to sowing, seeds were imbibed on moist filter paper in plastic petri dishes at 2-4° C for 48 hours and then at 16-18° C for 24 hours. The cold pre-treatment was found to result in uniform germination. At the time of sowing the swollen embryo had ruptured the testa but the radicle had not begun to elongate. Thus there was minimal disturbance to the developing plant. Excess seeds were sown per pot and thinned to a common number of uniform plants after approximately two weeks.

All experiments were conducted in glasshouses without supplementary lighting or heating. During the summer months of November to March the glasshouses were covered with 70% shadecloth which, together with evaporative air conditioners, maintained daytime soil temperature between 25 and 28° C.

Mass screening

When screening a large number of genotypes or segregating generations, plants were grown at a high density in either wooden boxes or plastic trays containing B enriched soil. Initially, wooden boxes of dimensions 1400mm x 800mm x 300 mm deep were used but later non-draining plastic trays of dimensions 400mm x 285mm x 120 mm and containing 10.5 kg air dry soil were adopted. The plastic trays were preferred as they required less soil per plant and they could be moved and so allow re-randomization during the course of an experiment. Examples of the two systems are given in Plate 2.

Pre-germinated seeds were sown at a spacing of either 5cm x 5cm or 5 cm x 3.5 cm at a depth of 1.0 - 1.5 cm. The plants were watered with distilled water when the soil surface dried. The frequency of watering varied with the season and age of plants. During the period October to March plants were generally watered daily and required 300ml $tray^{-1}$. Plants were assessed and harvested as indicated for individual experiments.

Plate 2. Procedures used for mass screening of varieties and for assessing segregating generations.

(a) A large box containing B amended soil. Each marker indicates a plot.

(b) A plastic tray containing B amended soil used for assessing segregating generations. The markers within the tray indicate the grid of parents and the markers at the back indicate individual F3 families. In the foreground, fourth plant from the left is a (Wl*MMC) parent displaying the "mid-leaf necrosis" symptom described in Chapter 4. Two weeks after sowing.

(c) Arrangement of plastic trays in the glasshouse. Trays in the foreground and at right 5 days after sowing and in the background three weeks after sowing.

(a)

(b)

(c)

The soil used in the plastic trays and for pot experiments was recycled. Used soil was sieved to pass a five mm screen to remove roots. The soil was then raked over on a concrete floor to mix soil from different trays and pots and to redistribute B which may have been leached through the soil profile.

3.3 CHEMICAL ANALYSES

3.3.1 Soil

The concentration of extractable B in soil samples obtained from the field was determined by the $CaCl_2$ - mannitol method of Cartwright *et al.* (1983). Twenty cm^3 of 0.01 M $CaCl_2$ + 0.05 M mannitol (pH 8.5) was added to 10 g of sieved soil in a 50 cm^3 polypropylene centrifuge tube. Sealed tubes were shaken end over end for 1 hr in a shaker rotating at 11 revolutions per minute. The suspensions were centrifuged at 3500 revs min^{-1} and the extracts filtered through Whatman No 42 filter paper into 15 cm^3 polycarbonate tubes. Samples were stored at 1 - 2°C until analysis by ICP-spectrometry.

This method, originally developed for alkaline soils, is no longer used by Cartwright and co-workers. It has been replaced by an extraction in hot $CaCl_2$ which has proved to be applicable for both alkaline and acidic soils (Cartwright, pers. comm.).

3.3.2 Plant material

Plant tissues and grain were digested by nitric acid (Zarcinas *et al.*, 1987) and the concentrations of B and 13 other elements were measured by ICP - spectrometry. Tissue samples harvested from both field and pot experiments were rinsed with de-ionized water, placed in paper bags and dried in a forced draught oven at 85° C for 48 hours. Samples of greater than one gram were ground in a stainless steel mill to pass a one mm screen. Zarcinas *et al.* (1987) found no variation in analytical values of B between ground and whole wheat grain, therefore samples of grain were digested without grinding. Analytical standards were included in each set of digests to allow detection of systematic variation within each set and to allow comparisons between sets.

Immediately prior to weighing for digestion, samples were oven dried for two hours at 60 - 80°C. One gram subsamples, or entire samples if less than one gram, were placed in aged 75 cm^3 pyrex tubes. Ten cm^3 70% HNO_3 (A.R. grade) was added and the tubes were allowed to stand overnight at room temperature in a Tecator Digestion System 40. The temperature was raised to 120° C for two hours and then to 140° C and maintained at this temperature until about 1 cm^3 of acid remained. After cooling, the samples were diluted to 20 ml with 1% V/V nitric acid. The digests of plant samples were filtered through Whatman No. 54 filter papers to remove amorphous siliceous residue and the grain digests were decanted directly into 15 ml polycarbonate tubes. Samples were stored at 1 - 2° C until analysis by ICP - spectrometry.

The concentations of 14 elements (Al, B, Ca, Co, Cu, Fe, K, Mg, Mn, Mo, Na, P, S and Zn) were simultaneously determined by a Labtest V-25 Inductively Coupled Plasma - Optical Emission Spectrometer (Zarcinas and Cartwright, 1983).

3.4 CYTOLOGICAL METHODS

The chromosome complements of pollen mother cells (PMCs) and of root tip meristematic cells were determined during monosomic analysis (Chapter 7). To determine chromosome pairing, PMCs were examined at metaphase I. Spikes at the early boot stage were collected and a single anther from a floret was squashed in aceto-orcein stain and examined microscopically to determine the stage of cell division. When an anther at 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.

The chromosome number of meristematic cells of the root tips was determined by collecting the tip 1cm from rapidly growing seedlings and counting the chromosomes of cells at the metaphase stage of mitotic division. To ensure rapid growth and therefore many dividing cells, seeds were imbibed on moistened filter paper in petri dishes at 2-4°C

for 48 hours and then transferred to an incubator at 24°C for 22 hours. Root tips were removed and place in water in vials which were stored in ice in a refrigerator for 24 hours. The root tips were then fixed in 3 absolute ethanol : 1 glacial acetic acid for 24 hours at 2-4°C, hydrolysed in 1N HCl at 60°C for 12 minutes and stained with Feulgen stain for 1-2 hours at room temperature. The stained root tips were placed in water and refrigerated at 2-4°C for 1-2 hours and then placed on a slide in 45% acetic acid and the meristematic region was teased apart to free the dividing cells. The excess root material was removed and the cells squashed and examined microscopically.

Chapter 4.

RESPONSE TO BORON FOR GENOTYPES OF DIFFERING TOLERANCE AND IDENTIFICATION OF SELECTION PARAMETERS

4.1 INTRODUCTION

Information on the detailed responses of wheat genotypes differing in tolerance to high concentrations of B is limited, particularly when comparing between the glasshouse environment and naturally occurring high concentrations of B in the field. As glasshouse studies are aimed at predicting field responses it is imperative to determine which characters are consistent with the field response.

This project investigates the response to high concentrations of B at two genotypic levels, namely for homozygous varieties and segregating generations. For the former, replicated factorial experiments may be conducted, but for the latter, response must be determined on a single plant basis. It is therefore necessary to identify plant responses which could accurately and efficiently be applied to individual plants of a segregating generation. Efficiency of selection would be increased by identifying characteristics in early growth stages correlated with the mature plant response to B.

Previous glasshouse experiments comparing response to high concentrations of B, between genotypes, have evaluated mature plants with little or no information on response at the vegetative stage (Mehrotra *et al.*, 1980; Paull, 1985; Paull *et al.*, 1988) and only limited comparison to field response (Paull, 1985; Paull *et al.*, 1988). The most pertinent findings are that more tolerant wheat genotypes accumulate less B in their shoots in the glasshouse (Paull, 1985; Paull *et al.*, 1988), while for a field experiment at a site with high concentrations of B in the sub-soil, the highest yielding genotypes were those with the lowest concentration of B in grain (Cartwright *et al.*, 1987). As outlined in the review of literature, numerous sampling procedures have been employed when determining the concentration of B in wheat tissues, but there is no information on the most appropriate procedure for comparisons between genotypes.

A glasshouse pot experiment and a field experiment at a site with high concentrations of B in the sub-soil were conducted to provide more detailed information on the response to B. The genotypes used for these experiments had previously been found to differ in response to B, either at maturity in pots (Paull, 1985) or in the field (Cartwright *et al.*, 1987).

The aims of the pot experiment were :

(1) assessing growth response at the vegetative stage in relation to response at maturity,

(2) identifying appropriate tissue sampling procedures for comparisons between genotypes.

Growth responses were measured both destructively and non-destructively as the latter would be more appropriate for progeny testing in genetic studies.

The location of high concentrations of B in the sub-soil precludes field experiments with high and low B treatments in close proximity. In the absence of a control treatment it is not directly possible to attribute differences in growth, between genotypes, to B. The field experiment was therefore conducted for the purpose of comparing alternative tissue sampling procedures for their ability to distinguish between genotypes differing in B accumulation.

These detailed experiments were conducted for a limited number of genotypes with the expectation that the responses identified would be useful for comparing a greater number and range of genotypes in later experiments.

4.2 MATERIALS AND METHODS

4.2.1. Genotypes

Genotype	Response to B	Reference
Halberd	Moderately tolerant	Paull (1985), Cartwright *et al.* (1987)
Olympic	Moderately tolerant	Paull (1985)
Warigal	Moderately sensitive	Cartwright *et al.* (1987)
Bindawarra	Moderately sensitive	Paull (1985), Cartwright *et al.* (1987)
(Wl*MMC)/W1/10	Sensitive	Paull (1985)

4.2.2 Pot Experiment

The three genotypes, Halberd, Warigal and (Wl*MMC), were grown at three B treatments and harvested at three stages of development to compare their responses during vegetative growth with that at maturity.

B treatments

B0, B20 and B60 soil, as described in Chapter 3, was weighed into 100 mm diameter non-draining pots (900 g pot^{-1})

Experimental design

The pots of the three genotypes, B treatments and stages of harvest, combined factorially, were arranged as a randomized complete block design with six replicates. Pots were labelled at the commencement of the experiment to denote the stage of development at which they were to be harvested. Two seeds were sown per pot and thinned to one plant after two weeks. Harvests were undertaken when the plants attained specific growth stages rather than on the same day, to allow analysis of tissues at a common stage of development. The stages of growth at which plants were harvested were :

(1) first node visible (Zadoks 31) ,

(2) boot stage (Zadoks 43) and

(3) maturity.

Assessment of response

The response of plants was assessed both destructively and non-destructively. Non-destructive parameters included plant height from the base of the plant to the tip of the youngest emerged blade (YEB), time from sowing to attainment of specific stages of development and symptom expression. Harvested plants were cut at ground level, rinsed in de-ionised water, oven dried and then weighed. The B concentration in whole shoots for the first two harvests and for straw and grain at maturity was determined by ICP spectrometry.

4.2.3 Field Experiment

Five genotypes known to differ in response to B were grown at a high B site. The B concentration in plants sampled twice during the growing season and in grain at maturity was determined. The experiment was duplicated by sowing at two dates to determine the effect of time of sowing on B concentration in plant tissues and to test whether the same tissue analyses were appropriate to both sowing dates. The two sowing dates were 20th May and 4th June, 1987.

Site

The experiment was conducted at Two Wells, a site with high concentrations of B in the sub-soil (Cartwright *et al.*, 1987). The site is described in Chapter 3.

Experimental design

The experiment was arranged as a randomized block design with nine replicates. Each plot consisted of a paired plot; plants of one plot were sampled for B determination and the adjacent plot was harvested to determine grain yield. The plots were arranged as described in Chapter 3 and the two blocks for sowing dates were separated by two border plots, approximately 1.5 metres.

Tissue sampling

The two experiments were sampled twice, namely 7th August and 4th September (79 and 106 days after sowing, respectively) for the early sown experiment and 4th and 23rd September (91 amd 110 days after sowing, respectively) for the later sown experiment. When sampled, plants were at the growth stages late tillering - stem extension (Zadoks 23-31), stem extension - early boot stage (37 - 41), late tillering - stem extension (23-31) and boot stage (41-45), respectively. Tissue samples consisted of 10 whole plants and 10 youngest emerged leaf blades (YEB's) per plot. Samples were rinsed with de-ionised water, oven dried and ground prior to determining B concentrations by ICP spectrometry. Grain from the plots harvested for yield determination was also analysed for B.

4.3 RESULTS

4.3.1 Pot experiment

Plant height

The height of seedlings from the base of the plant to the tip of the YEB was measured four, five and six weeks after sowing for plants labelled for harvest at the first node visible stage. At week six the B0 plants were at the stem elongation stage of development. The effects of genotype, B treatment and the genotype x treatment interaction were all highly significant ($P<0.001$) for the three measuring times (Fig. 4.1).

The heights of Warigal and (Wl*MMC) were significantly reduced at B60, relative to the B0 control, for the three measuring times, but the height of Halberd was not affected by B treatments. The B20 treatment did not affect seedling height. The heights of the three genotypes at the B0 control were not significantly different four weeks after sowing but at weeks five and six the height of Warigal was significantly less than Halberd or (Wl*MMC).

Figure 4.1 Height (cm), from the base of plants to the tip of the youngest emerged leaf for Halberd, Warigal and (Wl*MMC) grown at the B0, B20 and B60 treatments.

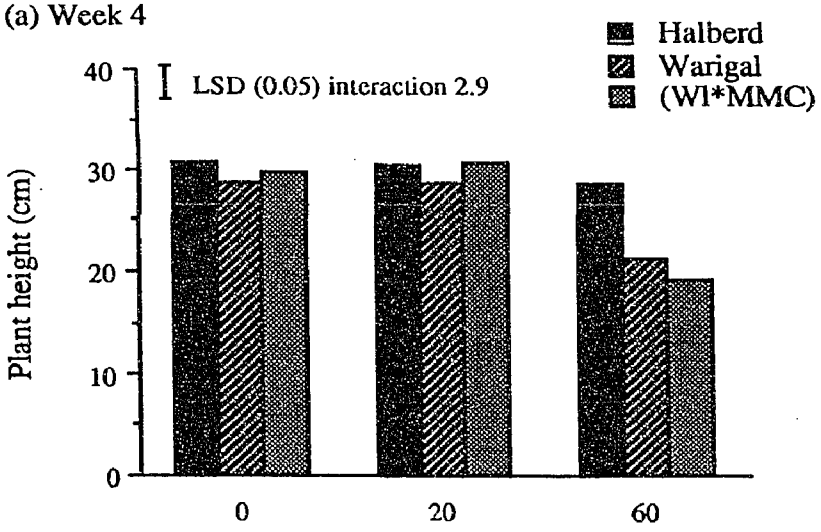
(a) four,

(b) five and

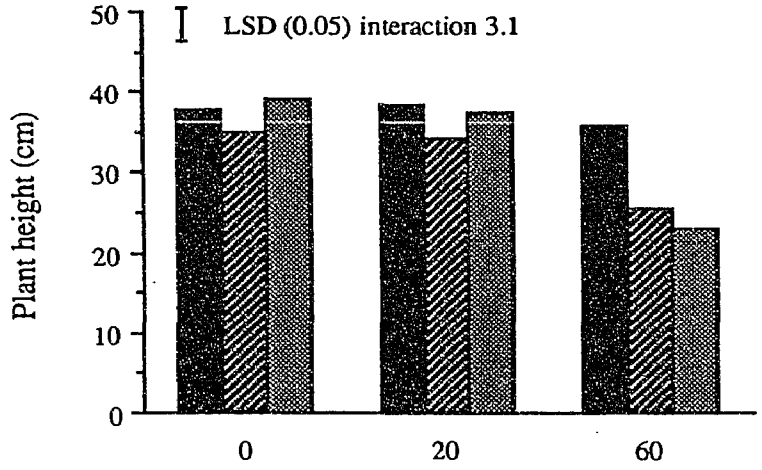
(c) six weeks after sowing

Vertical bars represent the LSD (0.05) for the genotype x treatment interaction.

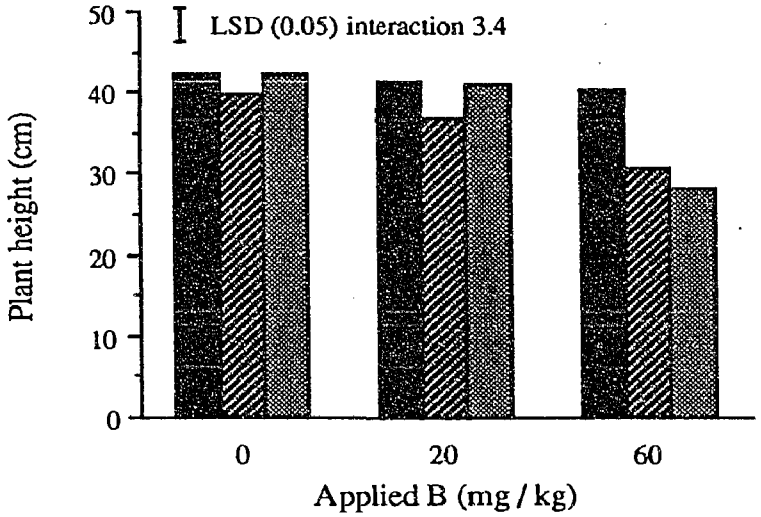
(a) Week 4



(b) Week 5



(c) Week 6



Rate of development

The time from sowing to the four growth stages commencement of tillering, first node visible, boot stage and ear emergence, was recorded (Fig. 4.2), a genotype being deemed to have reached a specific growth stage when half the plants reached that growth stage. Tillering was the most sensitive aspect of growth to B treatments and the initiation of tillers of all genotypes was delayed at the B60 treatment. However, the delay varied between genotypes and at B60 tillering of Halberd was delayed by only five days relative to B0 while tillering of Warigal and (Wl*MMC) was delayed 14 and 16 days, respectively. The total number of tillers per plant was also recorded (Fig. 4.3). The total number of tillers for (Wl*MMC) was less than for the other genotypes at B0, even though tillering of all three commenced at the same time. The commencement of tillering may therefore be more appropriate than total number of tillers for assessing the response of genotypes to high concentrations of B.

The development of the primary shoot of Halberd and Warigal was not affected by the B treatments but development of the primary shoot of the sensitive (Wl*MMC) was delayed by 12 - 14 days for the three stages measured.

Symptom expression

The symptoms of B toxicity of wheat initially consist of chlorosis, developing into necrosis, progressing from the leaf tip and margin towards the midrib and leaf base (Plate 1).

The expression of symptoms of B toxicity was determined for the three youngest leaves (YEB, YEB+1 and YEB+2) of the primary shoot five and eight weeks after sowing (Table 4.1). The rating of the severity of symptom expression was adapted from the scale used by Kluge and Podlesak (1985) for asessing B toxicity of barley.

A symptom not previously described was recognized on (Wl*MMC) at B60. This consisted of necrosis within the otherwise green leaf tissue approximately one third of the leaf length from the tip and will be referred to as "mid-leaf necrosis". The leaf became very weak at this point and bent over (Plate 3). This region eventually became engulfed in the necrosis developing from the leaf tip. In subsequent experiments this symptom was

Figure 4.2 Time (days) from sowing to the growth stages

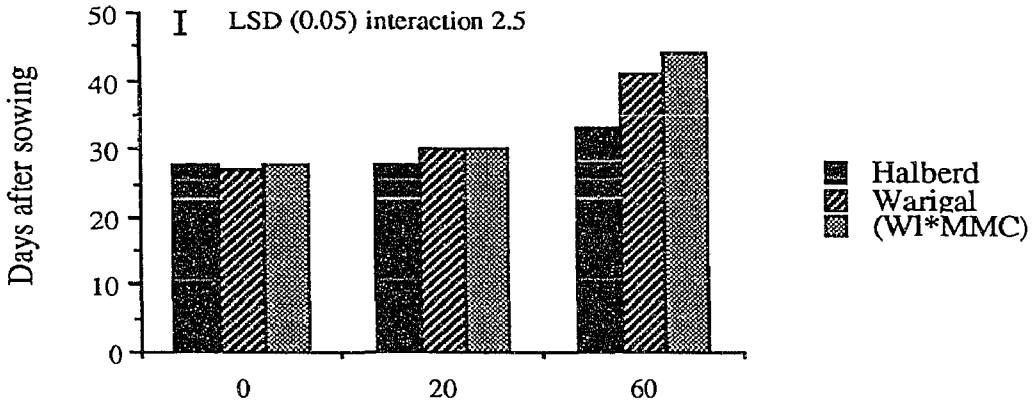
(a) commencement of tillering,

(b) first node visible,

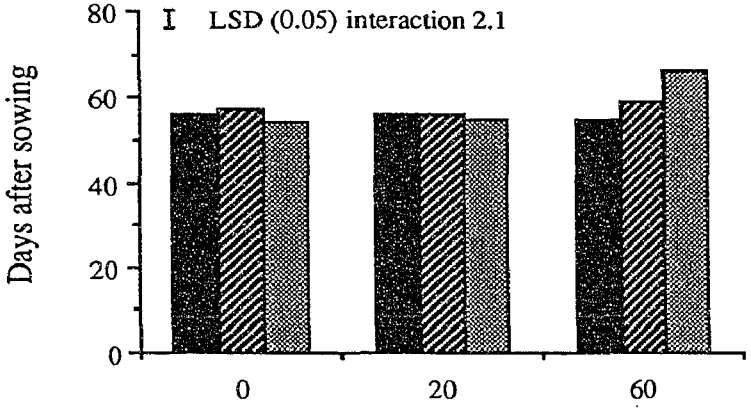
(c) boot stage and

(d) ear emergence for Halberd, Warigal and (Wl*MMC) grown at the B0, B20 and B60 treatments. Vertical bars represent the LSD (0.05) for the genotype x treatment interaction.

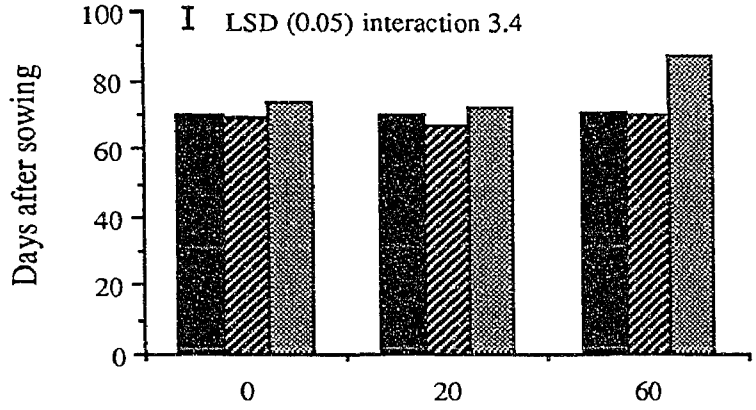
(a) Tillering



(b) First node



(c) Boot stage



(d) Ear emergence

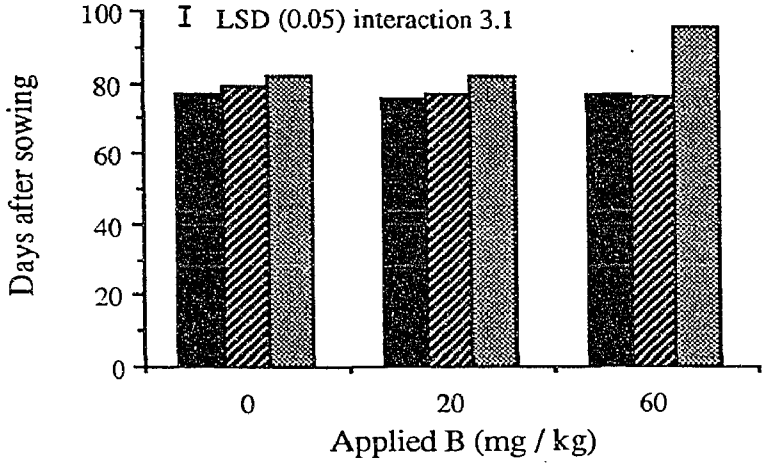


Figure 4.3 Number of tillers per plant to eight weeks after sowing for

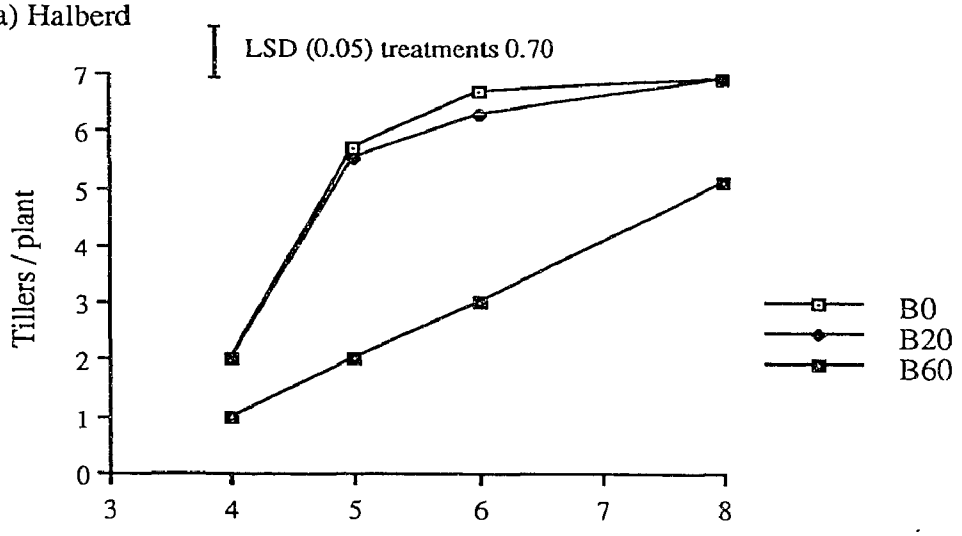
(a) Halberd,

(b) Warigal and

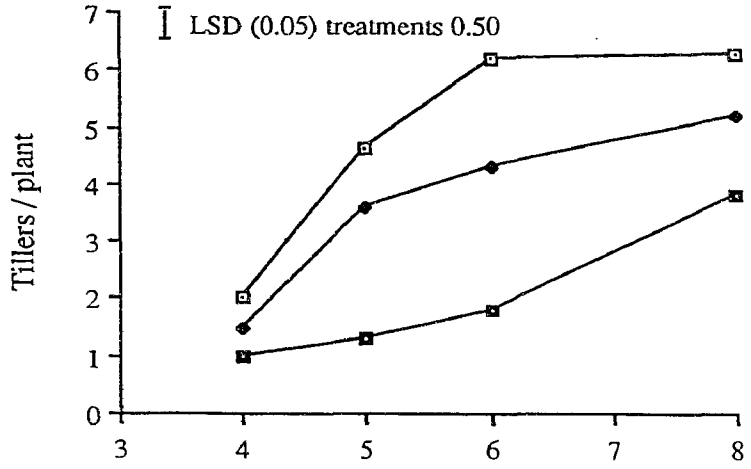
(c) (W1*MMC) grown at the B0, B20 and B60 treatments.

Vertical bars represent the LSD (0.05) for the treatment effect.

(a) Halberd



(b) Warigal



(c) (Wl*MMC)

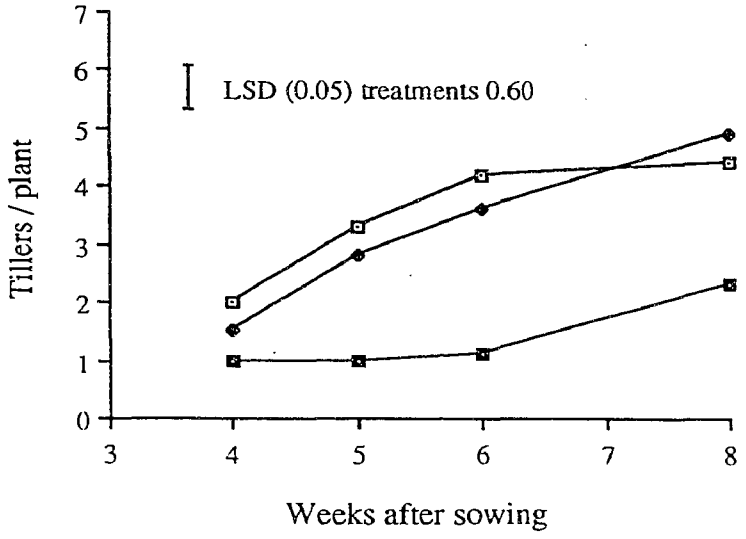


Table 4.1 Foliar symptoms of B toxicity five and eight weeks after sowing. Three genotypes were grown at three B treatments and the severity of symptoms was scored for the three youngest leaves. Values are the means of six replicates.

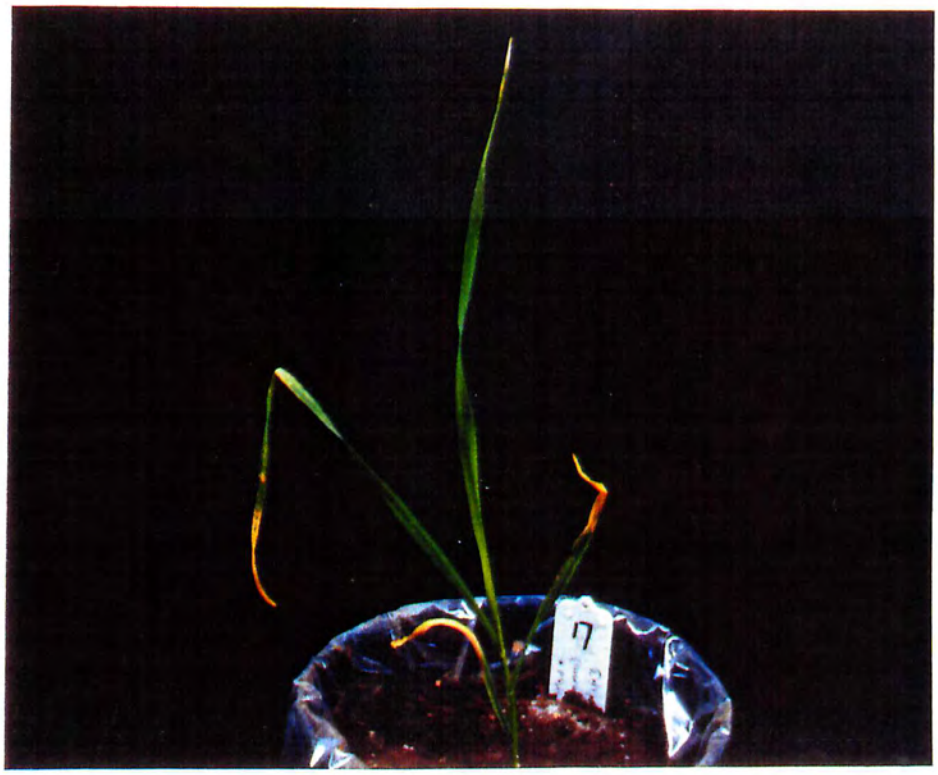
Genotype	Treatment	Symptom of B toxicity[a]		
		YEB[b]	YEB+1	YEB+2
Week 5				
Halberd	B0	9	9	9
	B20	9	9	9
	B60	9	8	8
Warigal	B0	9	9	9
	B20	9	9	9
	B60	8	6	6
(Wl*MMC)	B0	9	9	9
	B20	9	9	7
	B60	5	2	1
Week 8				
Halberd	B0	9	9	9
	B20	9	8	7
	B60	7	6	6
Warigal	B0	9	9	9
	B20	8	8	7
	B60	7	6	6
(Wl*MMC)	B0	9	9	9
	B20	8	7	6
	B60	5	4	3

[a] Symptom of B toxicity adapted from Kluge and Podlesak (1985)

Visual rating	Description of damage
9	no symptoms
7	tip necrosis <1cm
5	1/4 blade severe chlorosis with > 1cm tip necrosis
3	1/2 blade necrosis
1	leaf dead

[b] YEB = youngest expanded blade

Plate 3. The "mid-leaf necrosis" symptom developed by sensitive genotypes in response to high concentrations of boron.



only observed for sensitive genotypes. It could not be induced, even by extremely high concentrations of B, on the more tolerant genotypes.

The symptoms of toxicity were more severe for the YEB+2 than the two younger leaves. There was considerable difference in the severity of symptom expression, among genotypes, and the difference was greatest for the YEB+2. Five weeks after sowing the YEB+2 of Halberd at the B60 treatment had tip necrosis of less than one centimetre while the equivalent leaf of (Wl*MMC) was dead. There was only a slight difference between Halberd and Warigal for symptom expression at the B60 treatment at week five and no difference at week eight.

Dry matter production

For both vegetative stage harvests there were significant genotype and B effects. The genotype x B interaction was statistically significant for the first node harvest but not at the boot stage. At maturity the interaction was significant for both total dry matter production and grain yield (Table 4.2). At the first node stage of development there was no difference in yield between genotypes at the B0 treatment. The yield of Halberd was not affected by the B20 treatment, however the yields of Warigal and (Wl*MMC) were significantly reduced, relative to B0. The yields of all genotypes were significantly reduced, but to differing extents, at the B60 treatment. Halberd was the most tolerant and (Wl*MMC) the most sensitive.

At the boot stage harvest the yields of all genotypes were significantly reduced at the B20 treatment. Warigal produced less dry matter at the B0 treatment than the other genotypes but despite its lower vigour at B0, yields of Warigal and (Wl*MMC) were similar, although less than that of Halberd, at the B20 and B60 treatments.

At maturity, the total dry matter production and grain yields of Halberd were similar at the B60 and B0 treatments and the yield at B20 was greater than at B0. The total dry matter production of Warigal was greater at B20 than at B0 but this effect was not observed for grain yield. The yields of Warigal and (Wl*MMC) were significantly reduced at B60 however Warigal was more tolerant than (Wl*MMC).

Table 4.2 Dry matter and grain yield at three stages of development. Three genotypes were grown at three B treatments and harvested as they attained specific stages of development.

Harvest	Genotype	Yield (g pot $^{-1}$)			
		B0	B20	B60	Mean
First node	Halberd	2.16	2.17	1.26	1.86
	Warigal	2.08	1.77	0.97	1.61
	(Wl*MMC)	2.24	1.63	0.61	1.49
	Mean	2.16	1.86	0.95	
	LSD (0.05) genotypes 0.20, treatments 0.20, interaction 0.35				
Boot stage	Halberd	4.55	3.93	3.10	3.86
	Warigal	3.97	3.55	2.18	3.23
	(Wl*MMC)	4.42	3.37	1.90	3.23
	Mean	4.31	3.62	2.39	
	LSD (0.05) genotypes 0.31, treatments 0.31, interaction n.s.				
Maturity					
Total dry matter	Halberd	8.72	9.62	8.51	8.95
	Warigal	9.21	9.66	7.57	8.81
	(Wl*MMC)	9.14	8.88	5.77	7.93
	Mean	9.02	9.39	7.28	
	LSD (0.05) genotypes 0.38, treatments 0.38, interaction 0.66				
Grain	Halberd	3.33	3.90	3.50	3.58
	Warigal	4.01	4.13	3.42	3.85
	(Wl*MMC)	3.90	3.68	1.66	3.08
	Mean	3.75	3.90	2.86	
	LSD (0.05) genotypes 0.24, treatments 0.24, interaction 0.42				

The yield at the B60 treatment relative to the yield at B0 increased for successively later harvests (Figure 4.4). For example, the relative yields (B60/B0) of Warigal at the first node, boot stage and maturity were 0.47, 0.55 and 0.82, respectively.

Components of grain yield

Analysis of the components of grain yield (Table 4.3) indicated that the very low grain yield of (Wl*MMC) at the B60 treatment resulted principally from low grain production on the secondary ears. The grain yield of the primary ear of (Wl*MMC) was also reduced, although not significantly, at B60 and this resulted from a reduction in the number of fertile spikelets and weight per kernel (data not presented). The reduced total grain yield of Warigal at B60 could be attributed to a reduction in yield of the secondary ears although the number of ears per plant was not reduced.

Concentration of B

The concentration of B in whole shoots at the vegetative stages and in straw and grain at maturity increased markedly at increasing levels of applied B (Table 4.4). The data required transformation prior to statistical analysis. A logarithmic transformation was used for shoots and straw data while the transformation logarithm (1+x) was used for grain. Highly significant genotype and B treatment effects and genotype x treatment interactions occurred for the four data sets.

The concentration of B in shoots and straw of (Wl*MMC), at the B20 and B60 treatments, was significantly greater than for Warigal and Halberd in all instances, while the B concentrations of shoots and straw for Warigal were either similar or greater than for Halberd.

At the B0 treatment, concentrations of B in shoots and straw were inconsitent between genotypes and stages of development. Contamination of two replicates of Warigal at B0 for the first harvest, either during growth or after harvest, was suspected as the concentrations of B for their plants was greater than 20 mg kg^{-1} while the values for the other four replicates were below 10 mg kg^{-1} so the anomalous values were omitted from the analysis. The concentration of B in shoots of (Wl*MMC) was the same as for

Figure 4.4 Relative dry matter (yield at B60 / yield at B0) for Halberd, Warigal and (Wl*MMC) at the three stages of development, first node visible, boot stage and straw and grain at maturity. Values are the means of six replicates.

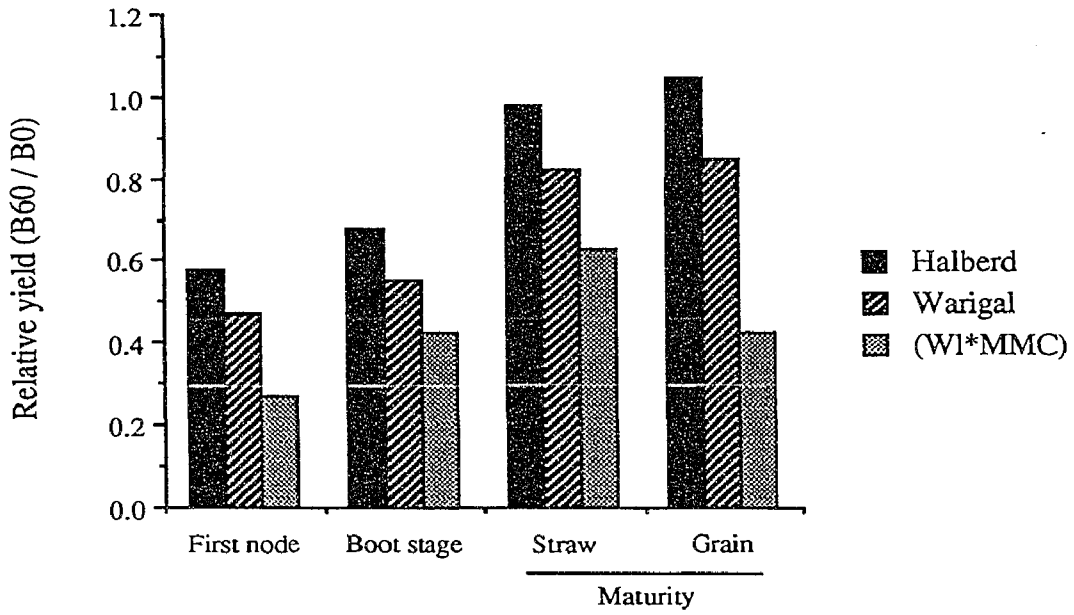

1.2
1.0
0.8
0.6
0.4
0.2
0.0
Relative yield (B60 / B0)
First node
Boot stage
Straw
Grain
Maturity
Halberd
Warigal
(Wl*MMC)
Stage of development at harvest

Table 4.3 Components of grain yield for three genotypes grown at three B treatments.

Component	Genotype	B0	B20	B60	Mean
Ears / plant					
	Halberd	2.6	2.8	2.5	2.6
	Warigal	3.0	3.0	2.7	2.9
	(WI*MMC)	2.3	2.3	1.6	2.1
	Mean	2.7	2.7	2.3	
	LSD (0.05) genotypes 0.44, treatments n.s., interaction n.s.				
Fertile spikelets main ear					
	Halberd	18.0	17.0	16.8	17.3
	Warigal	17.2	17.4	14.7	16.4
	(WI*MMC)	18.3	16.7	15.4	16.8
	Mean	17.8	17.0	15.6	
	LSD (0.05) genotypes n.s., treatments 0.90, interaction n.s.				
Grain number main ear					
	Halberd	42.6	43.2	47.0	44.3
	Warigal	39.2	42.6	35.2	39.0
	(WI*MMC)	51.5	52.8	42.6	49.0
	Mean	44.4	46.2	41.6	
	LSD (0.05) genotypes 5.1, treatments n.s., interaction n.s.				
Grain yield main ear (g)					
	Halberd	1.68	1.75	1.88	1.77
	Warigal	1.80	2.06	1.75	1.87
	(WI*MMC)	2.16	2.09	1.57	1.94
	Mean	1.88	1.97	1.73	
	LSD (0.05) genotypes n.s., treatments 0.21, interaction n.s.				
Grain yield other ears (g)					
	Halberd	1.67	2.15	1.64	1.82
	Warigal	2.23	2.07	1.75	2.02
	(WI*MMC)	1.71	1.51	0.13	1.12
	Mean	1.87	1.91	1.17	
	LSD (0.05) genotypes 0.25, treatments 0.25, interaction 0.43				

Table 4.4 Concentration of B in shoots and grain of three genotypes grown at three B treatments and harvested at three stages of development. Significance values refer to transformed data. (log_{10} for first node, boot stage and straw and log_{10} (1+x) for grain) in brackets.

Harvest	Genotype	B conc (mg kg^{-1})						
		B0		B20		B60		Mean
First node	Halberd	8.5	(0.87)	68.1	(1.83)	402.1	(2.59)	(1.76)
	Warigal	8.6	(0.94)	84.4	(1.92)	483.1	(2.68)	(1.83)
	(WI*MMC)	7.4	(0.87)	136.7	(2.08)	1076.6	(2.89)	(1.95)
	Mean		(0.89)		(1.94)		(2.77)	
	LSD (0.05) genotypes 0.08, treatments 0.08, interaction 0.14							
Boot stage	Halberd	5.6	(0.73)	78.6	(1.89)	371.3	(2.56)	(1.73)
	Warigal	5.0	(0.69)	80.7	(1.90)	498.8	(2.68)	(1.76)
	(WI*MMC)	4.1	(0.61)	122.5	(2.08)	804.9	(2.89)	(1.86)
	Mean		(0.68)		(1.96)		(2.71)	
	LSD (0.05) genotypes 0.07, treatments 0.07, interaction 0.12							
Maturity								
Straw	Halberd	13.6	(1.12)	128.8	(2.11)	862.0	(2.94)	(2.06)
	Warigal	16.5	(1.21)	152.9	(2.17)	918.0	(2.96)	(2.11)
	(WI*MMC)	17.2	(1.23)	244.8	(2.39)	1061.8	(3.02)	(2.21)
	Mean		(1.19)		(2.22)		(2.97)	
	LSD (0.05) genotypes 0.06, treatments 0.06, interaction 0.10							
Grain	Halberd	1.07	(0.313)	4.10	(0.706)	21.45	(1.348)	(0.789)
	Warigal	1.20	(0.342)	3.10	(0.611)	17.35	(1.261)	(0.738)
	(WI*MMC)	1.29	(0.360)	3.40	(0.641)	16.25	(1.235)	(0.745)
	Mean		(0.338)		(0.653)		(1.281)	
	LSD (0.05) genotypes 0.030, treatments 0.030, interaction 0.052							

Halberd at the first node harvest, significantly less than Halberd at the boot stage and significantly greater than Halberd in the straw. Thus, the greater concentration of B in shoots for (Wl*MMC), measured at the applied B treatments, was not evident at a nil applied B treatment.

The concentrations of B in shoots were similar between the first node and boot stage harvests, while B concentrations of straw were higher than for the vegetative shoots and in particular for Halberd and Warigal. The difference in B concentration between Halberd and Warigal and the sensitive (Wl*MMC) was greater for the first two harvests than for straw at maturity.

The concentration of B in grain was significantly influenced by the B treatments, while significant differences also occurred between genotypes. At the B20 and B60 treatments the concentration of B in the grain of Halberd, which had the lowest B concentrations for shoots and straw, was significantly greater than for grain of Warigal and (Wl*MMC).

4.3.2 Field Experiment

Significant differences in grain yield resulted between genotypes while the mean yield over all genotypes was significantly reduced for the later sowing (Table 4.5). The interaction between genotypes and sowing date was not statistically significant, however, and this was probably due to a large standard error for the experiment (coefficient of variation = 22%). The trends in yield between the two sowing dates showed that the yield of the B sensitive (Wl*MMC) was most affected by the later sowing, while yields of Warigal and Bindawarra were also reduced at the later sowing. Only the B tolerant varieties, Halberd and Olympic, were not affected by the later sowing.

The concentration of B in whole shoots, YEB's and grain differed between genotypes for all comparisons (Table 4.6). In all cases B concentrations were lowest for Halberd and Olympic and highest for Bindawarra and (Wl*MMC). The concentration of B in grain was much lower than in leaves and shoots and this is consistent with previous reports (Gupta, 1971; Cartwright *et al.*, 1984).

Table 4.5 Grain yield for five genotypes grown at Two Wells and sown on 20th May (Sowing 1) and 4th June (Sowing 2), 1987.

Genotype	Grain yield (g plot $^{-1}$)		
	Sowing 1	Sowing 2	Genotype mean
Halberd	518	521	520
Olympic	485	502	494
Warigal	611	523	567
Bindawarra	527	447	487
(WI*MMC)	506	354	380
Mean	529	469	

LSD (0.05) genotypes 73, sowing dates 46, interaction n.s

Table 4.6 Concentration of B in shoots, YEB's and grain for five genotypes grown at Two Wells. The experiments were sown on two dates (S1 and S2) and tissues of plants from each sowing block was harvested twice (H1 and H2) for B determinations.

Tissue analysed	B conc (mg kg^{-1})					
	Halberd	Olympic	Warigal	Bindawarra	(WI*MMC)	Mean
Shoots						
S1 H1[a]	14.2	16.3	19.8	21.4	25.2	19.4
S1 H2	27.9	34.2	45.9	57.1	56.4	44.3
Mean	21.1	25.3	32.9	39.3	40.8	
LSD (0.05) genotypes 2.4, harvests 1.5, interaction 3.4						
S2H1	24.3	29.2	36.5	43.7	43.9	35.5
S2H2	25.3	36.1	50.2	75.5	72.5	51.9
Mean	24.8	32.7	43.4	59.6	58.2	
LSD (0.05) genotypes 6.1, harvests 3.9, interaction 8.6						
YEB's						
S1H1	14.1	16.8	21.9	27.5	28.1	21.9
S1H2	30.5	48.3	70.1	84.3	119.5	70.5
Mean	22.3	33.1	46.0	55.9	73.8	
LSD (0.05) genotypes 9.4, harvests 6.0, interaction 13.4						
S2H1	43.6	48.4	73.1	84.6	99.3	69.8
S2H2	85.9	74.3	143.2	145.8	183.9	126.6
Mean	64.8	61.4	108.2	115.2	141.6	
LSD (0.05) genotypes 12.1, harvests 7.7, interaction 17.1						
Grain						
S1	3.1	3.1	3.9	5.5	5.8	4.3
S2	2.6	3.0	3.9	6.2	6.3	4.4
Mean	2.9	3.0	3.9	5.9	6.1	
LSD (0.05) genotypes 0.3, sowing n.s., interaction 0.4						

[a] S1 = sowing 1 (20th May), S2 = sowing 2 (4th June), H1 = harvest 1, H2 = harvest 2 S1H1 7th August, S1H2 4th September, S2H1 4th September, S2H2 23rd September.

Within each time of sowing the concentration of B in shoots and YEB's increased from the first to the second harvest and were highest for Sowing 2 Harvest 2 (Table 4.6). The second harvest for the first sowing (S1H2) and the first harvest for the second sowing (S2H1) were conducted on the same day. The concentration of B in whole shoots was significantly greater for S1H2 than S2H1, but the B concentration in YEB's was the same for both (statistical analysis not included in Table 4.6). Time of sowing did not affect the concentration of B in grain over all genotypes but the concentration for Halberd was lower and the concentrations for Bindawarra and (Wl*MMC) were higher at the second sowing.

The relative difference in B concentration between tolerant and sensitive genotypes increased from the first to the second sampling. For the early sowing the relative difference between genotypes was greater for the YEB's than for whole shoots, however this was not so for the later sowing. The relative difference for concentration of B in the grain between Halberd and (Wl*MMC) was comparable to the difference for shoots.

Five genotypes were considered too few to calculate correlations between the concentrations of B in shoots, YEB's and grain and between the concentration of B in tissues and grain yield for the sowing and sampling times. Therefore the results of individual plots, or 45 samples, have been used to determine these correlations. The correlations between all tissues for all sampling times were significantly correlated within both sowing dates (Table 4.7), therefore either whole shoots, YEB's or grain could be analysed to determine differences in B concentrations between genotypes.

The concentrations of B in tissues were not significantly correlated with grain yield for the first sowing, however at the second sowing, the B concentration of all tissues were significantly correlated with grain yield (Table 4.7). This is consistent with the yields of all genotypes being similar at the first sowing but reduced yields of the more sensitive genotypes at the second sowing.

Table 4.7 Correlation coefficients (r) for comparisons of B concentration between whole shoots, youngest expanded leaf blades (YEB's) and grain and for comparisons between concentration of B in tissues and grain yield for two harvest and two sowing dates for five varieties grown at Two Wells. Each variable consists of 45 observations.

Variable		Tissue analysed				Grain yield
		Shoots	YEB's		Grain	
		H2	H1	H2	Maturity	
Sowing 1						
Shoots	H1	0.76	0.87	0.67	0.81	0.18
	H2		0.72	0.80	0.79	0.12
YEB's	H1			0.62	0.80	0.25
	H2				0.74	0.00
Grain						-0.03
Sowing 2						
Shoots	H1	0.85	0.88	0.87	0.89	-0.36
	H2		0.88	0.84	0.89	-0.52
YEB's	H1			0.85	0.86	-0.58
	H2				0.80	-0.43
Grain						-0.55

LSD (0.05) r=0.29, (0.01) r=0.38

4.4 DISCUSSION

4.4.1 Pot experiment

The yield results at all harvests confirmed the relative responses of the three genotypes. Halberd was the most tolerant, Warigal intermediate and (Wl*MMC) the most sensitive to high concentrations of B. The response to B could be related to concentration of B in shoots with the B concentration being lower for more tolerant genotypes and this is consistent with the results of Paull (1985), Paull *et al.* (1988) and Nable (1988).

The parameters which would be most appropriate to use as selection criteria for assessing the relative response of genotypes to B would be those which :

(1) have a minimum difference between genotypes under conditions of normal B supply but are maximised in the presence of excessive supply of B,

(2) can be determined at an early stage in development and,

(3) if they are to be applied to a segregating generation or for progeny testing, the measurement should be non-destructive.

The non-destructive measurements of plant height, rate of development and symptom expression ranked genotypes in the same order as determined by dry matter production. Plant height was able to distinguish between Halberd and the two more sensitive genotypes four, five and six weeks after sowing. Although Halberd is a tall wheat and (Wl*MMC) is a semi-dwarf (*Rht1*) there was no difference in height between the two genotypes, at the B0 treatment, four, five or six weeks after sowing. Therefore, the reduced height of the B sensitive (Wl*MMC) at the B60 treatment could not be attributed to the semi-dwarf trait. The difference in height among the genotypes at B0 increased with time, therefore to minimize differences in height at the control, use of plant height as a selection parameter should be restricted to plants prior to stem elongation.

The rate of development of plants was affected by B treatments but of the four stages of development measured, initiation of tillers was the most sensitive. Tillering of even the tolerant variety, Halberd, was delayed by the B60 treatment. Delay in tillering at B60 did not distinguish between the levels of tolerance for Warigal and (Wl*MMC), however development of the main shoot was delayed at B60, relative to the B0 control for

(Wl*MMC) whereas it was not for Warigal. The delay in tillering due to high levels of B appears to be a suitable parameter for distinguishing between tolerant and moderately sensitive genotypes. Rate of development of the main shoot might not be applicable for comparing between genotypes in these two categories, however it may be useful for distinguishing between moderately sensitive and sensitive genotypes.

The three genotypes used for this experiment were similar for time from sowing to boot stage and ear emergence at B0, but this would not be the situation for genotypes differing in photoperiod or vernalization requirements. The utility of length of time to various developmental stages as an indicator of B tolerance over a diverse set of genotypes would probably be restricted to those expressed during early vegetative stages of growth.

The severity of expression of symptoms of B toxicity reflected the concentration of B in the shoots. The concentration of B in shoots of (Wl*MMC) was significantly greater than for Halberd and Warigal, and the symptoms for (Wl*MMC) were also more severe than for the other genotypes. There was only a small difference in symptom expression between Halberd and Warigal, although symptoms were more severe for Warigal in the earlier weeks (Table 4.1). The concentration of B in shoots was either similar to Halberd or slightly less (Table 4.4). The difference in symptom expression was greater for the YEB+2 than for the YEB. The uneven distribution of B and the resulting differences in symptom expression between leaves (Kluge and Podlesak, 1985) requires that equivalent leaves are used when comparing genotypes for severity of symptom expression.

The effect of the B treatments upon dry matter production differed between the times of harvest, however ranking of genotypes was constant with Halberd the most tolerant, Warigal moderately sensitive and (Wl*MMC) sensitive. As plants were harvested when they attained the specified stages of development to allow analysis of tissues of the same physiological age, and development was delayed by B treatments, comparisons for dry matter production underestimate the effect of B treatments upon yield. At the B0 control, plants of the three genotypes reached the first node stage within three days of each other, but Warigal reached the boot stage five days before (Wl*MMC).

The significantly lower yield of Warigal at the control for the boot stage harvest may therefore have been due to differences in time to harvest rather than growth rates. At the B60 treatment, (Wl*MMC) was harvested approximately 10 and 17 days after the other two varieties for the first node and boot stage harvests, respectively. Had all plants been harvested at the same time, the differences in yield during vegetative growth between the moderately sensitive Warigal and the sensitive (Wl*MMC) would have been much greater.

The apparent compensation in growth in response to the B60 treatment at the later harvests, and in particular at maturity, was a result of a delay in development and maturity of secondary tillers due to the B treatment. As the plants were supplied with water and nutrients until maturity, a lower growth rate due to B toxicity may not necessarily have resulted in a reduction in total dry matter or grain production. Manchanda and Yadav (1978) reported differences in critical concentrations of B in the soil and plants for different stages of development and this may have arisen for similar reasons. In the semi-arid regions of South Australia, however, water stress increases with temperature and day length and there would be little opportunity for an extended period of growth to compensate for a yield reduction arising from B toxicity.

The difference in B concentration of shoots, between Halberd and (Wl*MMC), was greater at the first node and boot stage harvests than for straw at maturity, therefore the most appropriate time to sample plants for tissue analysis would be during the vegetative stage. As the B concentration of tissues did not alter between the first node and boot stages it would be acceptable to harvest all plants at the same time when they are between these stages of development, rather than as they attain a specific stage of development. This would have the added advantage of being able to compare yield on a more uniform basis and would result in larger differences in yield between tolerant and sensitive genotypes, as discussed above.

The comparison between genotypes for concentration of B in grain for the pot experiment was not consistent with results obtained in the field for the current and previous experiments (e.g. Cartwright *et al.*, 1987; Paull, 1985). The difference in response between cultural conditions is probably related to the restricted root volume and

unlimited moisture supply during grain filling for plants grown in pots. At maturity it was observed that the majority of roots were growing between the soil and the pot and it is probable that the B concentration of the soil solution in this environment is lower than within the soil mass. No measurements were made of the distribution of roots on the perimeter of and within the soil mass but a previous experiment has shown that the roots of Halberd are able to penetrate further into soil of high B concentration than more sensitive genotypes (Paull, 1985). It is therefore possible that the roots of the three genotypes occupied different environments during grain filling and the high concentration of B in the grain of Halberd reflects a greater proportion of roots within the high B soil mass.

The B concentrations of shoots at the B0 treatment did not conform to the ranking of genotypes at the higher B treatments. This is in contrast to the results of Nable (1988) and Paull (1985) who reported wheat and barley genotypes of low B accumulaton at high levels of applied B also had lower B concentrations at low B supply but is in line with results obtained by D.B. Moody (pers. comm.) for wheat grown in pots at a range of B treatments.

This experiment was not intended for identifying critical concentrations of B in either the plant tissues or the soil. Indeed, the complex interactions between the effect of B on development, the stage of harvest for determination of B and the unlimited supply of water to plants until maturity suggest that it might not be possible to determine a universal critical concentration of B for toxicity. This may to some extent explain the conflict in critical values that have been derived by other authors (see Table 2.3). As an example, at the boot stage the concentration of B in shoots of Halberd at the B20 and B60 treatments were 78.6 and 371 mg kg^{-1}, respectively (Table 4.4). The dry matter yield of Halberd at the boot stage was significantly reduced by the B20 treatment (Table 4.2) and so the critical diagnostic toxic concentration of B in whole shoots would be less than 78.6 mg kg^{-1}. On the other hand, the grain yield of Halberd at maturity was not significantly reduced at B60 relative to B0, although it was less than for the B20 treatment. The critical concentration of B in the boot stage shoots for predicting yield reductions at maturity, or the prognostic critical toxic concentration, would therefore be greater than 78.6 mg kg^{-1},

and authors who compared the B60 with the control treatment rather than the treatment producing the highest yield would set the critical value at greater than 371 mg kg^{-1}.

Grain derived from the plants of this experiment was tested for the effect of B in the grain on germination and early seedling development (Nable and Paull, 1989) and results are summarized in Appendix B. The B treatment from which the seed was derived had no effect on seedling emergence, seedling height, dry matter production or the concentration of B in the shoots of four week old plants. Thus, grain can be obtained from plants grown in B enriched soil and B in the grain will have no influence on the vigour of the progeny.

4.4.2 Field Experiment

The yield data suggest that when sown later, B tolerant varieties have a comparative advantage at high B sites, however as there was not a control experiment at a nearby low B site this result should be treated with caution. The tall varieties Halberd and Olympic are inherently lower yielding than the semi-dwarf varieties. Hence, there was probably also loss of yield due to B toxicity for the sensitive varieties at the early sowing. The varieties were rated for maturity and when Halberd was at the boot stage (Zadoks 41), Warigal was at stage 39 and the other three genotypes were at stage 37. While the sensitivity of Bindawarra and (Wl*MMC) to the later sowing might be related to their later maturity and hence the possibility of moisture stress during grain filling, Olympic was equally late but not as sensitive to late sowing.

The results of tissue and grain analysis demonstrated a very significant effect of time of sampling upon the concentration of B in shoots and YEB's. The increase in B concentration for the later samples would result from the uneven distribution of B in the soil profile (Cartwright *et al.*, 1984). The proportion of the root system in the high B zone in the sub-soil, and therefore plant uptake of B, would increase with time. This is in marked contrast to the situation for pot experiments where the entire root system is, at least initially, subjected to the same concentration of B.

Irrespective of the tissue and time of sampling, the concentrations of B in the tolerant varieties Halberd and Olympic were significantly lower than for the more

sensitive genotypes, including Warigal. The relative difference in B concentration of shoots, between Halberd and Warigal, ranged from 29% for the first sampling of the early sown experiment to 98% for the second sampling of the late sowing. This is in contrast to the pot experiment where the concentration of B was similar for Halberd and Warigal at several treatments and the maximum relative difference between the two was 34% for the boot stage harvest at the B60 treatment.

The relative difference, between genotypes, for B concentration in shoots and YEB's was greater at the second than the first sampling for both sowing times. Tissue analysis for selecting between genotypes differing in accumulation of B would therefore be more efficient if conducted when plants were at or near the boot stage. Neither shoots nor YEB's resulted in consistently better discrimination between genotypes. Alternatively, grain could be analysed as the relative differences between tolerant and sensitive genotypes for concentration of B in the grain were of the same order as for shoots. Grain has the added advantages of being easy to collect, either from field experiments or for regional surveys, is robust and requires minimal processing prior to digesting.

The large fluctuation in concentration of B in whole shoots and YEB's over the growing season has significant implications for the determination of critical concentrations of B in shoots and assessing the probability of B toxicity affecting a crop. A low concentration of B in tissues at an early stage of development would give no indication of the probability of B toxicity occurring later in the season. Recent observations (D.B.Moody, pers. comm.) and experiments (R.O.Nable, pers. comm.) have demonstrated that the B concentration of shoots and YEB's of wheat grown under high B conditions, either in the field or in pots, is significantly reduced by precipitation of 25 mm, a result which is consistent with the finding of Oertli (1962) that B in leaves is subject to leaching and may also be lost by guttation. Thus, the time of sampling in relation to recent precipitation will also confound the interpretation of B concentration of shoots. Grain may prove to be less subject to sampling variation and so be more reliable for assessing the B status of soil as it affects plants.

A further complicating factor for interpreting data of concentration of B in tissues is the apparent interaction between tolerance to B and the time of sowing. As there was no difference in yield between genotypes for the early sowing, there was no correlation between concentration of B in tissues and grain yield, however for the later sowing all correlations between B concentration and grain yield were significant and negative. The second sampling of the first sowing and the first sampling of the second sowing were conducted on the same day. Despite similar concentrations of B in YEB's for both samples and a higher concentration of B in shoots of the earlier sown plots, reduced grain yields of the more sensitive genotypes resulted only at the second sowing.

For a nutritional deficiency, early detection is advantageous as it allows corrective measures to be taken and so minimize yield loss. This is not the situation for B toxicity as there are no means of ameliorating the soil conditions. Rather, information on the B status of a current crop could be used for selecting a variety with the appropriate level of tolerance for future cultivation.

Chapter 5.

RESPONSE OF HISTORICALLY IMPORTANT AUSTRALIAN WHEAT VARIETIES TO HIGH CONCENTRATIONS OF BORON

5.1 INTRODUCTION

Historically, the Australian wheat industry has been dominated by relatively few varieties with most belonging to one of several broad families (Macindoe and Walkden-Brown, 1968; Wrigley and Rathjen, 1981). Examples of the dominant wheat varieties, by family groups, include (1) Federation, Insignia, Heron and Halberd (2) Nabawa and Bencubbin, (3) Gabo and Gamenya and (4) Condor. The extent of the dominance of these varieties is evident from the data of area of cultivation by states and for Australia between 1929 and 1965 (Macindoe and Walkden-Brown, 1968). The leading varieties in Australia, South Australia and Victoria for selected years between 1929 and 1965 and the leading varieties in all mainland states between 1979 and 1989 are presented in Appendix C. The predominance of varieties of common ancestry would suggest that either the breeding of wheat in Australia has been based on a narrow gene pool or the members of the dominant families possess a character (s) which is subject to significant selection pressure.

Despite the introduction of the semi-dwarfing genes into Australian wheat varieties, with the release of Kite, Condor and Egret in 1973, the tall variety Halberd has remained dominant in South Australia, and to a lesser extent in Western Australia and since its release in 1969 (Appendix C). Halberd and the advanced breeding line (Wq*KP)*WmH)/6/12, which has Halberd as a parent, were found to be more tolerant to high concentrations of B than several other varieties and advanced breeding lines from the Waite Institute wheat breeding program (Paull, 1985; Paull *et al.*, 1988).

A series of experiments was conducted to determine the response to B for historically important Australian wheat varieties and the varieties currently grown in South Australia. This was undertaken with the following objectives :

(1) to determine whether tolerance to B may have been a factor in contributing to the dominance of particular wheat varieties;

(2) to compare the tolerance to B of varieties with the tolerance of their parents. For a character under multi-gene control and without B tolerance as a major selection force it would be expected that the offspring would tend to the mean of the parents, whereas if tolerance is under major gene control, offspring would be similar to one of the parents, and the tolerant parent if tolerance was selected for, albeit unrecognized;

(3) to determine whether there have been Australian wheat varieties with a greater level of tolerance than the most tolerant of current Australian wheats (i.e. Halberd level). Such Australian adapted varieties, if indeed they occur, would be preferable as sources of B tolerance compared to unadapted types from overseas and possibly landrace introductions;

(4) to determine the B tolerances of varieties currently grown in South Australia and so allow more objective recommendation of varieties for regions with high concentrations of B in the soil;

(5) to compare the responses to B, recognized in the previous experiment (Chapter 4), between glasshouse and field environments over a larger number of genotypes.

The wheat varieties used in experiments, together with their pedigrees, state of selection and year of release are presented in Table 5.1. The accession number is included for all varieties for which seed was obtained directly from the Australian Winter Cereals Collection.

Table 5.1 Varieties used in experiments with their pedigree, year of release and state of selection. The Australian Winter Cereals Collection (AUS) accession number is included for varieties obtained directly from the AWCC

Variety	Pedigree	Release	Selection	AUS
Aldirk	Dirk/2*Gabo	-	NSW	
Aroona	WW-15/Raven	1981	SA	20992
Banks	PWTH/Condor sib//2*Condor	1979	Qld	20599
Bayonet	Pitic 'S'/Glaive	1984	SA	22256
Bencubbin	Nabawa/Gluyas Early	1929	WA	
Bindawarra	Mexico 120/Koda/2/Raven	1980	SA	20621
Bobin	Thew/Steinwedel	1925	NSW	1977
Condor	Penjamo 62/4*Gabo 56/2/TZPP/Nainari 60/4/ 2*Lerma Rojo/2/Norin 10/Brevor 14/3/3*Andes	1973	NSW	16036
Cook	Timgalen/Condor sib//Condor	1977	Qld	20275
Cranbrook	WE/2/Ciano 'S'/Nordeste 66/3/Zambezi	1985	WA	
Currawa	Northern Champion/Cretan//little Club	1912	Vic	2229
Dagger	Sabre/Mec 3/2/Insignia	1984	SA	22255
Dundee	Hard Federation/Cleveland//Sands	1927	NSW	
Egret	Heron/4/2* Lerma Rojo//Norin 10/Brevor 14 /3/3* Andes	1973	NSW	
Etawah	Introduction	-	India	2335
Federation	Purple Straw/Yandilla	1901	NSW	218
Festiguay	Festival/Uruguay C10837	1963	NSW	227
Gabo	Gular?/Gaza//Gular? (see page 142)	1945	NSW	246
Gallipoli	Club wheat/Yandilla King	1917	Vic	2475
Gamenya	Kenya 117A/2*Gabo//Mentana/6*Gabo	1960	NSW	256
Ghurka	Gallipoli/3/Currawa//Indian 4E/Federation	1924	Vic	2494
Gluyas	Ward's Prolific seln.	1894	SA	
Halberd	Scimitar/Kenya C6042/Bobin/2/Insignia 49	1969	SA	11612
Heron	R.D.R./4*Insignia 49	1959	NSW	322
Improved Fife	Red Fife seln. introduction	-	USA	2635
Indian 4	Introduction	-	India	4642
Indian F	Introduction	-	India	4862
Insignia	Ghurka/Ranee	1946	Vic	2642
Insignia 49	Gabo/4*Insignia	1951	SA	2646
Katyil	AUS 10894/4*Olympic	1982	Vic	
Kenya C6042	Introduction	-	Kenya	5775
Kenya Farmer	Gaza/2*Bobin//Button/Kenya 73D211C	-	Kenya	6121

Kite	Norin 10/Brevor 14/2/4*Eureka 2/3/T-A/3* Falcon/4/T-A/4*Falcon/5/T-A/5*Falcon (T-A = Thatcher/*Ag. elongatum*)	1973	NSW	16035
Lance	Collafen/Raven	1978	SA	20592
Machete	Sonora 64/2/TZPP/Yaqui 54/3/*Gabo/4/Madden	1985	SA	23038
Madden	Gamenya/Gabo*3/Khapstein	1973	WA	
Major	Federation/Wallace	1915	Vic	2929
Marshall's No.3	Ward's Prolific seln.	1890	SA	
Mec-3	Sonora 64	-	Mexico	
Meering	Condor seln.	1984	Vic	
Mexico 120	Yaktana 52//Norin 10/Brevor 14	-	Mexico	
Miling	Complex cross. Bencubbin, Charter, Sword, Kenya C6041 Mexico, Gamenya	1982	WA	
Millewa	Sonora 64/Yaqui 50E/2/Gaboto/Mexico 8156	1979	Vic	
Olympic	Baldmin/Quadrat	1956	Vic	3117
Oxley	Penjamo 62/4*Gabo 56/2/TZPP/Nainari 60/4/ 2*Lerma Rojo/2/Norin 10/Brevor 14/3/3*Andes	1974	NSW	16461
Pinnacle	Pindar seln.	1946	Vic	3168
Purple Straw	Introduction believed to be from Tuscany	1860	-	Many
Quadrat	Ghurka/3/Currawa//Major/Gallipoli	1941	Vic	3213
Ranee	Indian F/Federation	1924	Vic	1001
Raven	Orfed, Uruguay, Mayo/4*?Dirk 48	1963	NSW / SA	
Sabre	Gabo/3/Nabawa/Dan/2/Dundee/4/Dundee/5/ Kenya C6042	1952	SA	1158
Schomburgk	W3589/Oxley/2/2*Warigal/3/2*Aroona	1986	SA	23325
Scimitar	Nabawa/Egyptian 4	1930	SA	1192
Siete Cerros	Penjamo 62 sib/Gabo 55		Mexico	
Spear	Sabre/Mec 3/2/Insignia	1984	SA	22254
Summit	Pindar/Thatcher//2*Insignia/3/Pinnacle	1965	Vic	1334
Sunstar	Condor/4/WW-15/3/W199/WC356/2/LaPrevision	1983	NSW	22177
Takari	Kite/3/Frocor/Kentana/2/2•Festiguay	1983	NSW	21905
Vulcan	Condor/Pitic 62//Condor sib	1985	Qld	23039
Ward's Prolific	Du Toit seln.	1890	SA	1630
Warigal	WW-15/Raven	1978	SA	20593
Warimba	Mengavi/Mexico 22A	1976	SA	
(Wl*MMC)	Warigal//8156/Mengavi/Crim	-	SA	-
WW-15	Lerma Rojo//Norin 10/Brevor 14/3/3*Andes	-	Mexico	
Yandilla	Improved Fife/Etawah	c 1900	NSW	1691
Yandilla King	Yandilla/Silver King	1907	SA	7037

5.2 RESPONSE OF HISTORICALLY IMPORTANT AUSTRALIAN WHEAT VARIETIES TO HIGH CONCENTRATIONS OF BORON IN THE FIELD

5.2.1 Introduction

A number of wheat varieties, which were chosen to represent the major historical groups of wheat in Australia, were grown at a high B site to assess their response to B and compare the response of related varieties.

5.2.2 Materials and Methods

Varieties

A total of 25 varieties, which could be grouped into five categories, were grown. These categories were :

(1) introductions and selections prior to the adoption of systematic cross-pollination and selection within the progeny (essentially pre-Farrer and before 1900);

(2) varieties developed by cross-hybridizaton which have the variety Federation as an ancestor;

(3) other widely grown tall varieties not directly related to Federation ;

(4) semi-dwarf parents introduced from CIMMYT, Mexico (Federation is a parent of Brevor and is therefore distantly related to all semi-dwarf wheat varieties based on the *Rht* 1 and *Rht* 2 genes derived from Norin 10/Brevor seln 14. Despite the common ancestor, semi-dwarf wheats shall be considered as a separate category from the varieties related to Federation which have been entirely selected in Australia.);

(5) the first semi-dwarf varieties released in Australia .

Seed for this experiment was initially derived from the AWCC and was obtained from stocks held at Waite Agricultural Research Institute and Roseworthy Agricultural College.

Experimental design

The experiment was conducted at Two Wells in 1985 and 1986 and sown according to the standard procedure for field experiments as described in Chapter 3. The experiments were sown on August 1st in both years. The experiments were arranged as randomized complete block designs of six replicates in 1985 and seven replicates in 1986. The B sensitive genotype, (Wl*MMC), was grown as a control grid and included every fifth plot. Results for (Wl*MMC) were excluded from statistical analysis, rather they were used to give an indication of edaphic variation.

In 1985, five whole shoots per plot were harvested on October 18th (78 days after sowing) when plants were at or near the boot stage (Zadoks 41-45). Samples were rinsed with de-ionized water, oven dried, ground in a stainless steel mill and analysed for concentration of B by nitric acid digestion and ICP-spectrometry.

The experiments were harvested at maturity and grain yield per plot and the concentration of B in the grain was determined for both experiments.

5.2.3 Results

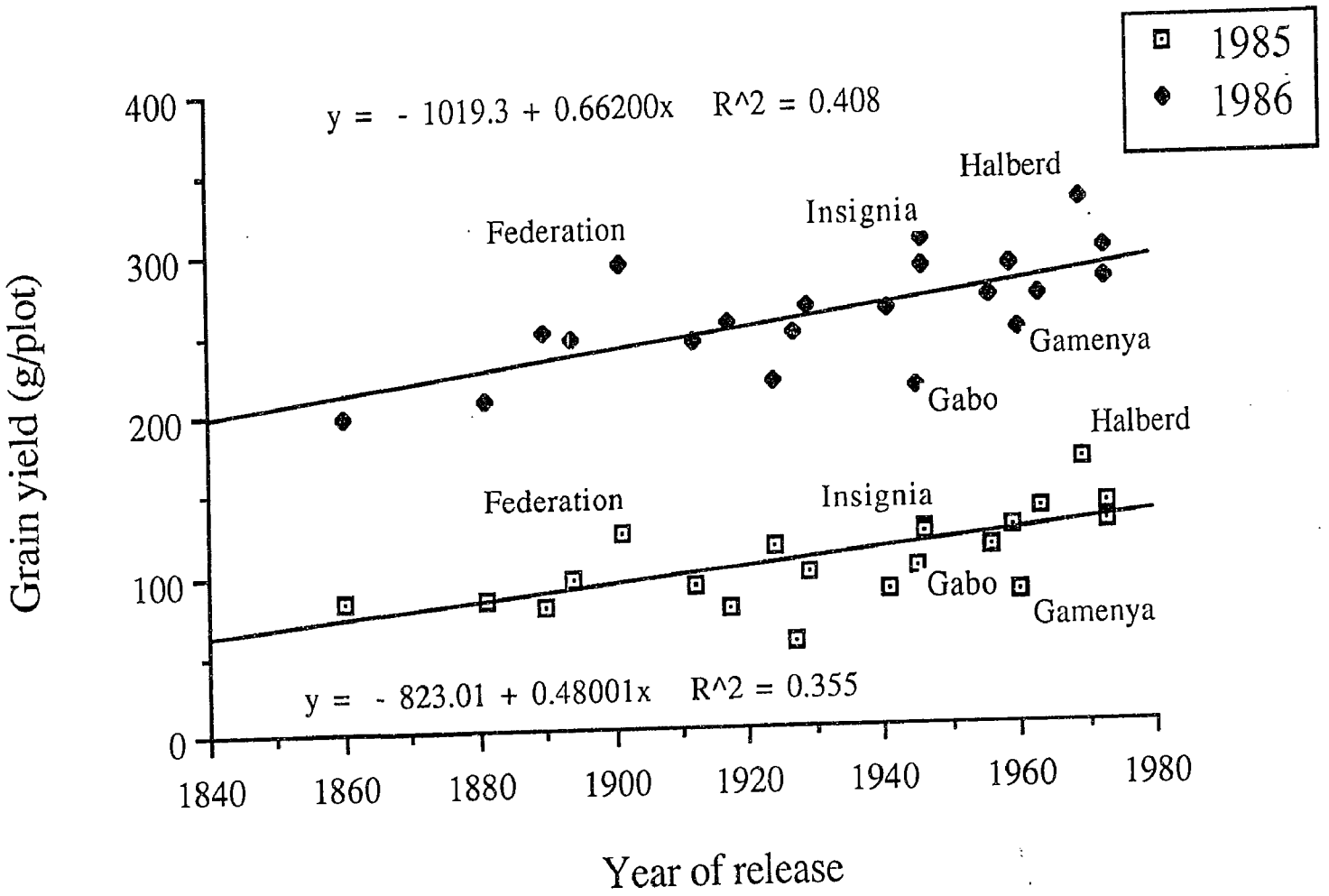
There were significant differences among varieties for grain yield (1985 and 1986) and concentration of B in the shoots (1985) and grain (1985 and 1986) (Table 5.2). Grain yields were lower in 1985 than 1986 and this may have resulted from either the lower rainfall, or the higher level of B toxicity as measured by the concentration of B in the grain, in 1985, or a combination of both. The concentration of other elements in shoots in 1985 and grain in 1986 are given in Appendix D.

Comparisons for B tolerance on the basis of grain yield are perhaps unreasonable considering the time scale over which the varieties were released. This factor is borne out by the significant regression between grain yield and year of release for the Australian varieties of these experiments (Figure 5.1). The increment in grain yield, derived from the slope of the regression line, was 0.48% and 0.66% per annum for the 1985 and 1986 experiments, respectively. The grain yields of Federation, Insignia and Halberd were above and Gabo and Gamenya below the regression line in both years.

Table 5.2 Concenration (mg kg^{-1}) of B in shoots and grain and grain yield (g $plot^{-1}$) for historically important Australian wheat varieties when grown at Two Wells in 1985 and 1986. (Wl*MMC) was included as a standard but excluded from statistical analysis.

Variety	B conc (mg kg^{-1})			Grn yield (g $plot^{-1}$)	
	Shoots 1985	Grain 1985	Grain 1986	1985	1986
Quadrat	36.5	8.47	4.82	85	261
Olympic	38.4	7.68	5.01	111	269
Currawa	39.6	8.43	5.46	90	243
Insignia	40.2	6.57	4.10	123	304
Halberd	41.7	6.32	4.17	165	328
Dundee	49.7	9.65	4.98	53	247
Federation	51.6	7.21	4.33	122	292
Heron	52.5	7.37	4.27	122	286
Pinnacle	57.5	8.69	4.13	121	286
Bencubbin	58.2	11.87	6.29	96	263
Ranee	60.0	10.24	5.74	114	217
Wards Prolific	65.9	15.45	8.59	82	207
Raven	68.3	11.83	6.22	133	268
Condor	70.6	14.73	7.86	124	297
Marshalls No3	73.0	15.17	7.51	78	249
Mec 3	75.4	13.70	7.01	142	250
Gallipoli	75.7	12.90	6.03	76	255
Gluyas	79.4	10.98	7.48	94	244
Purple Straw	79.8	14.02	6.88	82	198
WW-15	82.5	15.45	8.20	105	303
Mexico 120	87.5	13.15	5.68	118	169
Gabo	87.8	15.39	7.87	100	212
Kite	87.8	16.27	9.65	136	278
Siete Cerros	88.1	14.70	7.37	83	188
Gamenya	89.4	15.99	8.84	82	246
Mean	65.5	11.69	6.34	105	254
LSD (0.05)	14.0	1.53	1.26	34	80
(Wl*MMC)	109.3	17.58	6.72	99	228

Figure 5.1 Relationship between date of release of varieties and grain yield (g plot $^{-1}$) for 20 varieties grown at Two Wells in 1985 and 1986. Yields are the means of six replicates in 1985 and seven replicates in 1986.


1985
1986
y = - 1019.3 + 0.66200x R^2 = 0.408
y = - 823.01 + 0.48001x R^2 = 0.355
Halberd
Insignia
Federation
Gamenya
Gabo
Halberd
Federation
Insignia
Gabo
Gamenya
Grain yield (g/plot)
Year of release
400
300
200
100
0
1840
1860
1880
1900
1920
1940
1960
1980

The concentrations of B in shoots and grain in 1985 and in grain between seasons were significantly correlated (Figure 5.2). Thus, B concentration in grain is a highly heritable character and, as found in Chapter 4, the concentration of B in grain reflects the uptake of B by the plant. B concentrations were consistently low for a number of varieties, including (by date of release) Federation, Currawa, Dundee, Quadrat, Insignia, Pinnacle, Olympic, Heron and Halberd. Concentrations of B in shoots have been included in the the pedigree diagram (Fig 5.3) where it can be seen that the low B varieties have either Federation or Currawa, or both of these varieties, in their ancestry. B concentrations of Bencubbin, Gabo and Gamenya and also the Mexican semi-dwarf introductions and the Australian semi-dwarf varieties, Condor and Kite (not included in Fig. 5.3, see Table 5.2), were high. There were no varieties which had a lower concentration of B than Halberd, while the B concentration in shoots and grain of (Wl*MMC) in 1985 was greater than for all other varieties in 1985.

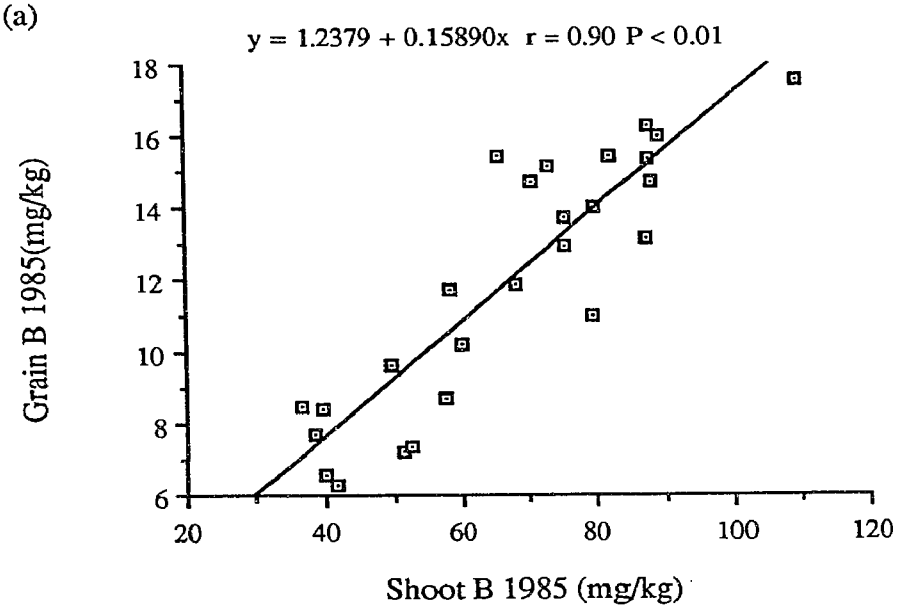
This experiment has demonstrated a marked familial effect upon the concentration of B in plant tissues and the response was consistent over seasons. The low B concentrations could be traced to Federation and Currawa, however the genetic source(s) of low B for these two varieties was not identified. As there was a significant effect of date of release of varieties upon grain yield, with the yields of the more recent varieties being greater, the correlation between concentration of B in tissues and grain yield was not determined for this experiment. However, tolerance to high concentrations of B has previously been shown to be associated with low concentrations of B in tissues for a limited number of genotypes grown in pots in a glasshouse (Paull, 1985; Paull *et al.*, 1988; Chapter 4) and these field experiments provide the basis for a comparison of the response to B, between the glasshouse and naturally occurring high B conditions in the field, over a larger number of varieties.

Figure 5.2 Correlation between concentration of B in shoots and grain for 26 varieties grown at Two Wells in 1985 and 1986.

(a) concentration of B in shoots, 1985 v B in grain, 1985 and

(b) concentration of B in grain, 1985 v B in grain, 1986.

(a)


y = 1.2379 + 0.15890x r = 0.90 P < 0.01
Grain B 1985(mg/kg)
Shoot B 1985 (mg/kg)
18
16
14
12
10
8
6
20
40
60
80
100
120

(b)

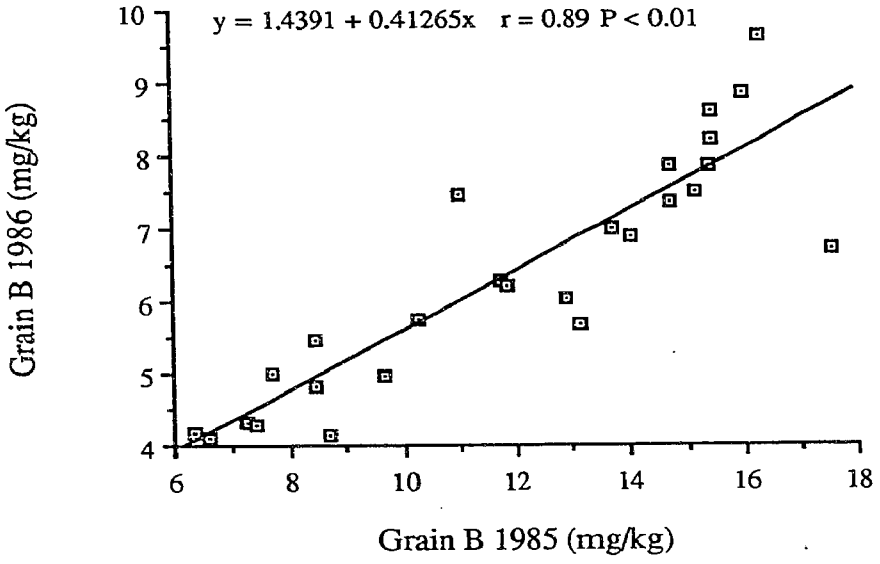
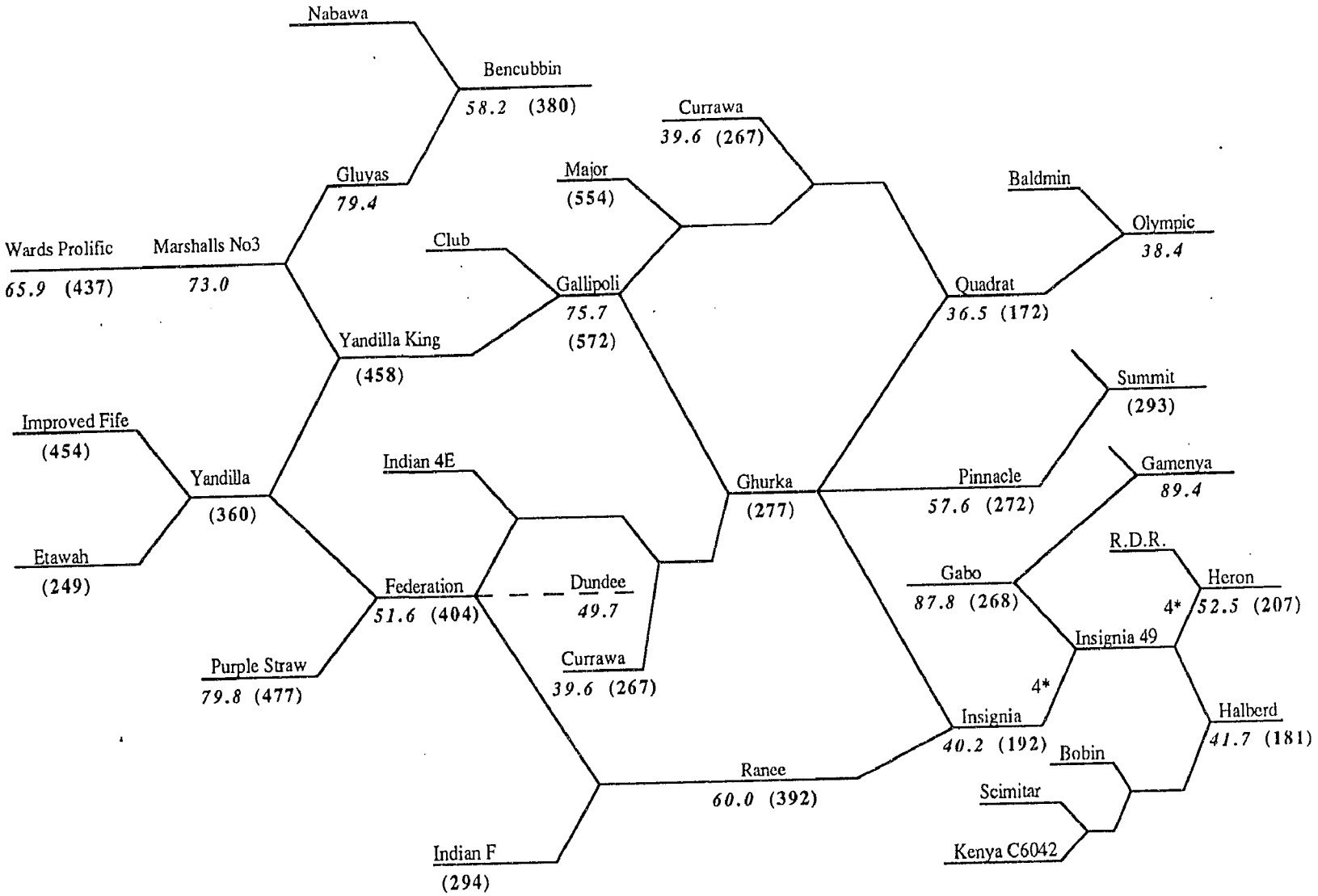

y = 1.4391 + 0.41265x r = 0.89 P < 0.01
Grain B 1986 (mg/kg)
Grain B 1985 (mg/kg)
10
9
8
7
6
5
4
6
8
10
12
14
16
18

Figure 5.3 Concentration of B in shoots (mg kg^{-1}) of historically important Australian wheat varieties. Data for varieties grown at Two Wells, 1985 in italics and for the B50 treatment of a pot experiment in brackets. Pedigree diagram based on that of Wrigley and Rathjen (1981).


Nabawa
Bencubbin
58.2 (380)
Currawa
39.6 (267)
Gluyas
79.4
Major
(554)
Baldmin
Olympic
38.4
Wards Prolific
65.9 (437)
Marshalls No3
73.0
Club
Gallipoli
75.7
(572)
Quadrat
36.5 (172)
Yandilla King
(458)
Summit
(293)
Improved Fife
(454)
Indian 4E
Gamenya
89.4
Yandilla
(360)
Ghurka
(277)
Pinnacle
57.6 (272)
R.D.R.
Etawah
(249)
Gabo
87.8 (268)
Heron
52.5 (207)
4*
Federation
51.6 (404)
Dundee
49.7
Insignia 49
Purple Straw
79.8 (477)
Currawa
39.6 (267)
4*
Insignia
40.2 (192)
Halberd
41.7 (181)
Bobin
Rance
60.0 (392)
Scimitar
Indian F
(294)
Kenya C6042

5.3 RESPONSE OF HISTORICALLY IMPORTANT AUSTRALIAN WHEAT VARIETIES TO HIGH CONCENTRATIONS OF BORON IN A POT EXPERIMENT.

5.3.1 Introduction

The field experiments conducted at Two Wells identified a number of varieties, of common ancestry, as having low concentrations of B in their tissues. The highly significant effect of date of release upon the yield of varieties precluded the calculation of meaningful correlations between B concentration in tissues and grain yield. In the absence of comprehensive data on the relationship between response to high concentrations of B in pots and in the field over a large number of varieties it was not possible to conclude with certainty that the varieties with lowest B concentrations were the most tolerant. A selection of the varieties grown in the field was therefore grown in a glasshouse pot experiment to determine their relative responses to B *per se*.

Several varieties not grown in the field experiment were also included to further investigate the significance and source(s) of the low B concentrations characteristic of the varieties derived from Federation and Currawa.

This experiment also allowed the testing of parameters identified in Chapter 4 as being indicative of the response to B over a larger number of varieties. The response of varieties could be compared between treatments in the pot experiment and between glasshouse and field conditions.

5.3.2 Materials and methods

Varieties

Twenty eight varieties or breeding lines were included in this experiment (Table 5.3), 18 of which were common to the experiments conducted at Two Wells in 1985 and 1986. The seed of all Australian varieties was obtained from the AWCC and full details of the varieties are found in Table 5.1.

Soil

B0, B50 and B100 soil was weighed into 200 mm diameter pots (3.6 kg pot-1).

Experimental design

The pots of the 28 varieties and three treatments were combined factorially and arranged as a randomized complete block design of three replicates. Four pre-germinated seeds were sown per pot and thinned to three plants after two weeks. Plants were grown to maturity.

The height of plants and number of tillers per plant were measured three and five weeks after sowing and the number of ears emerged was recorded weekly. When the main shoots of the control plants were at or near the boot stage, the first tiller was removed from each plant at the B0 and B50 treatments. All samples were collected on the same day. The three tillers per pot were bulked and analyzed for concentration of B. The B100 treatment was not sampled as not all plants had produced tillers. Plants were harvested at maturity and oven dried prior to weighing. Total dry matter yields, including the weight of the tiller sampled for tissue analysis, and grain yields were determined.

5.3.3 Results

At maturity there were highly significant variety and treatment effects and variety x treatment interactions for both total dry matter yield (Table 5.3) and grain yield (Table 5.4). As genotypes were of different vigour and produced different yields at the control, results are also expressed as the yield at the applied B treatments relative to the yield at B0 (Tables 5.3 & 5.4). Genotypes are ordered according to relative total dry matter yield at B100. The grain yield of several varieties was greater at B100 than B50. This could be accounted for by the combined effects of harvesting one tiller per pot at the B0 and B50 treatments, but not at B100, and delayed development and compensatory growth of plants at B100.

In general, the relative response of varieties was similar for grain and total dry matter yield. The correlation between the two parameters at B100, excluding the sensitive (Wl*MMC) and Kenya Farmer, was $r = 0.81$ ($P < 0.001$) and for the full set of

Table 5.3 Total dry matter yield (g pot^{-1}) and relative dry matter yield (yield at B50 / yield at B0) and (B100 / B0) for the historically important Australian varieties grown in a pot experiment at three B treatments. Varieties are ordered according to relative dry matter yield (B100/B0).

Variety	Total dry matter yield (g pot^{-1})				Relative yield	
	B0	B50	B100	Mean	B50	B100
Pinnacle	22.3	23.1	19.6	21.7	1.04	0.88
Quadrat	22.6	20.1	18.9	20.5	0.89	0.84
Halberd	22.8	20.1	18.9	20.6	0.88	0.83
Summit	22.4	20.7	18.7	20.6	0.92	0.83
Heron	22.0	19.9	18.0	20.0	0.90	0.82
Ghurka	22.6	19.3	18.6	20.2	0.85	0.82
Improved Fife	28.1	25.2	22.6	25.3	0.90	0.80
Yandilla King	26.4	20.9	20.8	22.7	0.79	0.79
Olympic	23.1	21.3	17.9	20.8	0.92	0.77
Condor	20.3	17.9	15.5	17.9	0.88	0.76
Insignia	23.6	21.0	17.8	20.8	0.89	0.75
Major	24.5	19.0	18.2	20.6	0.78	0.74
Purple Straw	25.8	22.5	19.0	22.4	0.87	0.74
Indian F	24.0	20.4	17.6	20.7	0.85	0.73
Federation	24.7	21.0	18.1	21.3	0.85	0.73
Gallipoli	25.6	18.4	18.7	20.9	0.72	0.73
Bencubbin	27.4	23.1	19.1	23.2	0.84	0.70
Currawa	23.4	20.0	16.1	19.8	0.85	0.69
Etawah	19.0	17.9	13.2	16.7	0.94	0.69
Ranee	26.1	21.1	17.9	21.7	0.80	0.69
Gabo	23.2	18.9	15.4	19.2	0.81	0.66
Aroona	24.9	16.8	15.5	19.1	0.67	0.62
WW-15	24.5	19.8	15.0	19.8	0.81	0.61
Wards Prolific	21.0	17.6	12.4	17.0	0.84	0.59
Yandilla	23.6	19.5	13.3	18.8	0.83	0.56
Siete Cerros	24.0	18.5	10.9	17.8	0.77	0.45
(Wl*MMC)	20.1	17.2	3.9	13.7	0.86	0.19
Kenya Farmer	24.7	3.6	0.2	9.5	0.15	0.01
Mean	23.7	19.5	16.1			

LSD (0.05) Varieties 1.6, Treatments 0.5, Interaction 2.8

Table 5.4 Grain yield (g pot^{-1}) and relative grain yield (yield at B50 / yield at B0) and (B100 / B0) for the historically important Australian varieties grown in a pot experiment at three B treatments. Varieties are ordered according to relative dry matter yield (B100/B0) in Table 5.3.

Variety	Grain yield (g pot^{-1})				Relative yield	
	B0	B50	B100	Mean	B50	B100
Pinnacle	7.04	6.41	6.06	6.50	0.91	0.86
Quadrat	6.47	4.92	5.18	5.52	0.76	0.80
Halberd	6.15	4.65	6.35	5.72	0.76	1.03
Summit	6.68	5.73	5.88	6.10	0.86	0.88
Heron	6.35	4.90	5.94	5.73	0.77	0.94
Ghurka	6.55	4.81	5.00	5.45	0.73	0.76
Improved Fife	5.06	4.73	3.77	4.52	0.93	0.75
Yandilla King	6.01	4.90	5.44	5.45	0.82	0.91
Olympic	7.05	5.35	5.24	5.88	0.76	0.74
Condor	7.57	6.70	4.63	6.30	0.89	0.61
Insignia	7.29	6.02	4.94	6.08	0.83	0.68
Major	6.43	3.85	4.58	4.95	0.60	0.71
Purple Straw	7.97	6.75	5.27	6.66	0.85	0.66
Indian F	5.84	3.97	5.52	5.11	0.68	0.95
Federation	8.50	5.84	6.07	6.80	0.69	0.71
Gallipoli	7.88	6.13	5.67	6.56	0.78	0.72
Bencubbin	6.92	5.12	5.47	5.84	0.74	0.79
Currawa	5.46	4.51	3.61	4.53	0.83	0.66
Etawah	6.28	5.39	4.29	5.32	0.86	0.68
Ranee	8.43	6.52	6.22	7.06	0.77	0.74
Gabo	7.07	4.85	4.04	5.32	0.69	0.57
Aroona	8.51	4.95	5.20	6.22	0.58	0.62
WW-15	9.19	6.13	5.72	7.01	0.67	0.62
Wards Prolific	7.14	3.56	3.53	4.74	0.50	0.49
Yandilla	6.94	5.35	3.48	5.26	0.77	0.50
Siete Cerros	9.54	6.34	3.69	6.52	0.66	0.39
(WI*MMC)	6.12	5.20	0.53	3.95	0.85	0.09
Kenya Farmer	6.52	0.29	0.00	2.27	0.04	0.00
Mean	7.03	5.14	4.69			

LSD (0.05) Varieties 1.07, Treatments 0.35, Interaction 1.85

genotypes was r = 0.93 (P < 0.001). Grain yield was more sensitive than total dry matter production to high concentrations of B and the mean relative yields of grain and total dry matter (B100/B0), over all genotypes, were 0.67 and 0.76, respectively. The grain yields of Yandilla King and Indian F, at B100, were higher than expected on the basis of total dry matter production. These two varieties were among the latest maturing, even at the control (see later, Table 5.6).

All of the historically important varieties were more tolerant than (WI*MMC), which was included as a sensitive standard, while Kenya Farmer was more sensitive than (WI*MMC). There were probably several levels of tolerance within the majority of the varieties but an attempt to divide them into classes on the basis of yield data would be based on quite arbitrary divisions.

The concentrations of B in tillers at the B0 and B50 treatments were logarithmically transformed to standardize residuals prior to statistical analysis. The variety and treatment effects and variety x treatment interaction were highly significant. While there were significant differences between varieties for the concentration of B in tillers at B0, the B concentrations at B0 and B50 were not significantly correlated (r = 0.24, P > 0.05). Therefore, the B0 results did not predict the response to high concentrations of B and discussion of B concentrations of tillers will be restricted to the B50 treatment.

The B concentrations in tillers are presented in Table 5.5 where varieties are ordered as for yield results (Tables 5.3 & 5.4). Concentrations for varieties in common with the pedigree diagram for the field experiment (Section 5.2) have also been included in Figure 5.3. The B tolerant genotypes were also the lowest for concentration of B in tillers at the B50 treatment and the B concentrations for Quadrat, Heron, Olympic, Insignia, Etawah, Ghurka, Pinnacle, Currawa and Gabo were not significantly greater than for Halberd. All of these low B varieties, with the exception of Gabo, are interrelated (Fig 5.3). The correlation between B concentration at B50 and dry matter yield at B100 relative to the B0 control (B100/B0) was r = 0.53 (P<0.01).

Among the more tolerant varieties with respect to dry matter yield at B100 were Improved Fife and Yandilla King, for which the B concentrations of tillers were much higher than would be expected on the basis of yield at the high B treatment. These two

Table 5.5 Concentration of B (mg kg^{-1}) in tillers at the B0 and B50 treatments for the historically important Australian wheat varieties grown in a pot experiment. Varieties are classified according to family relationships and ordered according to relative dry matter yield (B100/B0) in Table 5.3. Significance levels refer to logarithmically transformed data in brackets.

Variety	Family[a]	B conc (mg kg^{-1})				
		B0		B50		Mean
Pinnacle	3	5.50	(0.74)	272	(2.43)	(1.59)
Quadrat	3	4.07	(0.61)	172	(2.20)	(1.41)
Halberd	3	6.54	(0.78)	181	(2.25)	(1.52)
Summit	3	4.92	(0.68)	293	(2.46)	(1.57)
Heron	3	6.18	(0.79)	207	(2.32)	(1.56)
Ghurka	3	4.07	(0.59)	277	(2.42)	(1.51)
Improved Fife	1	6.38	(0.80)	454	(2.65)	(1.73)
Yandilla King	4	5.17	(0.70)	458	(2.66)	(1.68)
Olympic	3	5.98	(0.77)	204	(2.29)	(1.53)
Condor	5	5.12	(0.70)	331	(2.51)	(1.61)
Insignia	3	3.95	(0.58)	192	(2.27)	(1.43)
Major	4	4.62	(0.67)	554	(2.73)	(1.70)
Purple Straw	1	9.49	(0.90)	477	(2.67)	(1.79)
Indian F	2	5.26	(0.71)	294	(2.46)	(1.59)
Federation	3	8.66	(0.92)	404	(2.59)	(1.76)
Gallipoli	4	4.70	(0.67)	572	(2.74)	(1.71)
Bencubbin	4	5.14	(0.71)	380	(2.57)	(1.64)
Currawa	3	5.27	(0.71)	267	(2.43)	(1.57)
Etawah	2	6.19	(0.79)	249	(2.38)	(1.59)
Ranee	3	6.71	(0.80)	392	(2.59)	(1.70)
Aroona	5	4.80	(0.66)	337	(2.51)	(1.59)
Gabo	4	4.98	(0.70)	268	(2.43)	(1.57)
WW-15	5	5.35	(0.72)	287	(2.46)	(1.59)
Wards Prolific	1	6.57	(0.82)	437	(2.63)	(1.73)
Yandilla	4	7.27	(0.86)	360	(2.56)	(1.71)
Siete Cerros	5	5.91	(0.76)	496	(2.69)	(1.73)
(Wl*MMC)	5	4.41	(0.63)	621	(2.79)	(1.71)
Kenya Farmer	4	8.19	(0.89)	476	(2.67)	(1.78)
Mean			(0.74)		(2.51)	

LSD (0.05) Varieties (0.14), Treatments (0.04), Interaction (0.19)

[a] Family classifications

1 19th C introductions and selections - non Indian,
2 Indian introductions,
3 Federation and Currawa and varieties with either Federation (and therefore Indian wheats) or Currawa or both varieties in their ancestry,
4 Tall varieties resulting from systematic breeding but not in category 3,
5 Mexican semi-dwarf introductions and derived varieties.

varieties were later maturing, as measured by time from sowing to ear emergence at the B0 treatment, than the other tolerant varieties (Table 5.6). Similarly, the B concentrations for Major, Purple Straw and Gallipoli, which were also among the latest maturing varieties, were higher than earlier maturing varieties of similar tolerance. On the other hand Etawah, which was early maturing and low in B, was among the more sensitive genotypes. These results suggest that, for pot experiments, later maturing genotypes are at a relative advantage in grain and total dry matter yields with respect to B tolerance. This is consistent with the result of Chapter 4 when growing plants to maturity reduced the relative difference in yield between tolerant and sensitive genotypes.

The genetic source(s) of the low concentration of B for the varieties in the pedigree leading to Halberd is not clear. With the exceptions of Currawa and Bencubbin the genotypes can be divided into five distinct groups based on their pedigrees. These are :

(1) nineteenth Century introductions or selections of non - Indian origin,

(2) Indian introductions,

(3) varieties derived from Federation (and therefore Indian wheats) and Currawa,

(4) tall varieties resulting from cross hybridisation and selection within the progeny and not belonging to category 3 and

(5) Mexican semi-dwarf introductions or varieties derived from a semi-dwarf genotype.

These classifications have been included in Table 5.5. Varieties that have both Federation and Currawa in their ancestries comprise the most tolerant group and in general were more tolerant than either Federation or Currawa. They also have the lowest concentrations of B their tissues. This suggests that alternative genes for tolerance to high concentrations of B may have been derived from Federation (presumably of Indian origin) and from Currawa and when combined result in enhanced tolerance to B.

5.3.4 Comparisons between responses at the vegetative stage and at maturity

The height of plants three and five weeks after sowing was measured (data not presented) while the effect of B treatments upon development was assessed at the stages of tiller initiation and ear emergence (Table 5.6). Varieties are ordered as for yield results.

The height of seedlings was reduced at the B100 treatment relative to the B0 control, but as there were significant differences in height at the control, heights at B100 have also been expressed as a percentage of the control. The correlations between relative height three weeks after sowing and relative dry matter yield (Fig 5.4a) and relative height and B concentration in tillers (Fig 5.4b) were determined. While the correlations between seedling height and yield and seedling height and B concentration were statistically significant, seedling height was not very efficient at discriminating among the more tolerant varieties, although Kenya Farmer was clearly identified as being the most sensitive variety. The correlation between relative seedling height in pots (B100/B0) and B concentration of shoots at Two Well in 1985 was also determined and was statistically significant (Fig 5.4c) and is discussed below.

The low efficiency of seedling height at predicting response may have resulted from the combined effects of genetic differences in height and the relatively small difference in response to B over the majority of varieties included in this experiment, relative to the total range in response to B which has been identified for wheat by Moody *et al.* (1988). All varieties, with the exception of (Wl*MMC) and Kenya Farmer would be classified as either moderately tolerant or moderately sensitive by the rating of Moody *et al.* (1988). It is probable that the efficiency of seedling height as a predictor of tolerance would increase as the genetic range in response among genotypes increased.

The absence of tillering at the B50 treatment three weeks after sowing and at B100 five weeks after sowing identified the more sensitive varieties (Table 5.6). By week five all varieties except Kenya Farmer had commenced tillering at B50. The late maturing variety, Gallipoli, which had a high relative yield for the concentration of B in tissues, had not tillered at either time of scoring, while Etawah, which was early maturing and of low relative yield despite a low concentration of B in tillers, had tillered (Table 5.6).

Ear emergence was delayed by the B50 and B100 treatments for all varieties except Olympic at B50. Ear emergence of (Wl*MMC) and Kenya Farmer was delayed to a greater extent than for other varieties, and at B100 Kenya Farmer died during vegetative growth. For the other 26 varieties there was no obvious relationship between tolerance to B and delay in ear emergence. Rather, the delay at B100 appeared to be related to the time

Figure 5.4 Correlations between relative seedling height (height at B100/height at B0) three weeks after sowing and

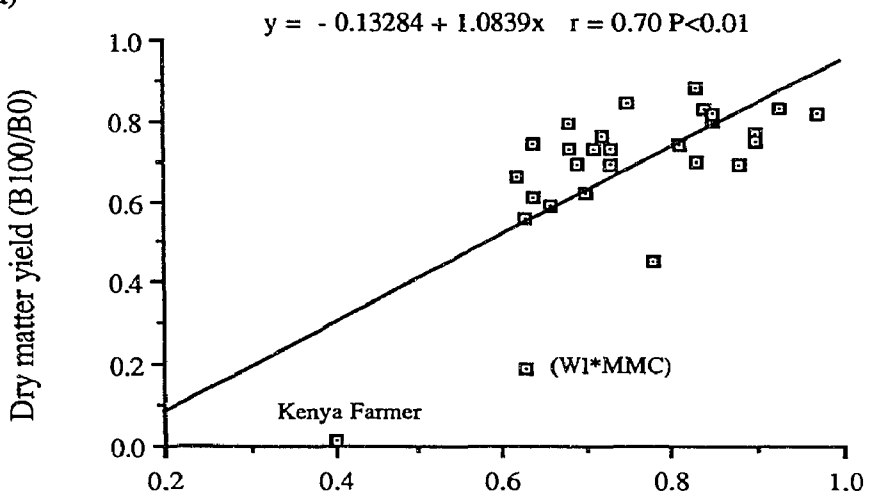
(a) relative dry matter yield (yield at B100/yield at B0) at maturity,

(b) concentration of B in tillers for plants grown at the B50 treatment and

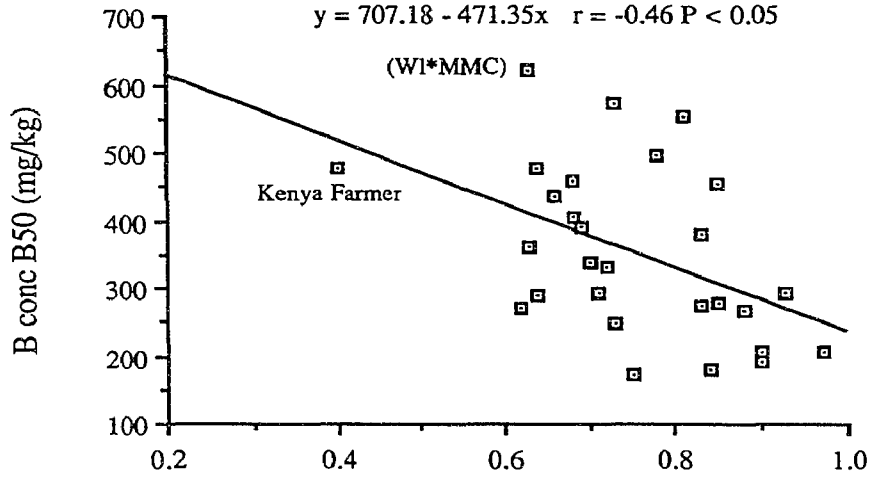
(c) concentration of B in shoots at Two Wells in 1985

for varieties of historically important Australian wheat varieties.

(a)


y = - 0.13284 + 1.0839x r = 0.70 P<0.01
Dry matter yield (B100/B0)
1.0
0.8
0.6
0.4
0.2
0.0
0.2
0.4
0.6
0.8
1.0
(W1*MMC)
Kenya Farmer

(b)


y = 707.18 - 471.35x r = -0.46 P < 0.05
B conc B50 (mg/kg)
700
600
500
400
300
200
100
0.2
0.4
0.6
0.8
1.0
(W1*MMC)
Kenya Farmer

(c)

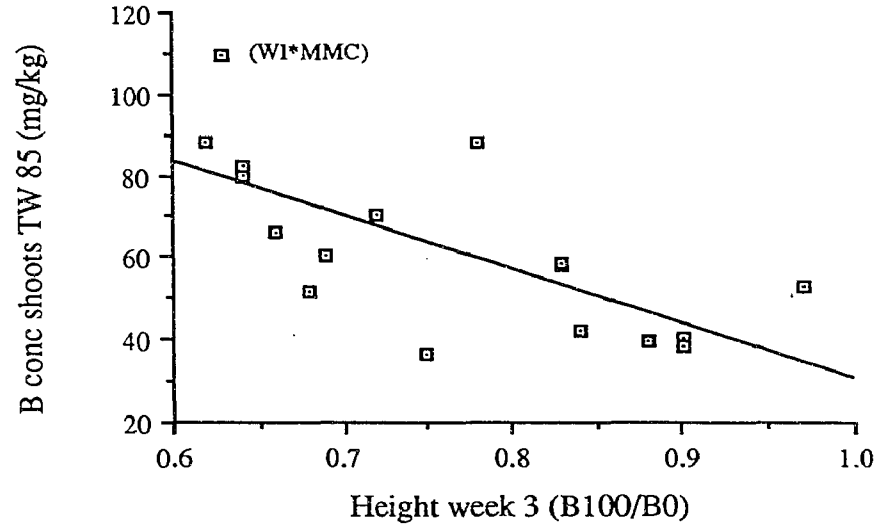

y = 162.51 - 131.36x r = -0.69 P < 0.01
B conc shoots TW 85 (mg/kg)
120
100
80
60
40
20
0.6
0.7
0.8
0.9
1.0
(W1*MMC)
Height week 3 (B100/B0)

Table 5.6 Effect of B treatments upon development of historically important Australian wheat varieties. The presence of tillers was scored three and five weeks after sowing for the B50 and B100 treatments, respectively. Days to ear emergence for the B50 and B100 treatments is expressed as days after the B0 control. Varieties are ordered according to relative dry matter yield (B100/B0) in Table 5.3.

Variety	Tiller initiation		Days to ear emergence		
	B50 Week 3	B100 Week 5	B0	B50	B100
Pinnacle	+	+	68	3	7
Quadrat	+	+	64	3	11
Halberd	+	+	55	3	13
Summit	+	+	63	3	4
Heron	+	+	60	4	7
Ghurka	+	+	57	7	14
Improved Fife	+	+	83	6	9
Yandilla King	+	+	74	2	13
Olympic	+	+	68	0	4
Condor	+	-	54	8	17
Insignia	+	+	62	2	7
Major	+	+	69	2	12
Purple Straw	+	+	71	7	14
Indian F	-	+	68	4	10
Federation	+	+	61	8	14
Gallipoli	-	-	72	5	16
Bencubbin	-	+	68	6	10
Currawa	+	+	63	2	7
Etawah	+	+	50	6	13
Ranee	+	+	66	2	6
Gabo	-	-	55	6	16
Aroona	+	+	47	8	18
WW-15	+	+	54	6	15
Wards Prolific	-	-	54	11	17
Yandilla	+	-	50	6	16
Siete Cerros	-	-	54	4	15
(Wl*MMC)	-	-	60	11	-
Kenya Farmer	-	-	61	24	-

to ear emergence at the control. In general, the delay in ear emergence was greater for early than late maturing varieties. The average delay in ear emergence, at B100, for varieties which reached ear emergence at B0 less than 60 days after sowing was 15.4 days (mean of 10 varieties) while for the later maturing group the average delay was only 9.4 days (mean of 16 varieties). Thus, the effect of B treatment on the rate of development as a predictor of response to B was more efficient at the tillering stage than at ear emergence. This finding is consistent with the result for the experiment in Chapter 4 with only three varieties.

5.3.5 Comparisons between responses to high concentration of B in pots and in the field

As the yield results at Two Wells were significantly influenced by the date of release of varieties, yield data at Two Wells are omitted from comparisons of response between the pot and field experiments. The concentration of B in shoots at Two Wells and at the B50 treatment of the pot experiment, together with the relative total dry matter yield (B100/B0), relative seedling height (B100/B0) and presence or absence of tillers at B100 are summarised in Table 5.7a. Parameters measured in the field and the pot experiments are compared by correlation coefficient and are included in Table 5.7b.

The concentration of B in shoots for the pot experiment was significantly correlated with concentrations of B in shoots and grain for the field experiments. Similarly, the relative total dry matter yields (B100/B0) of the pot experiment were significantly correlated with concentrations of B in shoots and grain for the field experiment and the varieties having the lowest concentration of B in shoots and grain at Two Wells were the most tolerant to B in the pot experiment. This finding should be equally applicable to the varieties grown at Two Wells but not included in the pot experiment.

The relative height of seedlings at the B100 treatment (B100/B0) was also significantly correlated with B concentrations of tissues at Two Wells (Fig. 5.4c; Table 5.7b) The correlation between seedling height in pots and B concentration at Two Wells was greater than between seedling height and mature response in the pot experiment. The

Table 5.7(a) Comparison between response to high concentrations of B at Two Wells in 1985 and in a glasshouse pot experiment for historically important Australian wheat varieties. Varieties are ordered according to relative dry matter yield (B100/B0) in the pot experiment.

Variety	B conc shoots (mg kg^{-1})		DM yield	Vegetative response[a]	
	TW'85	Pot B50	B100/B0	Hght Wk3 B100/B0	Till Wk5 B100
Pinnacle	57.5	272	0.88	0.83	+
Quadrat	36.5	172	0.84	0.75	+
Halberd	41.7	181	0.83	0.84	+
Heron	52.5	207	0.82	0.97	+
Olympic	38.4	204	0.77	0.90	+
Condor	70.6	331	0.76	0.72	-
Insignia	40.2	192	0.75	0.90	+
Purple Straw	79.8	477	0.74	0.64	+
Federation	51.6	404	0.73	0.68	+
Gallipoli	75.7	572	0.73	0.73	-
Bencubbin	58.2	380	0.70	0.83	+
Currawa	39.6	267	0.69	0.88	+
Ranee	60.0	392	0.69	0.69	+
Gabo	87.8	268	0.66	0.62	-
WW-15	82.5	287	0.61	0.64	+
Wards Prolific	65.9	437	0.59	0.66	-
Siete Cerros	88.1	496	0.45	0.78	-
(WI*MMC)	109.3	621	0.19	0.63	-

[a] Vegetative responses in the pot experiment; Relative plant height (B100/B0) 3 weeks after sowing and +/- tillers at B100 5 weks after sowing.

Table 5.7(b) Correlation coefficients (r) for comparisons between plant growth parameters measured at high B concentrations in pots and the field.

Pot variable	B conc Two Wells		
	Shoots '85	Grain '85	Grain '86
B conc shoots B50	0.76	0.59	0.47
Dry matter yield B100/B0	-0.75	-0.71	-0.54
Height week 3 B100/B0	-0.74	-0.74	-0.68

LSD (0.05) r = 0.47, (0.01) r = 0.59

reason for this may lie in the interaction between time of maturity, delay in development and compensatory growth at high B treatments for pot experiments which together have the effect of reducing the difference in response between moderately sensitive and moderately tolerant varieties in pots.

The incidence of tillering at the B100 treatment five weeks after sowing coincided with genotypes with lower concentrations of B in shoots at Two Wells. All varieties, except Purple Straw and WW-15, with greater than 60 mg kg^{-1} in shoots at Two Wells had not tillered at B100, five weeks after sowing. There are many lines of Purple Straw and while the accession grown in the pot experiment is known the identity of the Purple Straw grown in the field is uncertain and the two may have been different. The significance of this will become apparent in a following experiment examining the variation in response to B for accessions of Purple Straw.

There was generally good agreement for the response of varieties between field and pot experiments, however the results for Federation and Gabo were inconsistent between cultural conditions. For the field experiments the concentration of B in shoots and grain of Federation was similar to Currawa, Dundee, Quadrat, Insignia, Heron and Halberd, while the B concentration of shoots of Federation in the pot experiment was approximately twice that of the most tolerant varieties. The converse was the case for Gabo. There is no ready explanation for the variable response between cultural conditions but the significance of Federation as a possible source of B tolerance, as suggested by the field results, was the subject of a more detailed experiment.

5.4 THE GENETIC SOURCE OF BORON TOLERANCE FOR AUSTRALIAN WHEAT VARIETIES.

5.4.1 Introduction

The experiments examining the B tolerance of wheat varieties of historical importance in Australia identified a genetically related group which were more tolerant with respect to yield in the pot experiment and of lower B concentrations in tissues when grown at high B supply in pots and in the field (Fig. 5.3, Table 5.5). These varieties have both Federation and Currawa in their ancestry but the initial source of their genetic tolerance is not known as their ancestors have not been investigated at this stage. In the field experiment the B concentrations in shoots and grain of Federation and Currawa were similar to their B tolerant descendants, however, several of these varieties were more tolerant than Federation and Currawa when grown in pots.

The results of the pot experiment suggest that Federation and Currawa might have alternative genes for B tolerance and when combined result in enhanced tolerance in progeny such as Insignia and Halberd. The B tolerance of Ghurka is central to the hypothesis of alternative sources of tolerance as it represents the combining of genes from Federation and Currawa. In the pot experiment Ghurka was one of the most tolerant varieties, both with respect to yield and the concentration of B in tillers. As there were a large number of varieties grown, the pot experiment had a low number of treatments and replicates and so the results are not particularly accurate. The method employed for sampling tissues for B analysis, namely harvesting one tiller per plant, and growing plants to maturity and so minimising differences in yield response between moderately tolerant and moderately sensitive genotypes (see Chapter 4) may also have limited the accuracy of the results. A more detailed study was therefore conducted for the varieties immediately before and after the combining of Federation and Currawa to test the hypothesis that alternative sources of tolerance were combined to give the level of tolerance observed in recent Australian varieties.

5.4.2 Materials and methods

Varieties

Federation, Currawa, Indian F, Indian 4, Ranee, Gallipoli, Ghurka and Insignia. All seed was obtained from the AWCC. Full details of these varieties are given in Table 5.1. (The line Indian 4E, which is a parent of Ghurka, is not held at the AWCC. The line Indian 4 was included in this experiment although it might not correspond to Indian 4E.)

B treatments

B0, B50, B100 and B150 soil was weighed into 125 mm diameter pots (850 g pot^{-1}).

Experimental design

The pots of the eight varieties and four B treatments were combined factorially and arranged as a randomized complete block design with five replicates. Three pre-germinated seeds were sown per pot and thinned to two plants after 12 days. Plants were harvested eight weeks after sowing, rinsed with de-ionised water and oven dried. Plants were weighed and the shoots of plants grown at the B50 treatment were analyzed for B concentration.

5.4.3 Results

The B treatments were deliberately chosen to include very high concentrations as it was expected that these levels would be required to distinguish between varieties at the tolerant end of the spectrum of response to B. The B treatments proved to be unexpectedly toxic and dry matter production of all varieties was significantly reduced at the B50 treatment, while at B150 the most tolerant varieties produced only 10% of the total dry matter of the B0 control.

The yield data (Table 5.8) were treated with a square root transformation before testing by analysis of variance. Currawa, Ghurka and Insignia were the most tolerant varieties and yielded significantly more than all other varieties except Federation at B100

Table 5.8 Dry matter yield (g pot^{-1}) for a pot experiment to identify the genetic source of tolerance to B for Australian wheat varieties. Significance levels refer to square root transformed data in brackets.

Variety	Dry matter yield (g pot $^{-1}$)								
	B0		B50		B100		B150		Means
Indian 4	2.68	(1.63)	1.72	(1.31)	0.53	(0.72)	0.19	(0.43)	(1.02)
Indian F	3.05	(1.74)	1.65	(1.28)	0.50	(0.70)	0.11	(0.31)	(1.01)
Federation	3.05	(1.75)	1.93	(1.39)	0.60	(0.77)	0.12	(0.34)	(1.06)
Ranee	2.94	(1.71)	1.76	(1.33)	0.40	(0.63)	0.06	(0.23)	(0.98)
Currawa	3.02	(1.74)	1.98	(1.41)	0.94	(0.97)	0.34	(0.58)	(1.17)
Gallipoli	3.06	(1.75)	1.52	(1.23)	0.30	(0.54)	0.06	(0.24)	(0.94)
Ghurka	3.22	(1.79)	2.08	(1.44)	0.81	(0.90)	0.44	(0.66)	(1.20)
Insignia	3.29	(1.81)	2.12	(1.45)	0.82	(0.90)	0.32	(0.57)	(1.18)
Means		(1.74)		(1.36)		(0.77)		(0.42)	

LSD (0.05) Varieties (0.07), Treatments (0.05), Interaction (0.15)

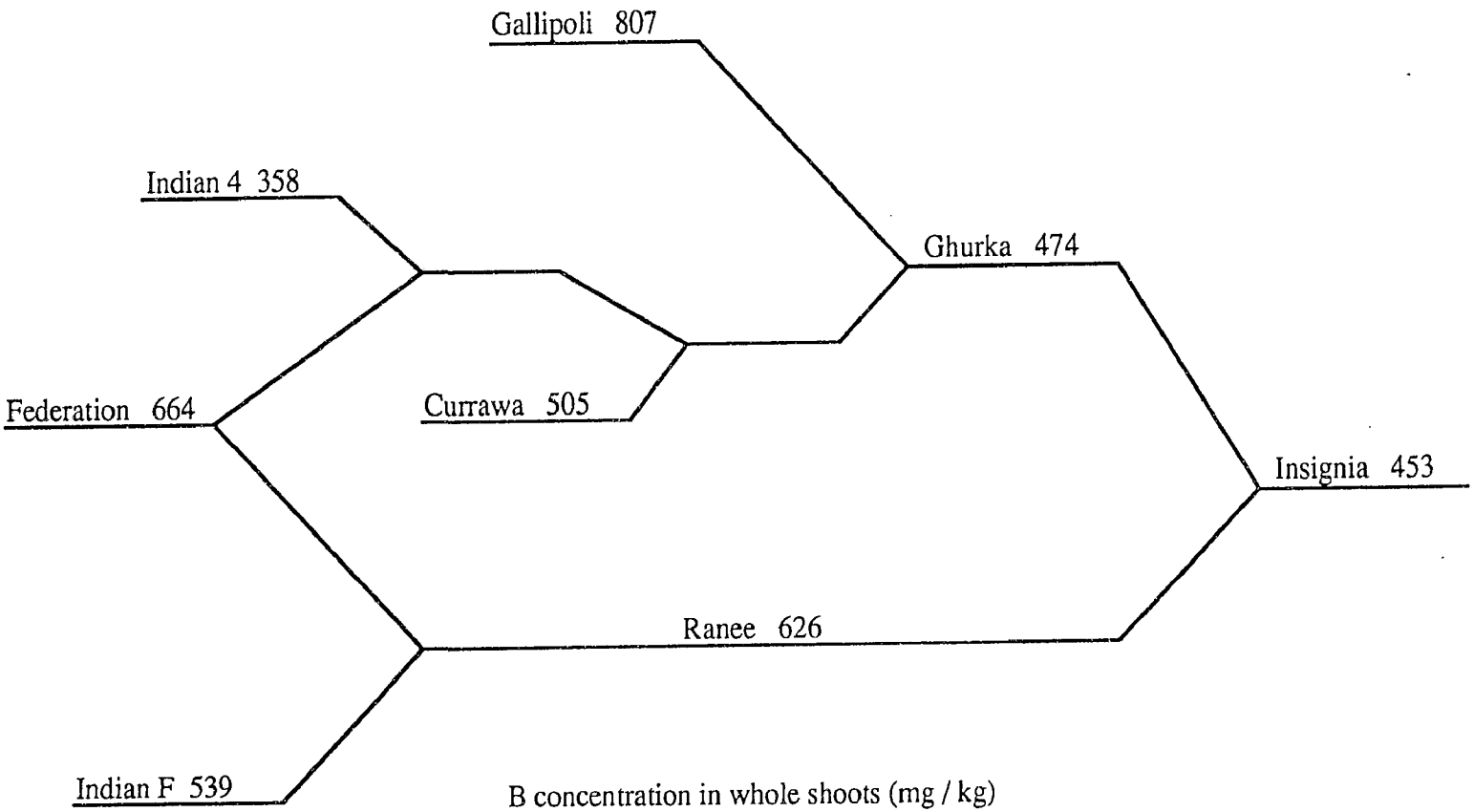
and Indian 4 at B150. Federation, Ranee and the two Indian introductions were intermediate while Gallipoli was the most sensitive variety.

The difference between varieties, for concentrations of B in the shoots, was highly significant and the results have been presented as a pedigree diagram to highlight the genetic relationships between varieties (Fig 5.5). Of the Australian varieties, B concentrations were lowest for Currawa, Ghurka and Insignia, while Federation and Ranee were intermediate and Gallipoli was significantly greater than all other varieties. The concentration of B in shoots of Indian 4 was significantly less than for all varieties except Insignia and Indian F was intermediate to Currawa and Ranee.

The rankings of all varieties, except Indian 4, were consistent for dry matter production and concentration of B in shoots. Indian 4 was intermediate with respect to yield but the B concentration in shoots was very low. The historical significance of the inconsistent response of Indian 4 is uncertain as there is doubt as to whether the particular line grown corresponds to Indian 4E which was a parent of Ghurka. There is no accession named Indian 4E in the AWCC.

Currawa, Ghurka and Insignia responded similarly and were more tolerant than Federation. There was no evidence of enhanced tolerance to B, above that of the parents, subsequent to the combining of Currawa and Federation and the level of tolerance to B exhibited by Ghurka and Insignia could be accounted for, in entirety, by Currawa. The source of tolerance exhibited by Currawa could not be studied as none of its parents are held by the AWCC.

Figure 5.5 Concentration of B in shoots (mg kg^{-1}) for varieties grown at the B50 treatment for an experiment to identify the source of B tolerance for Australian wheat varieties.



B concentration in whole shoots (mg / kg)

LSD (0.05) 97

5.5 TOLERANCE OF THE PARENTS OF HALBERD TO HIGH CONCENTRATIONS OF BORON

5.5.1 Introduction

Halberd and ancestral varieties Insignia and Ghurka have a similar level of tolerance to B and, so far, it has been presumed that tolerance to B was transmitted to Halberd from Insignia, via Insignia 49, although Insignia 49 was not included in the previous experiments. The parents of Halberd, together with Halberd and Insignia, were compared for response to B to confirm that the B tolerance of Halberd was derived from the common set of varieties previously identified as being tolerant to B, rather than one of its other parents.

5.5.2 Materials and methods

Varieties

Insignia, Insignia 49, Bobin, Scimitar, Kenya C6042 and Halberd. All seed was obtained from the AWCC. Full details of these varieties are given in Table 5.1.

B treatments

B0, B25, B50, B100 and B150 soil was weighed into 125 mm diameter pots (850 g pot -1)

Experimental design

The pots of the six varieties and five B treatments were combined factorially and arranged as a randomized complete block design with three replicates. Three pre-germinated seeds were sown per pot and thinned to two plants after two weeks. Whole shoots were harvested at ground level six weeks after sowing. Shoots were rinsed with de-ionized water, oven dried and weighed. Plants grown at the B50 treatment were analyzed for concentration of B.

5.5.3 Results

The varieties differed for response to B and four levels of response could be identified among the parents of Halberd. The yield data were treated with a square root transformation prior to analysis of variance. Halberd, Insignia 49 and Insignia responded similarly and were significantly more tolerant than the other varieties with the possible exception of Bobin (Table 5.9). The ranking of the other parents of Halberd, in order of tolerance, was Bobin, Scimitar and Kenya C6042. Kenya C6042 was extremely sensitive and developed the "mid-leaf necrosis" symptom at the B50 and higher treatments.

The concentration of B in shoots at the B50 treatment (Table 5.9) reflected the response for yield and confirmed that the B tolerance of Halberd was derived from Insignia.

Table 5.9 Dry matter yield (g pot^{-1}) at five B treatments and B concentration of whole shoots at the B50 treatment for Halberd and its parents. Significance levels for the yield results refer to the square root transformed data in brackets.

Variety	Dry matter yield (g pot $^{-1}$)										B conc(mg kg^{-1})
	B0		B25		B50		B100		B150	Means	B50
Halberd	2.93	(1.71)	2.50	(1.57)	1.53	(1.23)	1.15	(1.07)	0.65 (0.80)	(1.28)	322.9
Insignia 49	2.43	(1.56)	2.23	(1.49)	1.77	(1.33)	1.05	(1.02)	0.50 (0.71)	(1.22)	366.1
Bobin	2.37	(1.53)	2.63	(1.61)	1.63	(1.28)	0.73	(0.85)	0.32 (0.56)	(1.17)	466.0
Scimitar	1.97	(1.40)	1.52	(1.22)	1.18	(1.09)	0.48	(0.69)	0.18 (0.42)	(0.96)	473.3
Kenya C6042	2.02	(1.42)	1.03	(1.01)	0.65	(0.67)	0.15	(0.38)	0.10 (0.31)	(0.76)	595.1
Insignia	2.55	(1.59)	2.00	(1.41)	1.87	(1.36)	0.98	(0.99)	0.57 (0.74)	(1.22)	361.1
Means		(1.53)		(1.38)		(0.96)		(0.84)	(0.59)		

LSD (0.05) Varieties (0.09), Treatments (0.08), Interaction (0.20) LSD(0.05)B conc 86.0

5.6 RESPONSE OF ACCESSIONS OF PURPLE STRAW TO HIGH CONCENTRATIONS OF BORON

5.6.1 Introduction

Major problems faced when attempting to trace the genetic source of a character include the loss of genetic material over time and uncertainty as to the line used for hybridisation, particularly when a landrace was used. Examples of such problems encountered in the present study include the absence of the parents of Currawa from the AWCC and the presence of two or more accessions for several of the varieties grown in the pot experiment of historically important varieties (Section 5.3). For that experiment the accession representing the variety was chosen arbitrarily so that if there was variation in response among the accessions of a variety, or landrace, the choice could represent a source of error.

The landrace Purple Straw, which is believed to be of Italian origin, was the most widely cultivated wheat in Australia from the 1860's to the 1890's and is an important ancestor of present day varieties through Federation and Marshall's No 3 (Wrigley and Rathjen, 1981). In the AWCC there are 74 accessions which are either direct selections of Purple Straw (43) or have Purple Straw as a parent (31). The variation in the response to high concentrations of B for the direct selections of Purple Straw is described to illustrate the uncertainty that can be encountered when alternative accessions exist.

5.6.2 Materials and methods

Genotypes

A total of 41 accessions, or direct selections, of Purple Straw, obtained from the AWCC, were grown at a high B treatment and their response was compared with Halberd.

Soil

A large wooden box (100 cm x 75 cm x 30 cm) containing soil enriched with B to a concentration of 150 mg kg^{-1} applied B was used for screening.

Experimental design

Lines were sown as single rows with five seeds per row. Seeds were spaced 3 cm apart within rows and 5 cm between rows. Halberd was included as a standard every fifth row and as only half of the lines were grown at a time, their response was compared to Halberd. The experiment consisted of two replicates. Plants were visually rated for overall vigour and symptom expression and the height from the base of the plant to the tip of the YEB was measured six weeks after sowing.

5.6.3 Results

A wide range in response to B occurred among the accessions and selections of Purple Straw. The results for visual rating and plant height are presented in Table 5.10 while Plate 4 provides a comparison between tolerant and sensitive accessions. The most tolerant lines were at least as tolerant as Halberd and the most sensitive (AUS 2145) developed the "mid-leaf necrosis" symptom characteristic of only the more sensitive wheats.

The variation in response among the accessions could account for the discrepency in results for Purple Straw between the earlier field and pot experiments where there was the possibility that different lines were grown. The accession grown in the pot experiment (AUS 883) proved to be among the more sensitive of the Purple Straw accessions.

It is not possible to draw any definite conclusions regarding the genetic contribution of Purple Straw to the B tolerance of Australian wheat varieties, however, there is certainly the possibility that Purple Straw was the source of B tolerance exhibited by the accession of Federation grown in the field experiment.

Table 5.10 Response of accessions of Purple Straw to high concentrations of B.

AWCC accession number	Visual rating[a]	Height % Halberd	AWCC accession number	Visual rating	Height % Halberd
183	2.5	63	2246	4	76
289	4	93	2247	3.5	76
335	2.5	62	2315	2.5	55
488	3	64	2386	3	59
883[b]	2.5	72	2525	2.5	59
884	3	82	2565	2	59
885	2	67	2909	4	94
886	3	56	3205	3	73
887	4	82	3220	3.5	75
888	3	91	3221	5	91
889	2.5	79	3466	4.5	100
890	3	67	3468	4	97
891	3	82	3469	3	75
892	2.5	68	3470	3	64
895	3	63	3471	3	66
896	5	100	3472	3.5	58
1153	3	64	6956	3	88
1661	2	53	10764	3.5	94
1679	3	73	20717	2.5	54
2145	1	38	21889	3	78
2245	3	80			

[a] Compared to Halberd, scale 1 (sensitive) - 5 (tolerant), Halberd = 4

[b] AUS 883 was used for pot experiment, Section 5.3

Plate 4. Accessions of Purple Straw grown at the B150 treatment and comparison with Halberd.
Left to right : Halberd, accession AUS 3221, AUS 887, AUS 884, AUS 2565 and AUS 2145.


AUS 3921
AUS 684
PURPLE STRAW SELECTIONS
BORON TOXICITY

5.7 RESPONSE OF THE MAJOR WHEAT VARIETIES CURRENTLY GROWN IN SOUTH AUSTRALIA TO BORON.

5.7.1 Introduction

The South Australian wheat crop consists of many varieties and in a typical season between 50 and 60 varieties are delivered to silos (Australian Wheat Board data). Despite the large number of varieties, relatively few are grown over a substantial proportion of the wheat growing areas. Between 1972 and 1987 Halberd was the dominant variety and regularly accounted for more than 30% of the State's total wheat production (Appendix B). The responses of the most widely grown varieties were assessed in a field experiment at a site with high concentrations of B in the sub-soil to provide the information which would allow objective recommendation of varieties for high B regions and also to determine whether any varieties are more tolerant than Halberd. A number of advanced lines of high yield potential from the wheat breeding programs at the Waite Agricultural Research Institute and Roseworthy Agricultural College were also included.

5.7.2 Materials and methods

Genotypes

The named varieties were selected on the basis of silo receivals in 1984/85 and 1985/86 and collectively accounted for approximately 99% of South Australia's wheat production. A total of 40 genotypes were grown in 1985 and 36 genotypes in 1986. Twenty nine genotypes were common to both seasons. Varieties and advanced lines grown are listed in Table 5.11. Details of pedigrees, site of selection and date of release for the named varieties are given in Table 5.1. Six varieties (Condor, Gabo, Halberd, Kite, Olympic and Raven) were common to the experiment of historically important varieties also grown at Two Wells in 1985 and 1986 (Section 5.2). Seed of named varieties was obtained from stocks maintained at the Waite Agricultural Research Institute and seed of advanced lines was obtained from their respective institutes.

Experimental design

The experiments were conducted at Two Wells in 1985 and 1986 in conjunction with the experiment of historically important wheat varieties (Section 5.2) and sown according to the standard procedures for field experiments as described in Chapter 3. The experiments were sown on August 1st in both years.

The experiments were arranged as randomized complete blocks of five replicates in 1985 and six replicates in 1986. The B sensitive genotype (W1*MMC) was grown as a control grid and was included every fifth plot. Results for (W1*MMC) were excluded from statistical analyses, rather they were used to give an indication of edaphic variation.

In 1985, five whole shoots per plot were harvested on October 18th (78 days after sowing) when plants were at or near the boot stage (Zadoks 41-45) and analyzed for concentration of B. Plots were harvested at maturity and grain yields and the concentration of B in the grain were determined.

5.7.3 Results

There were significant differences among varieties for concentration of B in shoots and grain and for grain yield in both seasons (Table 5.11). The concentrations of B in shoots and grain were significantly correlated within and between seasons (Fig 5.6) as was the situation for the experiment of historically important varieties. The concentration of B in tissues was also significantly correlated to grain yield and B concentrations were lowest for the highest yielding varieties (Fig 5.7). The concentrations of other elements in shoots in 1985 and grain in 1986 of the named varieties are given in Appendix D.

The results for varieties in Table 5.11 are ranked by concentration of B in whole shoots for the 1985 experiment. Halberd had the lowest concentration of B in shoots in 1985, and in grain in 1985 and 1986, but the B concentrations for several other varieties, Olympic, Katyil, Spear, Dagger and Millewa, were not significantly greater than for Halberd. Significantly, all but Millewa have ancestors in common with Halberd and are extensions of the family derived from Federation and Currawa. Spear and a sister selection Dagger have become widely grown and Spear has recently overtaken Halberd as the most widely adopted variety in South Australia (Appendix C).

Table 5.11 Concentration (mg kg^{-1}) of B in shoots and grain and grain yield (g $plot^{-1}$) for important South Australian wheat cultivars and advanced breeding lines when grown at Two Wells in 1985 and 1986. (Wl*MMC) was included as a standard but excluded from statistical analysis.

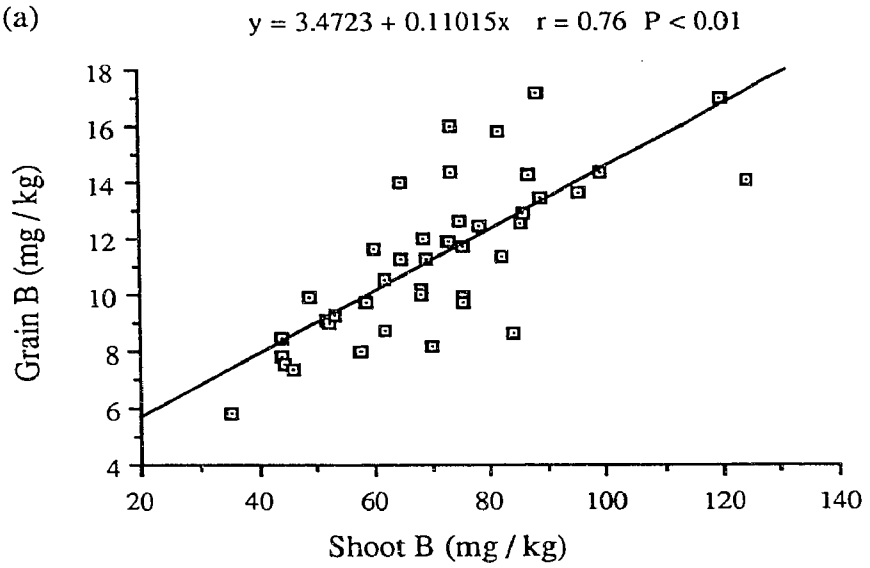
Variety	B conc (mg kg^{-1})			Grain yield (g $plot^{-1}$)	
	Shoots 1985	Grain 1985	Grain 1986	1985	1986
Halberd	35.6	5.84	3.32	291	320
Olympic	44.2	8.48	5.27	291	229
Spear	44.2	7.84	4.57	235	288
Katyil	44.8	7.59	4.37	149	272
Aroona	46.0	7.39	6.07	214	275
Raven	51.8	9.08	-	176	-
Dagger	52.4	9.03	4.95	196	277
Takari	57.5	7.98	5.79	180	218
Millewa	58.4	9.76	5.08	145	316
Sunstar	60.0	11.63	9.15	165	239
Banks	62.0	10.51	-	154	-
Vulcan	64.8	13.99	9.38	206	206
Cook	64.9	11.30	10.01	190	213
Aldirk	68.2	10.19	5.92	195	220
Condor	68.8	12.01	8.82	181	234
Meering	69.1	11.27	6.77	186	243
Kite	73.7	15.96	10.02	152	245
Madden	73.7	14.35	-	138	-
Cranbrook	74.9	12.68	6.36	171	242
Sabre	75.4	11.77	8.48	130	
Warigal	75.5	9.72	6.56	222	322
Schomburgk	75.5	9.89	5.80	227	263
Festiguay	78.4	12.42	7.91	177	217
Gabo	81.9	15.84	-	126	-
Bayonet	82.0	11.40	6.53	134	233
Oxley	85.5	12.51	7.71	175	231
Lance	86.0	12.88	6.43	207	232
Miling	87.1	14.30	8.66	149	234
Bindawarra	88.3	17.15	8.64	185	266
Egret	89.1	13.41	6.14	155	268
Machete	95.5	13.61	7.14	152	276
Warimba	124.8	14.13	6.20	92	202
WARI lines					
(MKR*Kite)	62.1	8.69	-	233	-
(Vd*MM**2)	70.0	8.22	4.28	203	326
Aroona PT7/5	73.0	11.93	-	226	-
(Pit*Fest)	120.3	16.97	-	164	-
(Wq*KP)*WmH)	-	-	4.14	-	448
(Tt2*Wl*3)*Aro)	-	-	4.14	-	291
(Wq*KP)*Bay)	-	-	5.23	-	434
RAC lines					
RAC-495	48.8	9.87	-	135	-
RAC-429	53.3	9.30	-	161	-
RAC-434-12	68.2	9.99	-	176	-
RAC-416	84.0	8.63	-	191	-
RAC-549	-	-	5.94	-	337
RAC-430	-	-	7.35	-	289
RAC-520	-	-	7.60	-	297
LSD (0.05)	30.0	4.20	1.95	62	99
(Wl*MMC)	99.4	14.38	7.35	125	208

Figure 5.6 Correlation between concentration of B in shoots and grain for South Australian wheat varieties and advanced breeding lines grown at Two Wells in 1985 and 1986.

(a) concentration of B in shoots, 1985 v B in grain, 1985 and

(b) concentration of B in grain, 1985 v B in grain, 1986.

(a)


y = 3.4723 + 0.11015x r = 0.76 P < 0.01
Grain B (mg / kg)
Shoot B (mg / kg)
18
16
14
12
10
8
6
4
20
40
60
80
100
120
140

(b)

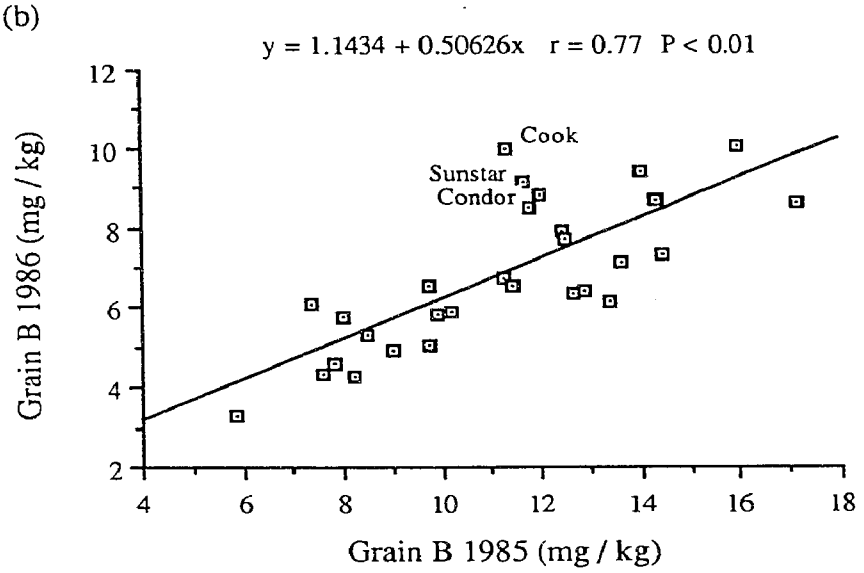

y = 1.1434 + 0.50626x r = 0.77 P < 0.01
Grain B 1986 (mg / kg)
Grain B 1985 (mg / kg)
Cook
Sunstar
Condor
12
10
8
6
4
2
4
6
8
10
12
14
16
18

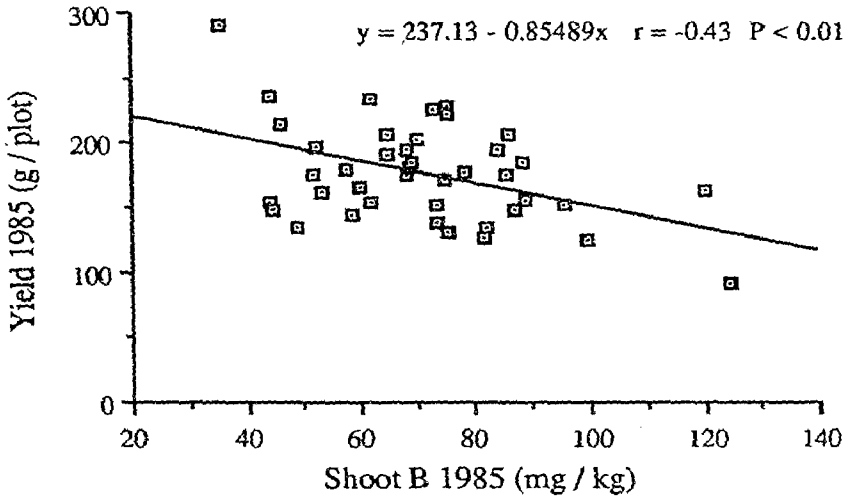
Figure 5.7 Correlations between concentration of B in tissues and grain yield for current South Australian wheat varieties and advanced breeding lines from the Waite Institute and Roseworthy Agricultural College wheat breeding program when grown at Two Wells.

(a) concentration of B in shoots at boot stage v grain yield at Two Wells, 1985,

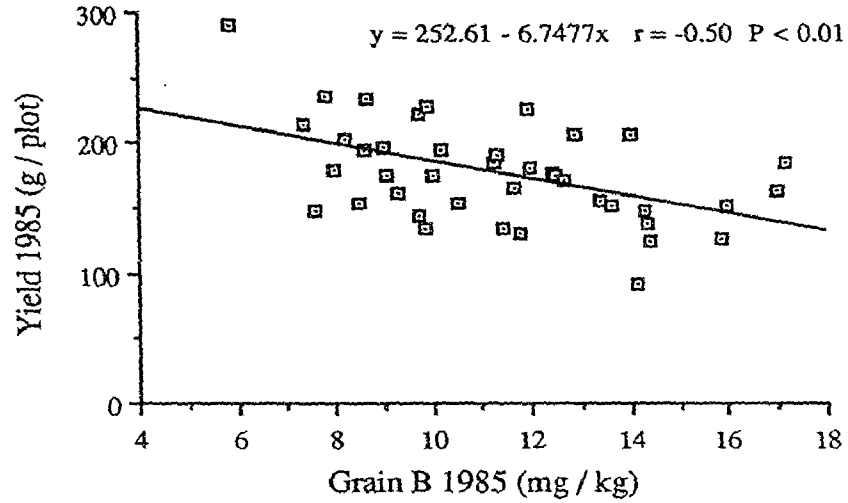
(b) concentration of B in grain, 1985 v grain yield at Two Wells, 1985 and

(c) concentration of B in grain, 1986 v grain yield at Two Wells, 1986 .

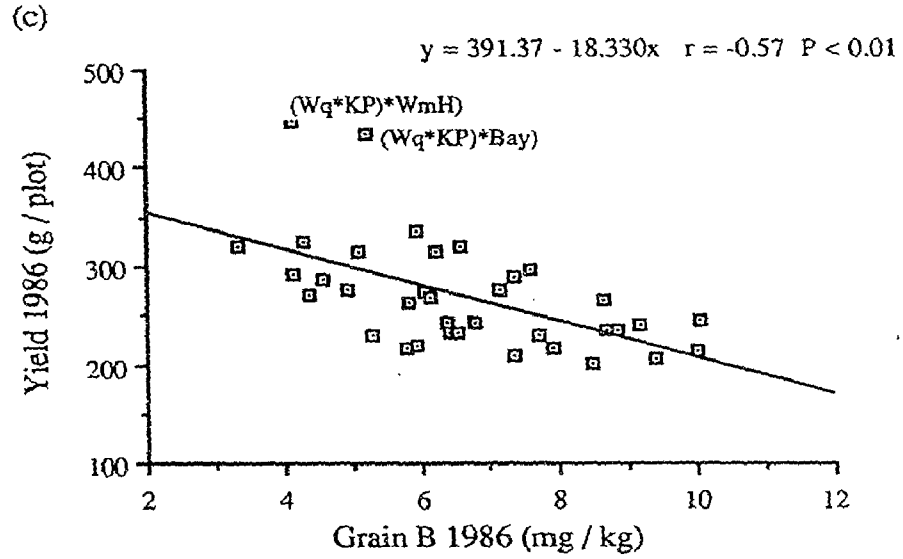
(a)


y = 237.13 - 0.85489x r = -0.43 P < 0.01
Yield 1985 (g / plot)
300
200
100
0
20
40
60
80
100
120
140
Shoot B 1985 (mg / kg)

(b)


y = 252.61 - 6.7477x r = -0.50 P < 0.01
Yield 1985 (g / plot)
300
200
100
0
4
6
8
10
12
14
16
18
Grain B 1985 (mg / kg)

(c)


y = 391.37 - 18.330x r = -0.57 P < 0.01
(Wq*KP)*WmH)
(Wq*KP)*Bay)
Yield 1986 (g / plot)
500
400
300
200
100
2
4
6
8
10
12
Grain B 1986 (mg / kg)

Condor has been the most important semi-dwarf variety in Australia, in particular in New South Wales, since its release in 1973. Condor has also been used extensively as a parent and in excess of 15 varieties with Condor, or the Condor sib, Oxley, as a parent have been released. Condor and related semi-dwarf varieties accounted for greater than 50% of the total area sown to wheat in New South Wales in 1985 (Fitzsimmons, 1987). Five varieties with Condor as a parent, together with Condor and Oxley, were included in these experiments and the concentrations of B in shoots and grain were similar for all these varieties.

The concentration of B in the grain of several of the Condor derived varieties was greater in 1986 than would be expected on the basis of the 1985 results. When the B concentration of grain for the 1986 experiment is plotted against the B concentration of grain in 1985, a group of varieties which were mid-range for B concentration in grain in 1985 were highest in 1986 (Fig 5.6). These varieties can be identified as Condor and its progeny Cook and Sunstar. When these three varieties are excluded from analysis, the correlation between concentration of B in the grain in 1985 and 1986 increases from $r = 0.77$ to $r = 0.86$. The concentration of B in the grain is therefore subject to environmental influences and not all varieties respond similarly. Nevertheless, varieties with the lowest concentration of B in shoots and grain were consistent between seasons.

A third group of varieties which are closely related and of recent importance in South Australia are those derived from (WW-15*Raven), namely Aroona, Warigal and Schomburgk. Warigal was the second leading variety in South Australia, behind Halberd, from 1982/83 until 1986/87 (Appendix C) and Aroona has also been among the leading varieties during this period. Schomburgk was released in 1986 and is still in the early stages of adoption. The concentrations of B in the shoots of Aroona and Raven were lower than for Warigal and Schomburgk in 1985, although this difference was not statistically significant, and the concentration of B in grain of the four varieties was similar in both seasons. While B concentrations in shoots of Aroona and Raven were not significantly greater than for Halberd in 1985, and B in grain of the four varieties were not significantly greater than Halberd in 1985, they were significantly greater than Halberd in 1986. The concentrations of B in shoots and grain of WW-15 were

significantly greater than for Raven in the experiment of historically important varieties grown at Two Wells in 1985 and 1986 (Table 5.2). It is therefore probable that the response of Warigal, Aroona and Schomburgk is derived from Raven which was one of the leading varieties in South Australia during the period 1965 - 1975.

Two advanced breeding lines from the Waite Agricultural Research Institute wheat breeding program produced exceptional yields and had low concentrations of B in the grain in 1986 (Fig 5.7c). These two lines have parents in common and one in particular, Wariquam (abbreviated as Wq), has the pedigree (Mexico 120*Quadrat). The high level of B tolerance of Quadrat was demonstrated by the experiments of historically important varieties. Despite their B tolerance and high yield potential neither line is to be released as a variety as they are below the required standard for grain quality.

5.8 DISCUSSION

The series of experiments investigating the level of tolerance to high concentrations of B for historically important Australian wheat varieties identified a group of related varieties which are tolerant to high concentrations of B. This family comprises the relatives of Halberd and includes a number of varieties which have been important Australia-wide, but to an even greater extent they have dominated South Australian and Victorian wheat production. No varieties more tolerant to high concentration of B than Halberd were identified.

Differences in yield response to high concentrations of soil B, among varieties in a pot experiment, were related to B concentrations in shoots and B concentrations were lower for the more tolerant varieties. The yield response and B concentration of shoots for the pot experiment were also significantly correlated with B concentrations of shoots and grain for the field experiment at a high B site. Thus genetic differences in B accumulation in the glasshouse environment reflects the field response.

The B concentrations of shoots and grain, and grain between seasons, were significantly correlated for the experiments of the historically important Australian varieties and of the major South Australian varieties conducted at Two Wells in 1985 and 1986. These results confirm those of Chapter 4 where only five genotypes were compared and demonstrate that B uptake is a highly heritable character and grain may be used for analysis when comparing B status among genotypes.

The previous chapter examined the response of a limited number of genotypes to high concentrations of B, in detail. Parameters which were identified as being potentially useful for distinguishing between genotypes for response to B included seedling height and delay in development. When applied to a greater number of varieties, relative height was not very efficient at distinguishing between the majority of genotypes in the pot experiment, although it readily identified the most sensitive genotype. Seedling height for the pot experiment was significantly correlated with B concentration in tissues for the field experiment (Fig 5.4c). The ability to tiller at high B concentrations was associated with tolerance to B in the pot experiments and low concentrations of B in shoots and

grain for the field experiments (Fig 5.7). Delay in development in response to B at the stage of ear emergence of the main shoot was able to identify the most sensitive genotypes but did not discriminate between the more tolerant varieties(Table 5.6). The results for effect of B on phenological development are consistent with the previous chapter and tillering is more sensitive than development of the main shoot to high concentrations of B.

The experiment of Chapter 4 also examined the effect of stage of development at time of harvest upon response to B. Results indicated that the difference between genotypes was maximized by harvesting plants during vegetative growth. As the development of plants was delayed by high levels of B, growing plants to maturity allowed compensatory growth by virtue of an extended growing period. A similar effect is believed to have occurred for the pot experiment with historically important varieties and the concentrations of B in shoots of the latest maturing varieties, including Improved Fife, Yandilla King, Major, Purple Straw and Gallipoli were higher than other earlier maturing varieties of similar relative yields. When the full significance of this result was recognized the procedure for pot experiments was altered so that harvesting was undertaken when control plants were at or near the boot stage. This procedure was used for the experiment comparing Federation and Currawa as possible sources of tolerance (Section 5.4) and the discrimination between genotypes, as expressed by relative yield at B100 compared with B0, was much greater, and more consistent with B concentrations of shoots, than for the same varieties when grown to maturity (Section 5.3). For example, relative yields (B100/B0) of Currawa, Ranee and Gallipoli were 0.77, 0.76 and 0.79, respectively when harvested at maturity but 0.31, 0.14 and 0.10 when harvested during vegetative growth.

The experiments were not able to identify the original non-Australian source of B tolerance for Federation and Currawa, the earliest Australian varieties tolerant to B. The results for Federation differed between the field (Section 5.2) and glasshouse (Sections 5.3 & 5.4) experiments and this may have been due to differences in seed sources between the experiments. Prior to the establishment of the Australian Winter Cereals Collection accessions of Federation (and many other varieties) were held by Australian

wheat breeding programs. These were supplied to the newly formed AWCC, where they were grown alongside each other and compared visually. For varieties which appeared uniform, either one line was chosen as typical, or seed from all lines was bulked. Varieties which varied morphologically were maintained as separate accessions. Seed obtained from the Waite Agricultural Research Institute and Sydney University was selected preferentially (M.MacKay, pers. comm.). The line of Federation grown in the field experiment was obtained from the Roseworthy Agricultural College germplasm collection and while this may be morphologically indistinguishable from other lines of Federation it could differ in response to B.

The parents of Currawa are no longer available, therefore the origin of tolerance of Currawa can not be identified. Similarly, the origin of tolerance for Federation is uncertain. Purple Straw, which is a parent of Federation, is a landrace and there is considerable variation in response to high concentrations of B among the accessions of Purple Straw and include types at least as tolerant as Halberd. Purple Straw is believed to have originated from Tuscany in Italy (Wrigley and Rathjen, 1981). High concentrations of boric acid occur in fumarole condensates in Tuscany (Kemp, 1956). The alternative source of tolerance are the Indian wheats which were used extensively by Farrer. Concentrations of B in shoots of both Etawah and Indian 4 were low and a high proportion of Indian wheats are tolerant to B (Moody *et al.*, 1988).

The similar level of tolerance to B for related varieties is consistent with response to B being under major gene control, however this result should not be considered as conclusive evidence and the mode of inheritance of response to B is studied systematically in the following chapter. A significant degree of B tolerance is unlikely to have persisted unless it was being selected for, particularly if it is under minor gene control. The State of selection is included in Table 5.1 for all Australian varieties grown in the experiments of historically important varieties and South Australian varieties. Most of the tolerant varieties have been selected in South Australia and Victoria and sites of selection of tolerant varieties include : Victoria - Dookie (Currawa), Werribee (Ghurka, Quadrat, Pinnacle), Horsham / Longerenong (Olympic, Katyil), Walpeup (Insignia) and South Australia - Roseworthy and Palmer (Halberd, Dagger, Spear), Waite (Insignia 49)

(Macindoe and Walkden-Brown, 1968; A.J.Rathjen, pers. comm.). The concentration of extractable B has been determined for soil samples from Horsham, Longerenong, Walpeup, Roseworthy, Palmer and Waite and concentrations in excess of 20 mgB kg^{-1} soil have been measured in the subsoil, for at least some samples, at all sites but Waite (B. Cartwright, pers. comm.). Insignia 49, which was selected at the Waite and Heron, which is one of the few tolerant varieties to be bred in New South Wales, were developed by backcrossing to a tolerant recurrent parent. This is in contrast to the other tolerant varieties selected at high B sites which resulted from single, top or complex crosses, often with more than one sensitive parent. The probability of transmission of B tolerance from parent to offspring is much lower where pedigree or progeny methods of selection are adopted rather than backcrossing. Thus, the site of selection appears to have been a significant factor in the breeding of tolerant varieties.

The significance of tolerance to high concentrations of B as a factor in determining the widespread adoption of a variety is a matter of speculation and the following discussion should be recognized as such. The varieties which have dominated Australian wheat production since 1900 are Federation, Nabawa, Bencubbin, Gabo, Insignia, Heron, Halberd and Condor. Nabawa was omitted from testing but all other varieties could be considered as possessing at least some degree of tolerance to B and all were more tolerant than the sensitive standard, (W1*MMC), included in all experiments. The concentration of B in shoots of the sensitive standard (W1*MMC), for the 1985 field experiments, was similar between the experiments of historically important varieties (Section 5.2) and varieties of importance in South Australia (Section 5.7) so it is possible to compare the concentrations of B in shoots between the two experiments. It is apparent that many varieties currently in production are more sensitive to B, or accumulate greater concentrations of B in shoots and grain, than the varieties which have been dominant throughout Australia. Evaluation of the current major Australian wheats has identified varieties as sensitive as (W1*MMC) including Hartog and Eradu (D.B. Moody, pers. comm.) and these sensitive varieties are widely grown in Queensland,northern New South Wales and Western Australia, respectively, but not South Australia or Victoria (Appendix C).

The distribution and predominance of varieties, by states, is more revealing as to the possible role of B tolerance in determining adoption of varieties. The area sown to the leading wheats in each state and in Australia for the period 1929 - 1965 is summarized by Macindoe and Walkden-Brown (1968) and areas for Australia, South Australia and Victoria are included in Appendix C. During the period 1937 - 1955 Bencubbin was the leading variety in Australia and occupied in excess of 30% of the total area sown to wheat in New South Wales and Western Australia in a number of years. The maximum area sown to Bencubbin in Victoria was 8.4% in 1946 and during the period of domination by Bencubbin the most widespread varieties in Victoria were the B tolerant lines, Ghurka (>45% of area, 1937 - 1940), Quadrat (>35% of area, 1948 - 1951) and Insignia (>40% of area, 1953 - 1965). Gabo succeeded Bencubbin as the leading Australian wheat but was not grown in Victoria to a significant extent. During this period B tolerant varieties regularly accounted for greater than 80% of the total area of wheat production in Victoria. The B tolerant varieties, Olympic and Halberd, were the leading varieties in Victoria during the 1970's (data not presented) but have since been replaced by less tolerant varieties following the introduction of stripe rust (*Puccinia striiformis*) into Australia in 1979.

The importance of B tolerant varieties in South Australia has been more recent than in Victoria and both Bencubbin and Gabo achieved greater than 20% of the South Australian wheat area. Insignia was the first B tolerant variety to achieve the dominant role in South Australia and since 1959 the leading varieties have been Insignia and Insignia 49 (1959 - 1967), Heron (1968 - 1971), Halberd (1972 - 1987) and Spear (1988 - present). The distribution of leading varieties, by counties, was published annually in the Statistical Register of South Australia, Department of Census and Statistics until 1975. This information has been used to produce maps comparing the distribution of B tolerant varieties with the concentration of B in barley grain for the 1983 South Australian crop (Cartwright and Hirsch, 1986) (Fig 5.8). The years and varieties chosen for comparisons were : 1960 (Insignia v Gabo), 1965 (Insignia + Heron v Gabo), 1970 (Insignia + Heron v Gabo + Gamenya) and 1975 (Halberd). These seasons were chosen for comparisons because, with the possible exception of Halberd,

sufficient time had elapsed from release to allow the varieties to establish niches within the wheat belt. In 1960, Insignia and Gabo were sown to approximately equal areas and therefore allowed a direct comparison of their distribution by area. After 1960 the tolerant varieties began to dominate to a greater extent and by studying their distribution, by counties, it would be possible to determine whether these varieties were being generally adopted or preferentially adopted in specific regions.

The regions where the B tolerant varieties have been most widely grown corresponds with the regions where the highest concentrations of B in grain of barley occurred (Fig. 5.8). Similarities in the distribution of Insignia and Heron in 1965 and 1970 and Halberd in 1975 can be recognized and the counties where these three varieties dominated to the greatest extent also corresponds to those where Insignia was preferred to Gabo in 1960. The greatest proportion of tolerant varieties occurs on Eyre Peninsula and in the county of Chandos in the Murray Mallee. High concentrations of B in barley grain were measured in the county of Jervois, the county with the largest area sown to wheat in South Australia, but the area sown to Halberd is lower than for most other counties of Eyre Peninsula. This is attributed to the coastal margin of Jervois being the highest risk region for stem rust (*Puccinia graminis*) to which Halberd is susceptible and there has been a deliberate campaign to discourage the cultivation of Halberd in the area.

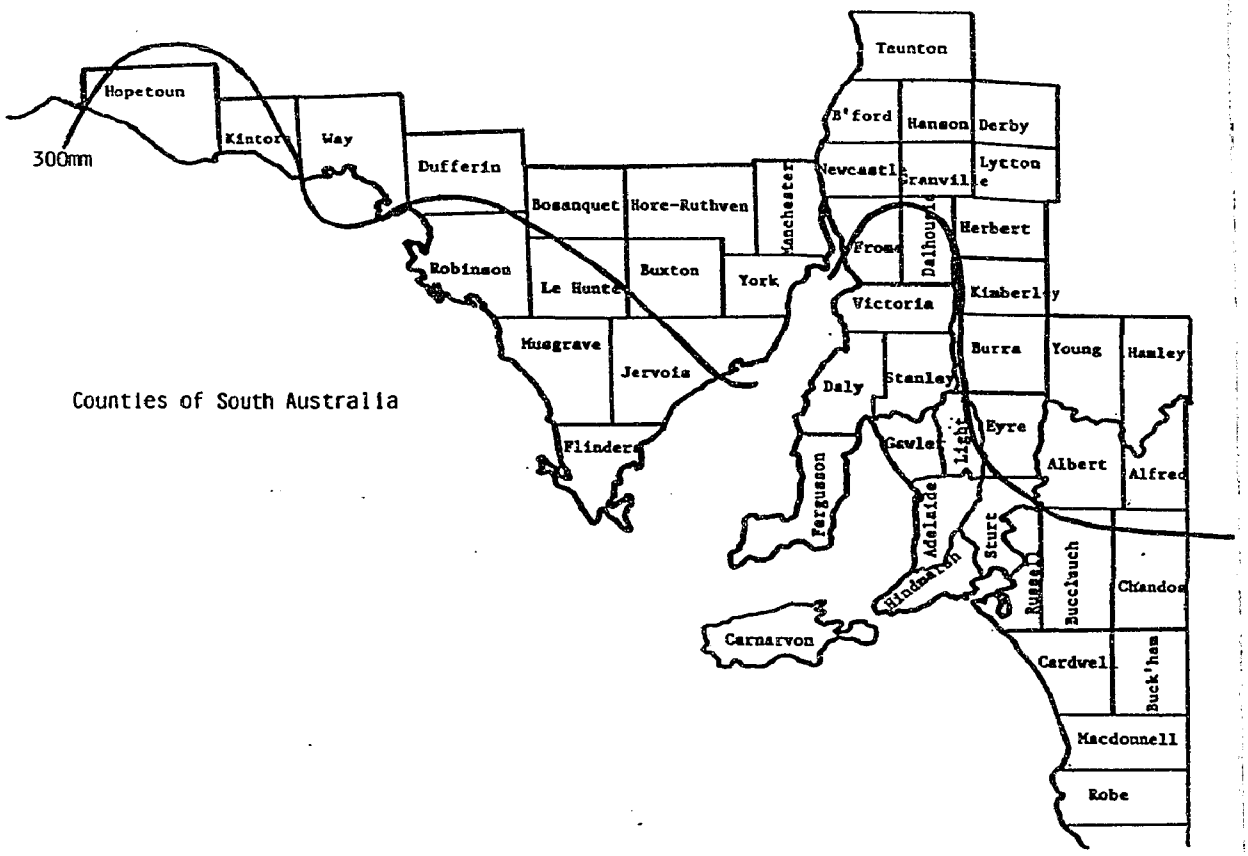
The apparent recent increase in B tolerance as a selection force in South Australian wheat cultivation might be related to the increased area sown to wheat on the Eyre Peninsula, the region where high concentrations of B in barley grain were most prevalent. The area sown to wheat in South Australia more than doubled during the period 1955 - 1987 from 651,000 ha in 1955 to 1,556,000 ha in 1987. The area of wheat on Eyre Peninsula increased from 211,000 ha to 801,000 ha in the same period and thus accounted for approximately two thirds of the total increase in area (Statistical Register of South Australia, 1956; South Australian Year Book, 1988).

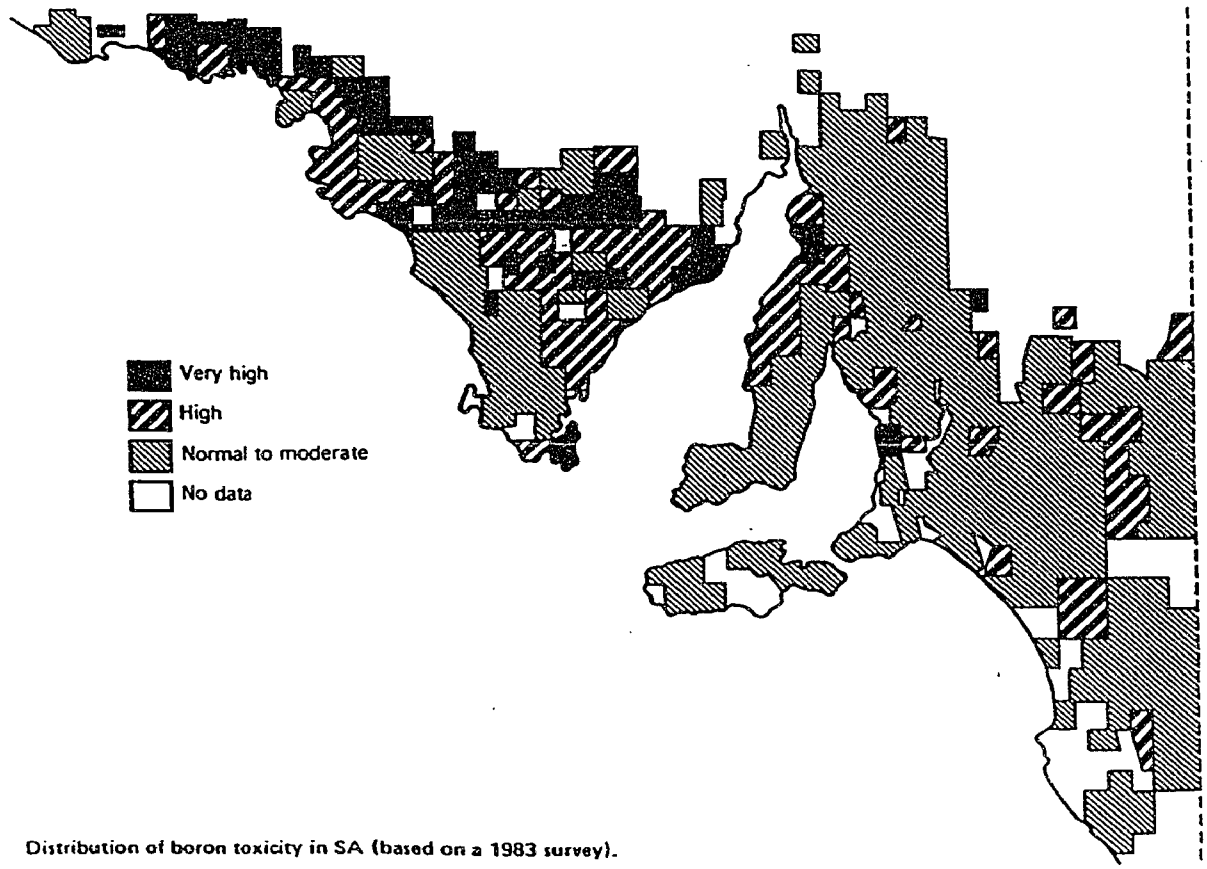
High concentrations of B are generally associated with the lower rainfall regions of the state, therefore an argument against B tolerance being the major selective force might be that the distribution of varieties has been determined by either drought tolerance or water use efficiency. In the Murray Mallee the two counties of Albert and Alfred are

Figure 5.8 Distribution of the dominant wheat varieties in South Australia, by counties, since 1960, in relation to the concentration of B in barley grain for the 1983 season (Cartwright and Hirsch, 1986). The distribution of each variety is expressed as a percentage of the total area sown to wheat for each county. Results are included only for counties in which more than 5,000 ha of wheat was grown. The 300 mm isohyet is indicated.

(a) The counties of South Australia within the wheat growing region.

(b) Relative concentrations of B in barley grain (Cartwright and Hirsch, 1986).



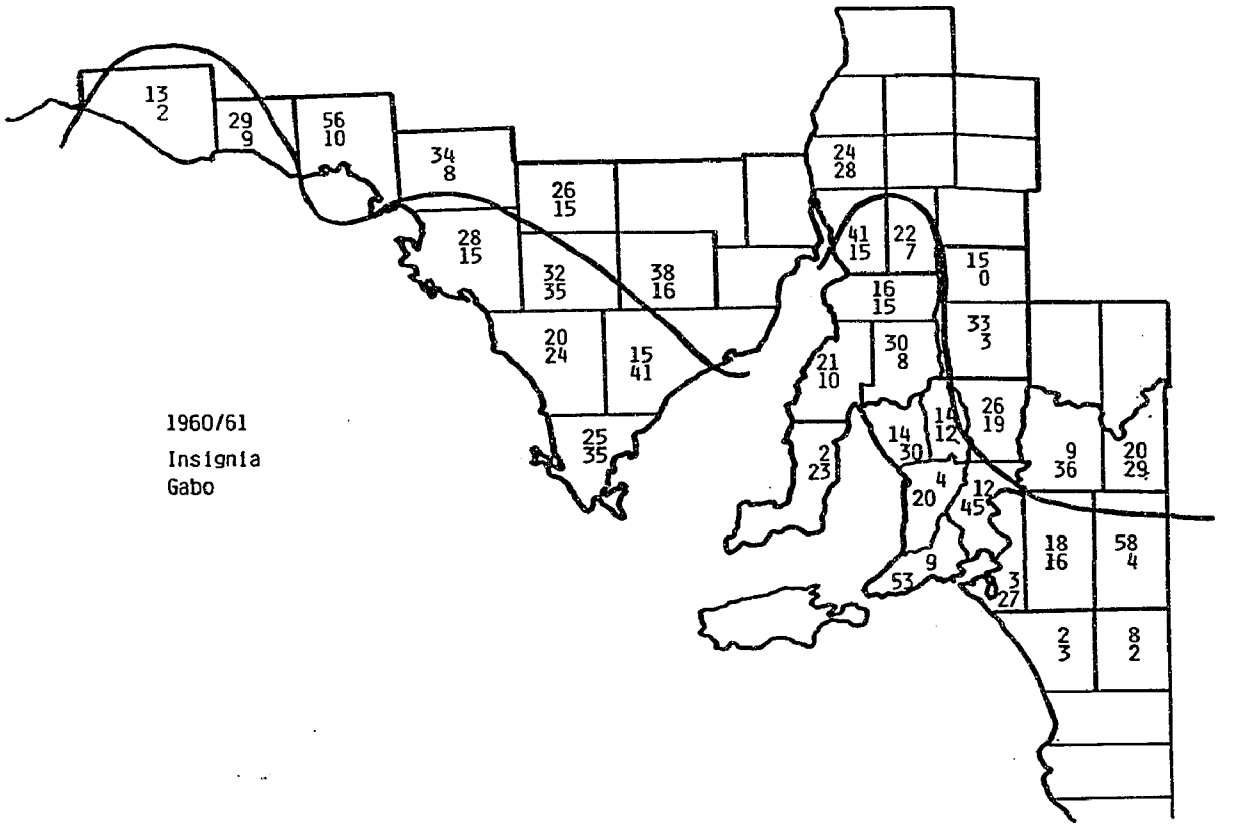


Distribution of boron toxicity in SA (based on a 1983 survey).

Figure 5.8 contd.

(c) distribution of Insignia (top number) and Gabo (bottom number) in 1960/61,

(d) distribution of Insignia and Heron, combined (top number) and Gabo (bottom number) in 1965/66,


1960/61
Insignia
Gabo
13 2
29 9
56 10
34 8
26 15
28 15
32 35
38 16
20 24
15 41
25 35
24 28
41 15
22 7
15 0
16 15
33 3
21 10
30 8
14 12
26 19
2 23
14 30
9 36
20 29
4
20
12 45
18 16
58 4
9
53
3 27
2 3
8 2

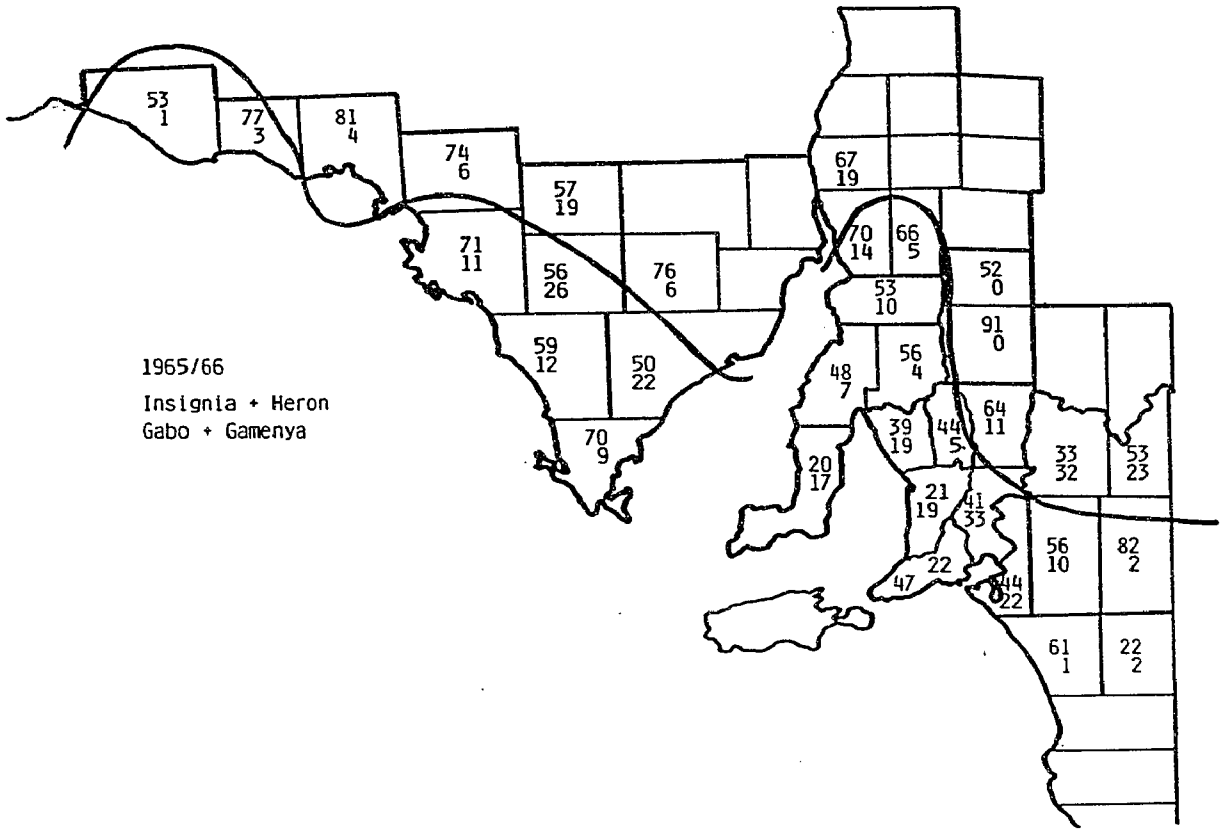
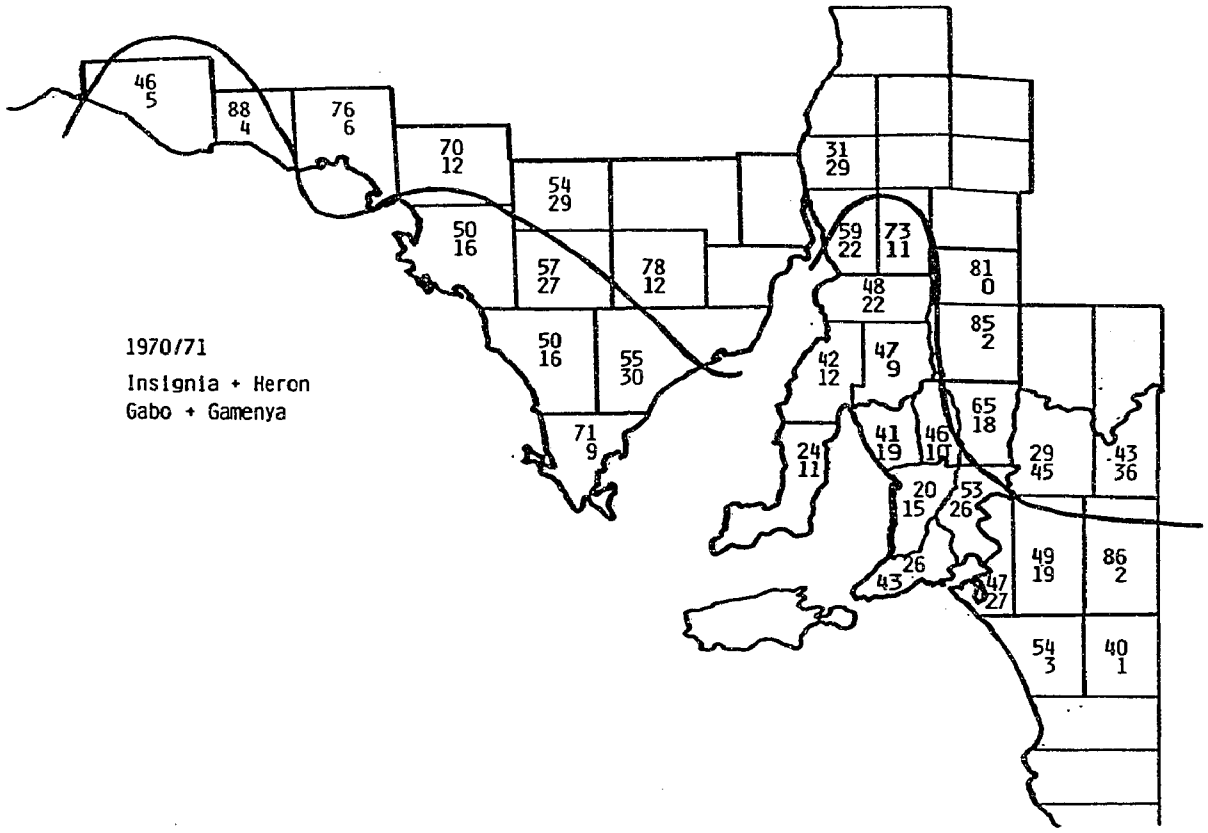

1965/66
Insignia + Heron
Gabo + Gamenya
53 1
77 3
81 4
74 6
57 19
71 11
56 26
76 6
59 12
50 22
70 9
67 19
70 14
66 5
52 0
53 10
91 0
56 4
48 7
64 11
39 19
44 5
33 32
53 23
20 17
21 19
41 33
56 10
82 2
22
47
44 22
61 1
22 2

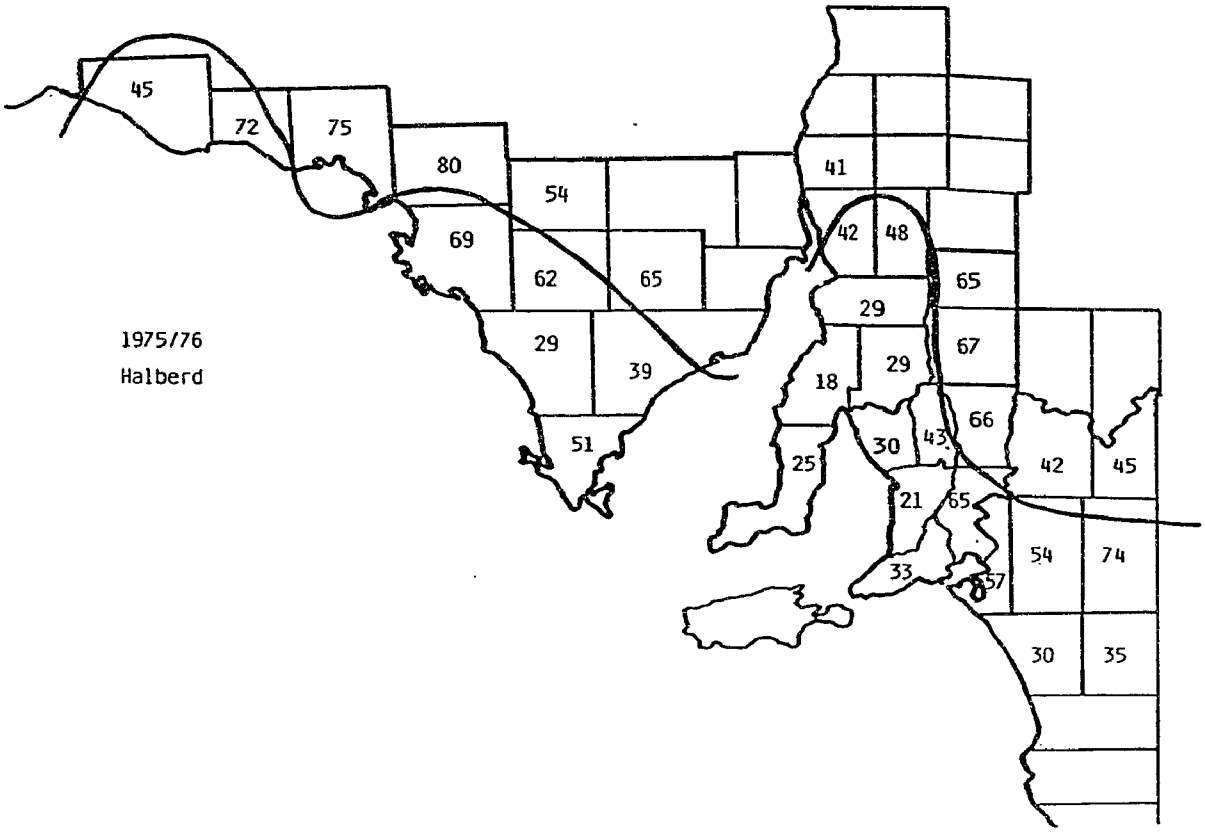
Figure 5.8 contd.

(e) distribution of Insignia and Heron, combined (top number) and Gabo and Gamenya, combined (bottom number) in 1970/71 and

(f) distribution of Halberd in 1975/76.



1970/71
Insignia + Heron
Gabo + Gamenya



1975/76
Halberd

outside the 300 mm isohyet. The proportion of B tolerant varieties grown in these counties is much lower than the adjacent higher rainfall counties of Chandos and Buccleuch and this is contrary to expectation if the distribution of varieties was determined primarily by response to moisture supply. The barley survey indicated that the B levels in grain from the former counties is below that in the counties where Halberd was a greater proportion.

The above observations regarding the distribution of B tolerant varieties and the incidence of high concentrations of B in grain in South Australia together with the occurrence of high concentrations of B in the soil at the sites of selection of B tolerant varieties are highly suggestive that tolerance to high concentrations of B is a major selection force in South Australia and Victoria. The reason that Halberd has persisted in South Australia despite the release of semi-dwarf varieties, commencing in 1973, could be attributed to tolerance to B. It is significant that Spear is the first semi-dwarf variety to exceed Halberd for production in South Australia as Spear is also tolerant to B and related to Halberd through having Insignia as a parent.

Chapter 6.

GENETIC CONTROL OF TOLERANCE OF WHEAT TO HIGH CONCENTRATIONS OF BORON

6.1 INTRODUCTION

There is no information on the genetic control of tolerance to high concentrations of B for any plant species, although major gene control has been reported for response to low concentrations of B in celery (Pope and Munger, 1953) and red beet (Tehrani *et al.*, 1971) and the translocation of B from roots to shoots under low B supply for tomato (Wall and Andrus, 1962). The manifestation of tolerance, by wheat, is reduced accumulation of B in shoots (Chapters 4 and 5; Paull, 1985; Nable, 1988). This may result from either reduced translocation from roots to shoots, or exclusion of B from the roots and the results of Nable (1988) indicate that the latter is the more probable mechanism. An understanding of the genetic control of tolerance of wheat to high concentrations of B would facilitate breeding for this character.

The method of analysis adopted for a genetic study will differ depending upon whether the variation is qualitative or quantitative in nature. For a qualitative character, discrete, alternative phenotypes can be readily recognized in a segregating population whereas this is not the case for a quantitative character when environmental effects are large in relation to the effect of single genes. Quantitative analysis gives a statistical measure of the combined effects of all segregating loci expressed as additive effects, dominance, epistasis, linkage and genotype - environment interactions, whereas qualitative analysis will provide information on the number of loci controlling the character, allelic variation at each locus, their dominance properties and whether loci interact (Snape, 1987). As a number of levels of tolerance to B can be recognized, in wheat, B tolerance can be considered as a quantitative character. Many of the approaches to the genetic analysis of quantitative characters of inbreeding species are reviewed by Baker (1984) and Snape (1987).

When deciding upon the means of analysis for a quantitative character two alternative assumptions can be adopted, namely, either :

(1) there are many genes each of small effect, or

(2) the character is under the control of a few major additive genes.

In deciding which of the two approaches to adopt for the study of tolerance of B, in wheat, a number of factors were considered including :

(1) a manifestation of tolerance to high concentrations of B, for wheat, is reduced accumulation of B in shoots. Major gene control of translocation of B to shoots has been identified for tomato, albeit under conditions of low B supply (Wall and Andrus, 1962).

(2) response of genotypes to high concentrations of B is highly correlated between cultural conditions and between seasons (Chapters 4 and 5). The differences in response, among genotypes, could therefore be ascribed principally to genetic rather than environmental effects. The small environmental effect upon a genotype's performance would increase the accuracy of identification of the genotype and so increase the likelihood of identifying major genes.

(3) the response of varieties tends to follow one of the parents. For instance, in general, the B concentration in shoots and grain of varieties derived from Currawa and Federation are low. This indicates that either the uptake / translocation of B is under the control of relatively few genes or, alternatively, if under the control of multiple additive genes, high concentrations of B have been a major, albeit unrecognized, selection force in Australian wheat breeding. Intense selection pressure may also apply for a character under major gene control.

(4) the number of characters which can be actively selected for in a breeding program is limited and the priority given to each will be determined by the balance of economic and genetic considerations. The probability of success in breeding for a specific character is inversely proportional to the number of genes by which it is controlled and so the question of greatest importance to a breeder is whether major genes can be identified.

On the basis of the above considerations it was decided that, rather than following a classical quantitative model for the study of tolerance to B toxicity, a more optimistic approach would be adopted with the attempted identification of major genes and their

chromosomal location. This would be undertaken by studies of F_1, F_2 and F_3 generations (Chapter 6) and by aneuploid methods (Chapter 7).

6.1.1 Genotypes

Five genotypes G61450, Halberd, Warigal, (Wl*MMC) and Kenya Farmer, which represented the known range in response to B, were chosen for genetic studies. These genotypes were chosen for the following reasons :

(1) Halberd is a member of the most successful family of Australian wheats and has persisted even after the introduction of semi-dwarf varieties (Chapter 5);

(2) Warigal and (Wl*MMC) are closely related but of contrasting response. Warigal was the second most widely adopted variety in South Australia, after Halberd, between 1982 and 1986 (Appendix C). The regions where Warigal was most widely adopted were outside the regions dominated by Halberd (Rathjen and Pederson, 1986);

(3) Kenya Farmer is extremely sensitive and intervarietal substitution lines of Kenya Farmer (syn. Kenya 833; Zeven and Zeven-Hissink (1976)) into Chinese Spring have been developed (Snyder *et al.*, 1963) and are the subject of study in Chapter 7. Kenya Farmer is also closely related to Gabo, which was a very successful Australian wheat and is an ancestor of Halberd and Warigal;

(4) Many genotypes more tolerant to high concentrations of B than Halberd have been identified at the Waite Institute (Moody *et al.*, 1988). G61450 was chosen to represent the most tolerant category as it had no apparent photoperiod or vernalization requirements and was therefore similar in maturity to the other genotypes selected. It was also not subject to lodging and was therefore amenable to glasshouse experiments.

(5) All genotypes, except G61450, would have a degree of homogeneity for background characters as there are several common varieties as ancestors.

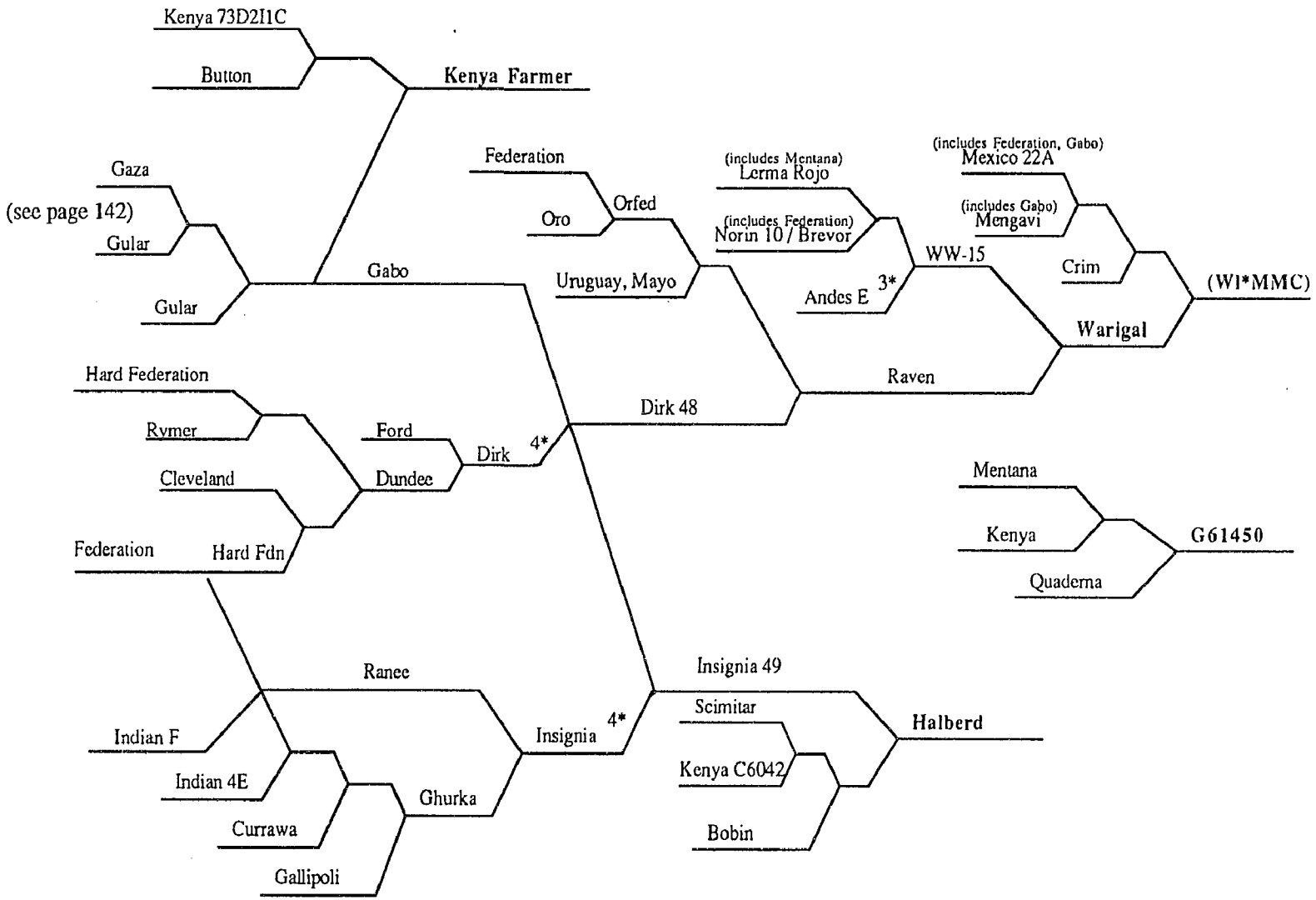
The pedigree diagrams for the selected genotypes are presented in Figure 6.1. Federation, which is a direct ancestor of Halberd, was a parent of Brevor, and therefore in the ancestry of all semi-dwarf wheats based on Norin 10 / Brevor seln. 14, including WW-15 and Mexico 22A (syn. Siete Cerros and 8156) which are parents of Warigal and

(Wl*MMC), respectively. Federation also occurs in the ancestry of Raven which is the other parent of Warigal. Gabo occurs in the ancestry of Warigal and Halberd and in Mengavi which is a parent of (Wl*MMC). The pedigree of Gabo was originally designated (Bobin seln.*Gaza)*Bobin seln.) (see Macindoe and Walkden-Brown, 1968) however this has been disputed and evidence from the use of biochemical markers suggests that Gular, rather than a Bobin selection, was a parent of Gabo (Wrigley and Shepherd, 1977). The pedigree of Kenya Farmer includes (Gaza*Bobin)* Bobin) (Zeven and Zeven-Hissink, 1976), but it will again be presumed that Gular, rather than Bobin was one of the parents. Mentana is a parent of G61450 and is an ancestor of both Warigal and (Wl*MMC) while all five genotypes have Kenyan wheats in their ancestries.

Seed of Halberd, Warigal and (Wl*MMC) was obtained from stocks held at the Waite Agricultural Research Institute while seed of G61450 and Kenya Farmer was obtained from the Australian Winter Cereals Collection. All genotypes were maintained as pure lines and multiplied under glasshouse conditions. The original sample of G61450 obtained from the AWCC (accession number AUS 6141)was a mixture of red and white grain, so all grain used for experiments and hybridizations was derived from a single white grain.

Note The actual parents of Gabo were Gaza and "Bobin" W39, an accession in the University of Sydney collection and believed to be closely related to Gular. A separate accession Bobin W360 also occurs in the collection (R.A. McIntosh, pers. comm.).

Figure 6.1 Pedigree diagrams depicting the relationships between the five genotypes used for genetic studies, based on Moss and Wrigley (1974) and Wrigley and Rathjen (1981) with additional information from Zeven and Zeven-Hissink (1976) and Wrigley and Shepherd (1977).


Kenya 73D2I1C
Button
Kenya Farmer
Gaza
(see page 142)
Gular
Gular
Gabo
Federation
Oro
Orfed
Uruguay, Mayo
(includes Mentana)
Lerma Rojo
(includes Federation)
Norin 10 / Brevor
Andes E
3*
WW-15
(includes Federation, Gabo)
Mexico 22A
(includes Gabo)
Mengavi
Crim
(WI*MMC)
Warigal
Raven
Dirk 48
Hard Federation
Rymer
Cleveland
Federation
Hard Fdn
Ford
Dirk
4*
Dundee
Mentana
Kenya
Quaderna
G61450
Ranee
Indian F
Indian 4E
Currawa
Gallipoli
Ghurka
Insignia
4*
Insignia 49
Scimitar
Kenya C6042
Bobin
Halberd

6.2 IDENTIFICATION OF OPTIMUM CONCENTRATIONS OF BORON FOR SELECTION BETWEEN GENOTYPES

6.2.1 Introduction

When conducting genetic studies for response to many environmental stress factors, all genotypes will be affected by the stress to some degree, regardless of their level of tolerance.

Choice of suitable levels of B treatments for screening segregating generations is important. The optimum treatment for comparing between genotypes is one which results in maximum difference between the parental genotypes with minimal effect on the more tolerant of the parents (Foy, 1976). If progeny testing is required a further criterion would be that the B level chosen permits sensitive genotypes to develop to maturity and produce grain. Assessment of response early in development would allow transplanting to low B potting mix to facilitate seed production.

The five genotypes chosen for this study were grown at a range of B treatments to determine the optimum concentrations of B to differentiate between the parents and for screening F_2 and F_3 generations. The F_1 hybrid of (G61450*Kenya Farmer) was also included to provide information on the response of the hybrid, relative to its parents.

6.2.2.Materials and methods

Genotypes

G61450, Halberd, Warigal, (Wl*MMC), Kenya Farmer and (G61450*Kenya Farmer) F_1 hybrid.

Soil

B0, B25, B50, B75, B100, B125 and B150 soil was weighed into 150 mm diameter pots (1.8 kg pot $^{-1}$). The soil used for this and subsequent experiments was obtained from an alternative source to that used for experiments of Chapters 4 and 5 and is referred to as Glenthorne III in Chapter 3.

Experimental design

The pots of the six genotypes and seven B treatments were combined factorially and arranged as a randomized complete block design with three replicates. Three pre-germinated seeds were sown per pot and thinned to two plants two weeks after sowing. The experiment was conducted for six weeks by which time the B0 plants were at or near the boot stage. Whole shoots were harvested at ground level, rinsed in de-ionized water, oven dried and weighed. Shoots derived from the B0 - B100 treatments, with the exception of Kenya Farmer at B75 and B100 and (Wl*MMC) at B100, were analyzed for B concentration.

6.2.3 Results

There were highly significant differences among genotypes for all parameters measured and the ranking of genotypes was consistent with the expectation based on previous observations. Toxic levels of B reduced dry matter yield, seedling height and tiller initiation and the concentration of B in shoots was increased. The responses of Halberd, Warigal and (Wl*MMC) are compared in Plate 5 and G61450, Kenya Farmer and their F_1 hybrid in Plate 6.

The data for dry matter production were treated with a square root transformation prior to statistical analysis. The optimum treatment for distinguishing between genotypes, with respect to yield, varied for different combinations of genotypes (Fig 6.2). The differences between (Wl*MMC) and Kenya Farmer and between Warigal and (Wl*MMC) were greater at the low treatments than at B100 and above while there was a greater difference between Halberd and Warigal at B100 than at B75. G61450 yielded significantly more dry matter than Halberd at the B100 and B125 treatments but there was no difference between the two genotypes at B150.

The heights of seedlings were measured two and four weeks after sowing and genotypes were ranked similarly as for dry matter yield (Fig. 6.3). Two weeks after sowing, genotypes were of two categories, namely the tolerant G61450 and Halberd and the sensitive Warigal, (Wl*MMC) and Kenya Farmer. The sensitive group diverged from

Plate 5. Comparison of the responses of three genotypes grown at seven boron treatments.
(a) Halberd,
(b) Warigal and
(c) (Wl*MMC).
Treatments from left to right : B0, B25, B50, B75, B100, B125 and B150.

(a)

(b)

(c)

Plate 6. Comparison of the responses of G61450, Kenya Farmer and the (G61450*Kenya Farmer) F_1 hybrid grown at seven boron treatments.

(a) G61450,

(b) (G61450*Kenya Farmer) F_1 hybrid and

(c) Kenya Farmer.

Treatments from left to right : B0, B25, B50, B75, B100, B125 and B150.

(a)

(b)

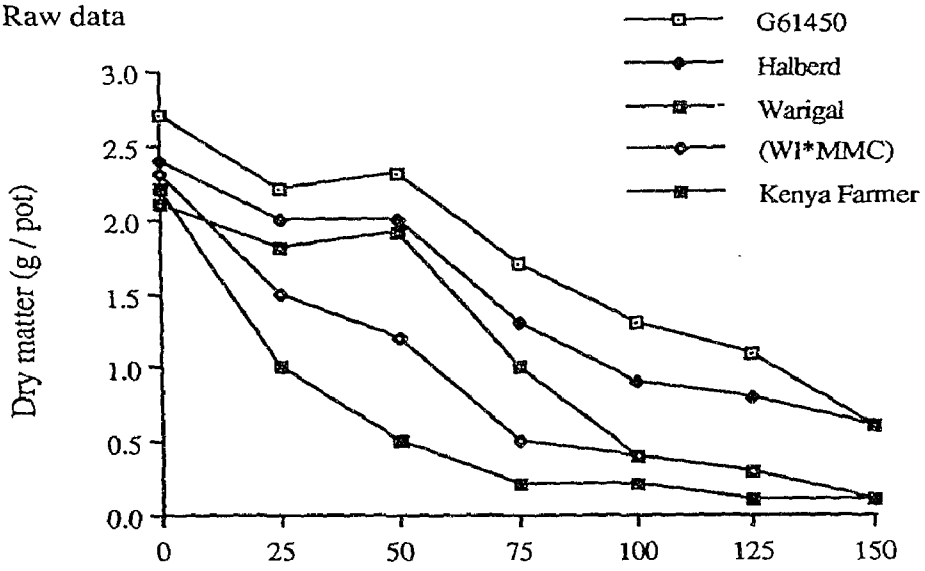
(c)

Figure 6.2 Dry matter production for five genotypes grown at seven levels of applied B.

(a) raw data

(b) square root transformed data. The vertical bar represents the LSD (0.05) for the genotype x treatment interaction.

(a) Raw data



(b) Transformed data

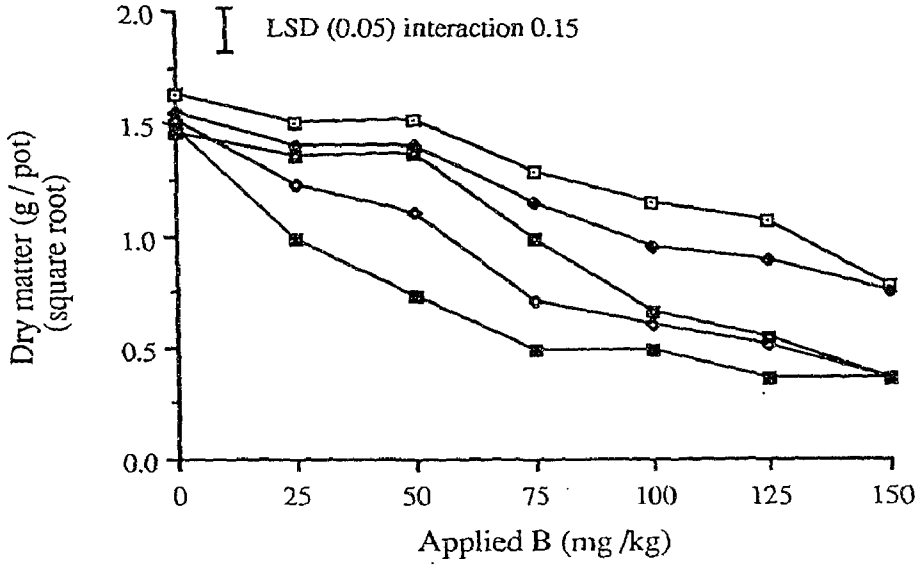


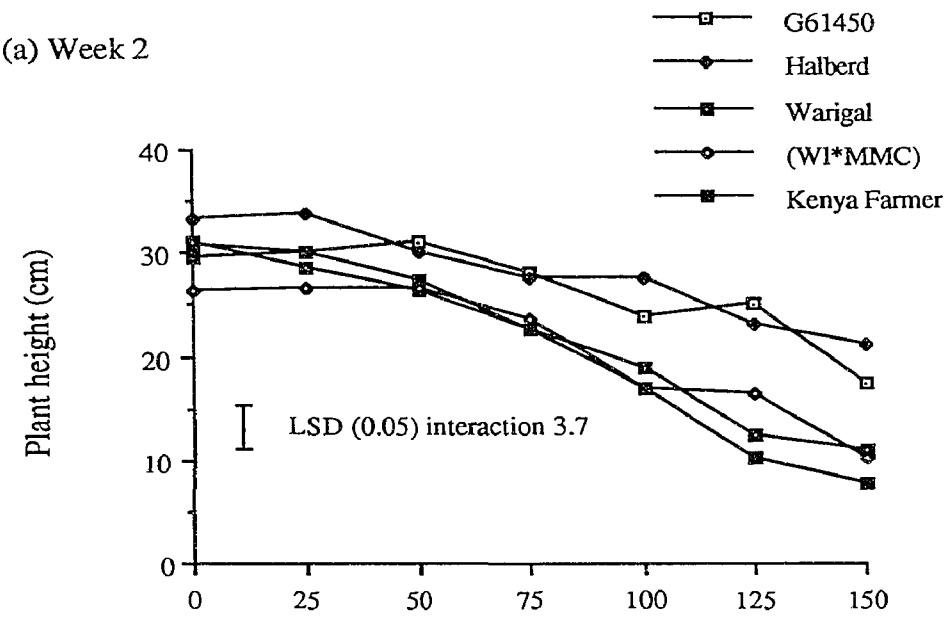
Figure 6.3 The height of seedlings (cm), from ground level to the tip of the youngest expanded leaf blade for five genotypes grown at seven levels of applied B.

(a) two weeks and

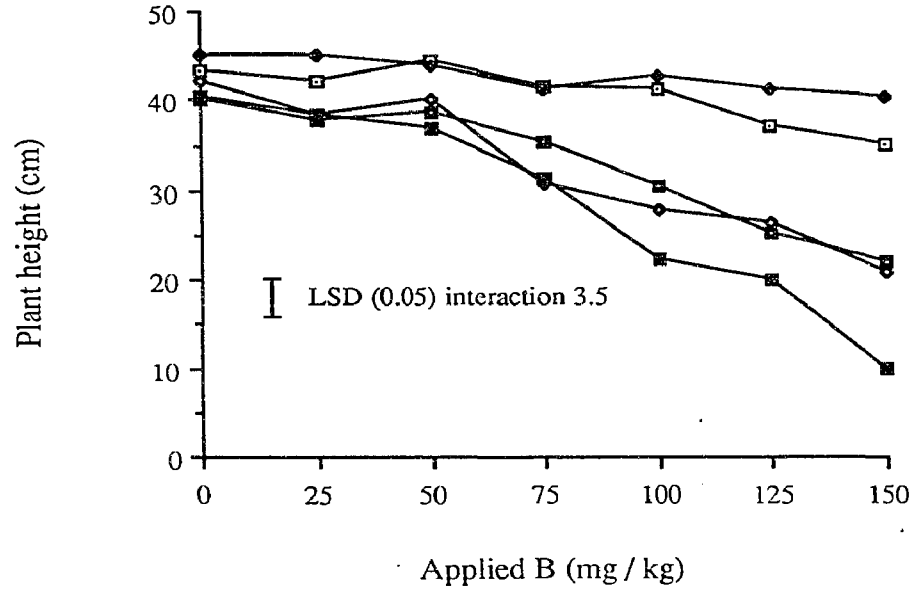
(b) four weeks after sowing.

The vertical bars represent the LSD (0.05) for the genotype x treatment interactions.

(a) Week 2



(b) Week 4



the tolerant genotypes at B75. Four weeks after sowing Kenya Farmer was more sensitive than Warigal and (Wl*MMC), with respect to height, at B100 and higher treatments.

At the B0 control the five standard genotypes and the F_1 hybrid commenced tillering between 20 (Warigal) and 25 days (G61450) after sowing. Initiation of tillers was delayed by increasing concentrations of B and at the time of harvest, no plants had tillered at B100. G61450 and Halberd were the only genotypes to tiller at B75 while Kenya Farmer failed to tiller at B50 (data not presented).

The concentration of B in shoots differed significantly between genotypes and concentrations were lower for the more tolerant genotypes (Table 6.1). The data for B concentration were logarithmically ($\log_{10}$) transformed, to standardize residuals, prior to statistical analysis. Plants of Kenya Farmer grown at the B75 and B100 treatments and (Wl*MMC) grown at B100 were not analysed for B concentrations due to insufficient plant material. Consequently, only the treatments for which the full set of results were obtained (i.e. B0, B25 and B50) were statistically analysed but the raw data for the more tolerant genotypes at B75 and B100 are also presented in Table 6.1. At the control, the B concentration of (Wl*MMC) was significantly less than for all genotypes except Warigal.

The discrimination between genotypes for B concentration of shoots varied with treatments. For example, the relative difference betwen Halberd and Warigal was greatest at B50 while the difference between Warigal and (Wl*MMC) was greatest at B25. Despite the extreme sensitivity of Kenya Farmer to high levels of B, the B concentration of shoots of Kenya Farmer was not significantly greater than the relatively more tolerant (Wl*MMC). This may have occurred because plants of Kenya Farmer developed less roots and there was less opportunity for B uptake.

The response of the F_1 hybrid of (G61450*Kenya Farmer) was intermediate to the parents for total dry matter production and concentration of B in shoots (Fig 6.4; Plate 6).

Table 6.1 Concentration of B in shoots (mg kg^{-1}) for five genotypes and the F_1 hybrid (G61450*Kenya Farmer) when grown at five B treatments. Significance values refer to the logarithmically (log_{10}) transformed data included in brackets.

Genotype	B conc in shoots (mg kg^{-1})							
	B0		B25		B50		B75	B100
G61450	15.9	(1.19)	154.3	(2.18)	392.3	(2.59)	696.7	1291.0
Halberd	13.3	(1.12)	169.3	(2.21)	696.7	(2.84)	1481.0	2223.7
Warigal	11.3	(1.03)	218.7	(2.34)	1285.0	(3.10)	1892.0	2615.7
(Wl*MMC)	8.8	(0.93)	364.0	(2.56)	1298.0	(3.11)	2061.3	
Kenya Farmer	16.8	(1.22)	388.7	(2.58)	1192.0	(3.07)		
(G61450*KF) F_1	16.8	(1.21)	199.3	(2.29)	680.0	(2.83)	1570.0	1983.3

LSD (0.05) Genotypes 0.01, Treatments 0.07, Interaction 0.18

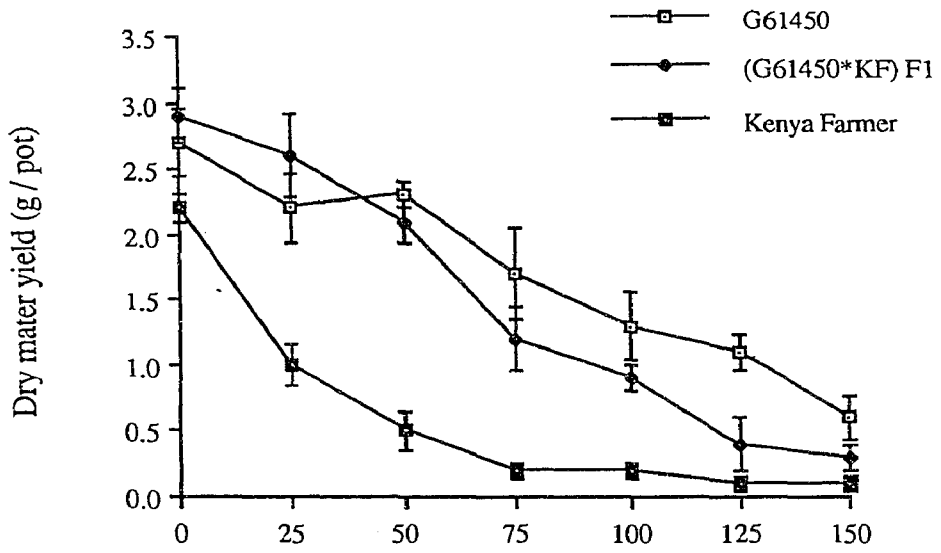
Figure 6.4 Response of G61450, Kenya Farmer and the (G61450 *Kenya Farmer) F_1 hybrid to applied B.

(a) dry matter yield (g pot^{-1})

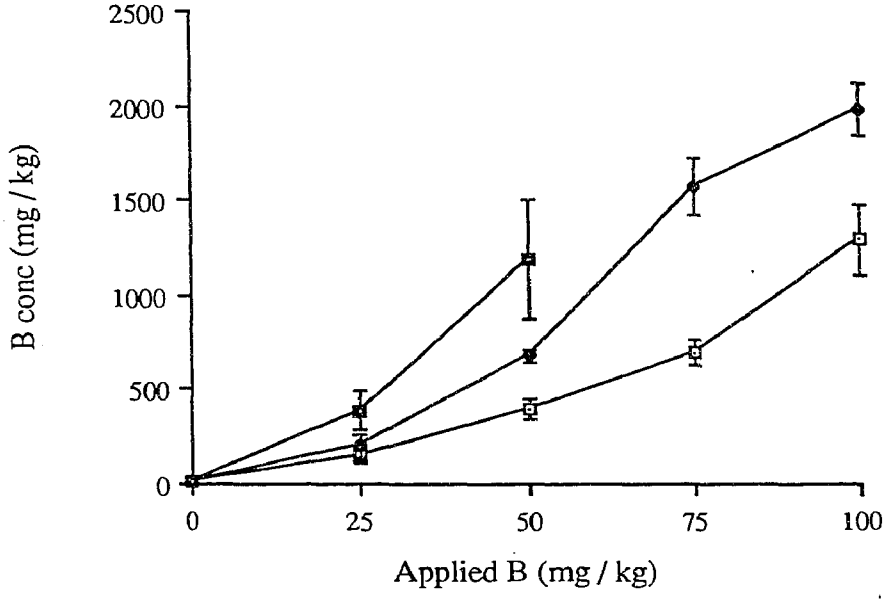
(b) B concentration (mg kg^{-1}) in whole shoots

The vertical bars represent the standard deviations of the means.

(a) Dry weight



(b) B concentration



6.2.4 Discussion

This experiment demonstrated that appropriate levels of B must be applied when comparing a number of genotypes of different tolerances to B. Lower concentrations of B are required for maximum discrimination between sensitive genotypes than when comparing between the more tolerant genotypes. Treatments which would be appropriate for screening segregating populations are B50, B75 and B100 when Kenya Farmer, (Wl*MMC) and Warigal are the more sensitive parents, respectively and this is demonstrated for the comparisons between the five genotypes at the B50 and B100 treatments (Plate 7) The optimum treatment for comparing between G61450 and Halberd is less certain. The difference between these latter varieties for dry matter yield was greatest at B100 and B125 but there was no difference in yield at B150. G61450 and Halberd responded similarly for seedling height and tillering pattern over all treatments.

The results for concentration of B in shoots indicate that while analysis of a single treatment may detect significant differences between tolerant and sensitive genotypes, the differences detected may vary with treatments. At the B25 treatment the concentration of B in shoots of Warigal was similar to Halberd and significantly less than for (Wl*MMC) but at B50, B concentrations of Warigal were significantly greater than for Halberd and similar to (Wl*MMC). Nable (1988) also reported differences in the discrimination between genotypes for concentration of B in shoots as the concentration of applied B increased. For example, at a concentration of 500μM B, in solution culture, the B concentrations of shoots of G61450, Halberd and Warigal were 50, 66 and 167 mg kg^{-1}, respectively, whereas at 5000μM they were 312, 620 and 734, respectively.

Analysis of shoots grown at a single level of applied B may be useful from the point of view of minimising the total number of chemical analyses, although this may occur at the expense of accuracy. The most appropriate treatments for identifying differences in B uptake, between genotypes, would appear to be B25 when comparing sensitive genotypes and B50 for the more tolerant genotypes. The reduced discrimination between genotypes at very high levels of B may result from a breakdown in the regulation of B uptake when plants are suffering severe toxicity or as a result of severe restriction of root proliferation. Analysis of the B0 treatment provided no information on the relative B

Plate 7. Comparison of the response of five genotypes to two B treatments.

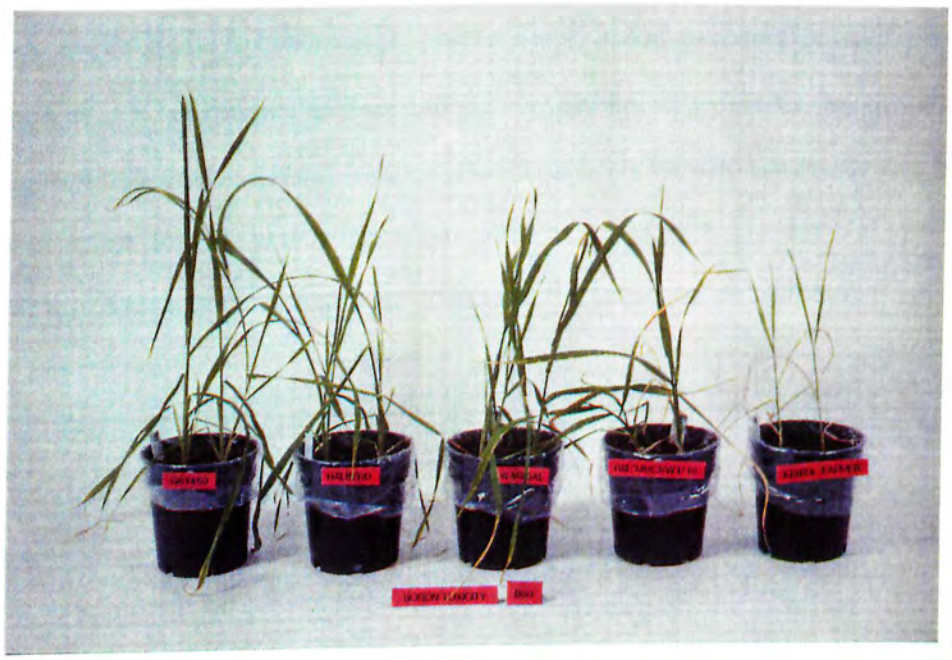
(a) B50 and

(b) B100.

Genotypes from left to right : G61450, Halberd, Warigal, (Wl*MMC) and Kenya Farmer.

Note the threshold effect; at B50 Kenya Farmer is considerably more sensitive than (Wl*MMC) while at B100 Warigal is more sensitive than Halberd.

(a)


GO1460
HALENO
WANGAI
BOREN TOXICITY
B103

(b)


GO1460
HALENO
WANGAI
BOREN TOXICITY
B103

uptake of genotypes at high levels of applied B and this result is consistent with those of Chapters 4 and 5, although contrary to the finding of Nable (1988) for plants grown in solution culture.

The response of the F_1 hybrid of (G61450*Kenya Farmer) varied from similar to G61450 at the lower B treatments to intermediate to the two parents at the higher treatments (Fig. 6.4). Response to high concentrations of B is therefore expressed as a partially dominant trait, for this particular combination of genotypes. The generality of this finding was tested for a wider group of genotypes in the following experiment.

6.3 RESPONSE OF THE F_1 HYBRIDS DERIVED FROM FIVE WHEAT GENOTYPES TO HIGH CONCENTRATIONS OF BORON.

6.3.1 Introduction

In the previous experiment the response to B for the F_1 hybrid of (G61450*Kenya Farmer) was intermediate to the two parents, both with respect to dry matter and for concentration of B in shoots. A further experiment using a greater number of genotypes was conducted to determine whether this is a general response or specific to the genetic combination studied. Reciprocal combinations were included to test for maternal effects upon the response to B. This experiment was grown to maturity with the intention of producing F_2 grain for testing for segregation under high B conditions, despite the results of Chapters 4 and 5 which demonstrated that maximum differences between genotypes occur during the vegetative stage of development.

6.3.2 Materials and methods

Genotypes

The five standard genotypes, G61450, Halberd, Warigal, (Wl*MMC) and Kenya Farmer were crossed in all combinations, including reciprocals. The F_1 hybrids and parents were compared for response to high concentrations of B.

Treatments

Parents and all F_1 hybrids were grown at B25 and B75 and the more tolerant genotypes (G61450, Halberd and Warigal) and their F_1 hybrids were also grown at B150. 150 mm diameter pots (1.8 kg soil pot $^{-1}$) were used for the B75 and B150 treatments while 100 mm diameter pots (400 g soil pot $^{-1}$) were used for the B25 treatment.

Experimental design

Pots were arranged by treatments to minimize shading by the more vigorous plants at the lower B treatments. Within treatments, pots were arranged as a randomized complete block design with four replicates. Three seeds were sown per pot and thinned to two plants after two weeks.

Plants grown at B25 were harvested four weeks after sowing and the shoots were analyzed for concentration of B. Plants at other treatments were grown to maturity, harvested at ground level, oven dried and weighed for total dry matter yield. Growth of plants was regularly recorded during vegetative growth.

Although the combination of parents and progeny was similar to a diallel cross of Experimental Method 1 (Griffing, 1956) and included parents and reciprocal F_1's, this experiment was not analysed by Griffing's method as too few parents were included in the crossing series for the analysis to be valid. Instead, the responses of F_1 hybrids have been compared with their parents to determine whether hybrids are regularly intermediate to their parents, as found in the previous experiment, or whether this effect differs with genetic combinations.

6.3.3. Results

The experiment was arranged by B treatments therefore results should only be compared within treatments. Significant differences in total dry matter yield, between parents, resulted at both of the treatments grown to maturity (Table 6.2). At B75, Halberd was the highest yielding of the parents and (Wl*MMC) and Kenya Farmer were the most sensitive. Warigal produced a similar amount of dry matter as G61450 at B75 but was more sensitive than G61450 and Halberd at B150.

The response of F_1 hybrids, with respect to their parents, varied with parental combinations (Table 6.2). There were no reciprocal differences, so there is no evidence of maternal effects upon response to B. Response to B, as measured by dry matter yield at the B75 treatment, appeared to be a dominant character for all combinations including

Table 6.2 Dry matter yield (g pot^{-1}) at maturity for five genotypes and their F_1 hybrids, including reciprocals, when grown at the B75 treatment; and for three genotypes and their F_1 hybrids grown at B150. P1 = Parent 1, P2 = Parent 2.

Parent 1	Parent 2	Dry weight (g pot^{-1})			
		P1	P2	P1*P2	P2*P1
B75					
G61450	Halberd	16.1	19.0	15.4	15.4
	Warigal		15.4	16.3	16.1
	(Wl*MMC)		10.4	15.5	14.0
	Kenya Farmer		0.4	14.5	13.7
Halberd	Warigal	19.0	15.4	16.5	16.3
	(Wl*MMC)		10.4	17.3	18.2
	Kenya Farmer		0.4	15.7	15.4
Warigal	(Wl*MMC)	15.4	10.4	15.4	15.3
	Kenya Farmer		0.4	13.8	14.3
(Wl*MMC)	Kenya Farmer	10.4	0.4	2.3	3.5
LSD (0.05)	2.7				
B150					
G61450	Halberd	11.3	11.4	8.1	9.1
	Warigal		0.1	9.6	5.5
Halberd	Warigal	11.4	0.1	1.0	1.1
LSD (0.05)	2.9				

G61450, Halberd and Warigal as a parent, although these three genotypes were virtually unaffected by the treatment and the yield of the moderately sensitive Warigal was the same as the tolerant G61450. The F_1 hybrid between (Wl*MMC) and Kenya Farmer was more sensitive than (Wl*MMC), the more tolerant of the two parents. At the B150 treatment, the F_1 hybrids of G61450 crossed with Halberd and with Warigal responded in a tolerant or intermediate manner while the F_1 hybrid between Halberd and Warigal was sensitive.

Growing plants to maturity has been shown to result in compensatory growth by slower development of more sensitive genotypes and this lengthened growing period reducing differences in response to B (Chapters 4 and 5). In anticipation of this effect, the response of F_1 hybrid plants was also recorded during the vegetative stage to provide further information on the mode of inheritance of tolerance to high concentrations of B.

The days from sowing to commencement of tillering and the number of tillers per plant seven weeks after sowing were recorded. There was no maternal effect upon response to B, with respect to tillering, therefore reciprocals have been combined to give eight replicates of each F_1 hybrid in Table 6.3.

Commencement of tillering at the B75 and B150 treatments was delayed to a greater extent for parental combinations including successively more sensitive genotypes (Table 6.3a). For example, Halberd and the F_1 hybrids of Halberd in combination with G61450 and Warigal commenced tillering at approximately the same time while the F_1 hybrids with (Wl*MMC) and Kenya Farmer were delayed six and ten days, respectively, in comparison to Halberd. The total number of tillers per plant seven weeks after sowing showed a similar response as the time from sowing to the commencement of tillering (Table 6.3b). The experiment of Section 6.2 found little difference among the five parents in time to tillering in the absence of applied B. Therefore, the response of the F_1 hybrids, with respect to tillering under high B conditions, could be attributed principally to differences in tolerance to B rather than genetic differences in tiller initiation *per se*.

Table 6.3(a) Days from sowing to commencement of tillering for five genotypes and their F_1 hybrids when grown at the B75 treatment, and for three genotypes and their F_1 hybrids at B150. Reciprocal hybrids have been pooled therefore the values are the means of eight replicates.

	Days to commencement of tillering				
	G61450	Halberd	Warigal	(Wl*MMC)	K F
B75					
G61450	26	26	27	31	30
Halberd		28	28	34	38
Warigal			39	35	50
(Wl*MMC)				76	-
Kenya Farmer					-
LSD (0.05) 5					
B150					
G61450	43	45	60		
Halberd		57	77		
Warigal			-		
LSD (0.05) 15					

Table 6.3(b) Number of tillers per plant for five genotypes and their F_1 hybrids seven weeks after sowing when grown at the B75 treatment. Reciprocal hybrids have been pooled therefore the values are the means of eight replicates.

	Tillers / plant				
	G61450	Halberd	Warigal	(Wl*MMC)	K F
G61450	7.0	5.6	5.9	3.9	3.5
Halberd		5.3	4.8	3.4	3.0
Warigal			2.5	2.6	1.6
(Wl*MMC)				1.0	1.0
Kenya Farmer					1.0
LSD (0.05) 1.5					

The results for commencement of tillering and number of tillers per plant are consistent with the F_1 hybrids being intermediate to the two parents for response to B and therefore with tolerance to B being inherited as a partially dominant character. The compensatory growth which occurs when plants with some degree of tolerance are grown to maturity (Chapter 4) would account for the discrepancy between response at the vegetative stage and at maturity.

The concentration of B in shoots of four week old F_1 hybrid plants, grown at B25, was generally intermediate to the two parents (Table 6.4), although there was no difference in B concentration between combinations including either (Wl*MMC) or Kenya Farmer as parents and this is consistent with the result of Section 6.2 where there was no difference in concentration of B in shoots of (Wl*MMC) and Kenya Farmer at B25. As with other parameters measured there was no difference between reciprocals for concentration of B in shoots.

The results of this experiment demonstrated for a greater number of genotypes that response to B is expressed as a partially dominant character. The changing response of the F_1 hybrids for different parental combinations and treatments would result from differences, between genotypes, in the critical toxic concentrations of soil B. When the B treatment is less than the critical concentration for the F_1 hybrid, the hybrid will respond similarly to the more tolerant parent and tolerance will be expressed as a dominant character. As the concentration of applied B exceeds the critical level the hybrid will respond in a progressively more sensitive manner and tolerance to B will be expressed as either a partially dominant or recessive character.

Table 6.4 Concentration of B (mg kg^{-1}) in whole shoots for five genotypes and their F_1 hybrids, including reciprocals, when grown at the B25 treatment.

Parent	B conc (mg kg^{-1})				
	G61450	Halberd	Warigal	(Wl*MMC)	K F
G61450	207	217	261	335	328
Halberd	216	246	318	341	361
Warigal	258	338	464	480	471
(Wl*MMC)	324	317	464	561	510
Kenya Farmer	296	398	400	557	568

LSD (0.05) 74

6.4 RESPONSE OF AN F_2 POPULATION TO TWO LEVELS OF BORON STRESS.

6.4.1 Introduction

The two previous experiments demonstrated that response to high concentrations of B is expressed as a partially dominant character with the phenotype of the heterozygous F_1 hybrid, relative to the two parents, varying with the degree of B stress. The response of the heterozygotes within a segregating generation might therefore be expected to vary, in relation to the homozygous parental types, for different concentrations of applied B. The response of an F_2 population at two B treatments was examined to test this hypothesis.

6.4.2 Materials and methods

G61450, Kenya Farmer and their F_2 progeny were grown in 100 mm diameter pots containing B75 and B150 soil (400 g soil pot $^{-1}$). One pre-germinated seed was sown per pot. Twenty pots of each parent and 120 pots of F_2 plants were grown at each treatment. Pots were arranged by treatments and the parental standards were distributed at random within the blocks. Pots were re-randomized regularly. The two blocks were located on the same bench in a glasshouse to minimize environmental variation between treatments. Plants were harvested at ground level five weeks after sowing, oven dried and weighed individually.

6.4.3 Results

The dry weights of the F_2 plants and parents are shown in Figure 6.5. The greater degree of toxicity at B150 is evident by the reduced yield of G61450 compared with B75. The mean, mode and maximum yields of the F_2 population were also reduced at B150. There was little change in the response of Kenya Farmer, between treatments, as the B75 treatment proved to be extremely toxic and plants died within three weeks of sowing.

There was also a change in distribution of the response of individual plants compared with the parents and in particular compared with G61450. At B75,

approximately two thirds of the F_2 plants yielded within two standard deviations of the mean of G61450 while at B150 only one quarter of the F_2 plants produced such yields. The linear scale used for plant dry weight in Figure 6.5 results in the impression of a considerable number of F_2 plants at B150 responding similarly to Kenya Farmer. This is in fact not the case and only seven plants yielded within two standard deviations of the mean of Kenya Farmer.

The very low frequency of F_2 plants which were as sensitive as Kenya Farmer demonstrates that the two parents differ at more than one locus with respect to control of response to B. If there had been a single gene difference between the two parents, one quarter of the F_2 plants would have been as sensitive as Kenya Farmer.

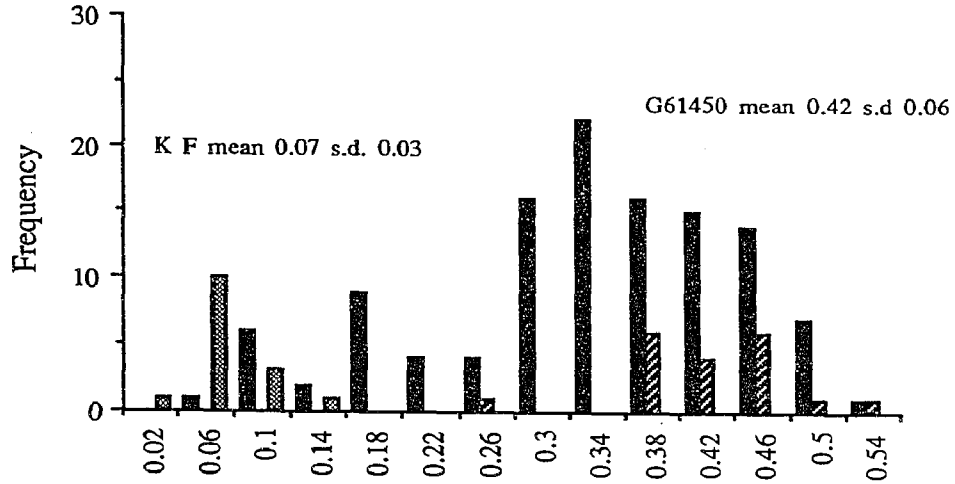
The change in the frequency distribution of dry matter production for the F_2 population is consistent with additive gene control. When the concentration of applied B is less than the threshold level for the heterozygous, or intermediate homozygous genotypes of an F_2 population, these genotypes will be phenotypically similar to the more tolerant parent. As the concentration of applied B is increased above the threshold level, the intermediate genotypes will respond as more sensitive than the tolerant parent. This principal is demonstrated in Figure 6.6 for the situation where the two parents differ at two loci with alternative alleles at each locus and based on the assumption of equal expression at both loci and a linear reduction in yield above a threshold level as indicated by Figure 6.2 and described by Bingham *et al.* (1985).

Figure 6.5 Dry matter yield (g) of individual plants for G61450, Kenya Farmer and the F_2 generation of (G61450*Kenya Farmer) grown at two B treatments.

(a) B75

(b) B150

(a) B75


30
20
10
0
Frequency
K F mean 0.07 s.d. 0.03
G61450 mean 0.42 s.d 0.06
0.02
0.06
0.1
0.14
0.18
0.22
0.26
0.3
0.34
0.38
0.42
0.46
0.5
0.54

(b) B150

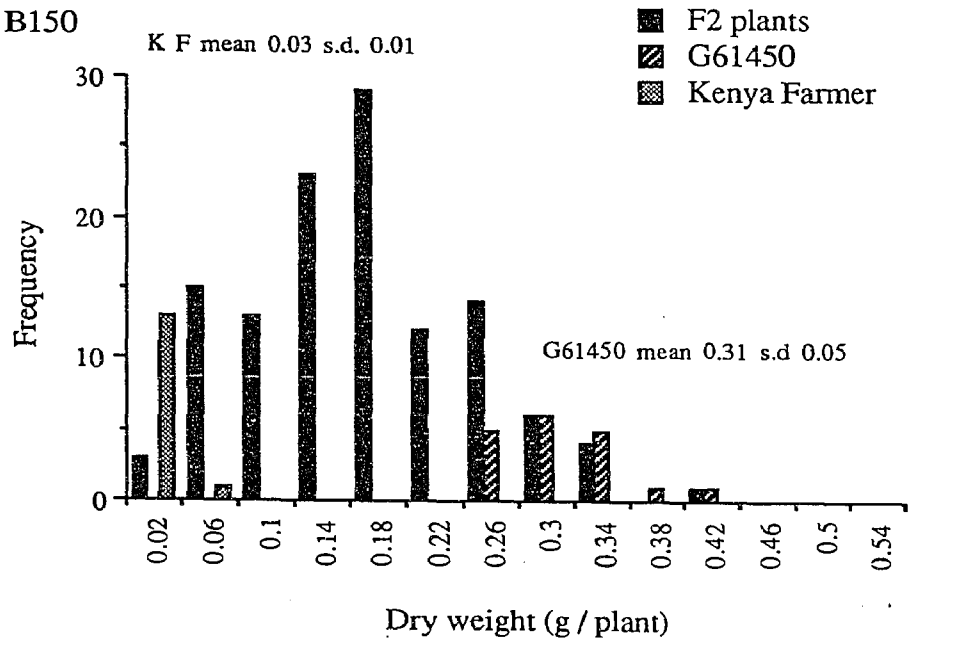
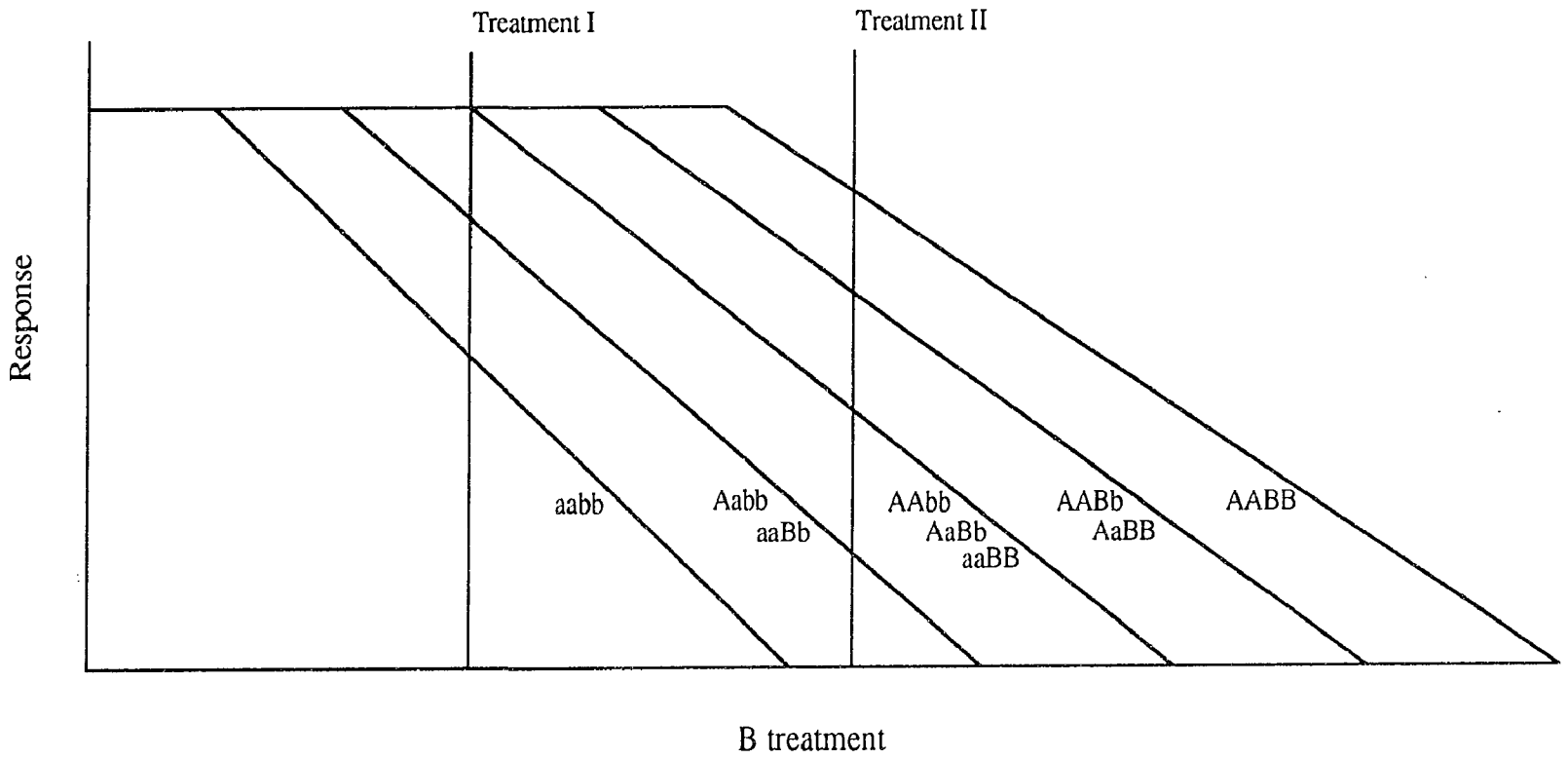

K F mean 0.03 s.d. 0.01
F2 plants
G61450
Kenya Farmer
30
20
10
0
Frequency
G61450 mean 0.31 s.d 0.05
0.02
0.06
0.1
0.14
0.18
0.22
0.26
0.3
0.34
0.38
0.42
0.46
0.5
0.54
Dry weight (g / plant)

Figure 6.6 Theoretical responses to applied B for five genotypes of contrasting tolerance to B. The model assumes additive genetic control and a linear reduction in yield above a threshold concentration. Two B treatments are indicated to demonstrate the differences in discrimination between genotypes at alternative levels of B stress. At Treatment I the genotypes AAbb, AaBb, aaBB, AABb and AaBB would respond in a similar manner to the most tolerant genotype, AABB. At Treatment II all genotypes would be more sensitive than AABB and the difference between aabb and Aabb or aaBb is less than between the other genotypes.


Treatment I
Treatment II
Response
aabb
Aabb
aaBb
AAbb
AaBb
aaBB
AABb
AaBB
AABB
B treatment

6.5 ESTIMATION OF THE NUMBER OF LOCI CONTROLLING RESPONSE TO HIGH CONCENTRATIONS OF BORON.

6.5.1 Introduction

The previous experiment demonstrated that response to high concentrations of B is under the control of several (or many) additive genes. This conclusion is based on the results obtained for the extreme comparison between tolerant (G61450) and very sensitive (Kenya Farmer) genotypes. A further experiment was conducted examining segregation in response to B for the progeny of less extreme parental combinations to determine whether individual major genes could be recognized.

A consequence of the additive nature of inheritance of response to B (i.e. more than two genes acting additively) is that it is unlikely that discrete classes of the two parental types will be observed in the F_2 generation. The proposed strategy for assessing segregation in response to B is to grow F_2 and F_3 generations at the lowest B treatment required to distinguish between the two parental types. The treatment will vary with parental combinations and will be chosen according to the more sensitive parent on the basis of the results of Section 6.2.

In the comparison of response of the F_2 generation of (G61450*Kenya Farmer) at the B75 and B150 treatments, the frequency of plants in the most sensitive category (i.e. similar to Kenya Farmer) was similar between treatments and so it appears that the response of sensitive genotypes is more stable, between treatments, than the intermediate genotypes. Therefore, plants in the segregating generations would be rated as being either as sensitive as the more sensitive parent or more tolerant than the sensitive parent. The genotypes of plants in the intermediate - tolerant category in the F_2 can be determined by progeny testing at the F_3 generation.

Five genotypes were used as parents and they were compared, together with three further genotypes studied in Chapter 7, at high concentrations of B under the conditions intended for screening of the F_2 and F_3 generations to ensure that they responded in a similar manner to the pot experiment described in Section 6.2.

6.5.2 Materials and methods

Genotypes

All combinations, excluding reciprocals, of the standard genotypes G61450, Halberd, Warigal, (WI*MMC) and Kenya Farmer, with the exception of (G61450* Kenya Farmer) were tested for response to B at the F_2 generation and selected crosses were tested at the F_3. All F_2 seed was obtained from F_1 plants grown in potting mix without applied B. F_3 seed was, in general, obtained from plants which had been tested at a high B treatment and then transplanted to normal potting mix. F_3 seed of (G61450*Halberd) and (G61450*Warigal) was obtained from unselected F_2 plants grown in normal potting mix.

Treatments

B enriched soil was weighed into non-draining trays, dimensions 400mm x 285mm x 120mm (10.5 kg soil tray $^{-1}$). For the initial screening of the parents B50, B75 and B100 treatments were used. The concentration of B applied to the soil varied between parental combinations and the treatment was selected according to the more sensitive of the two parents. The treatments were B50, B75 and B100 when Kenya Farmer, (WI*MMC) and Warigal were the more sensitive parents, respectively, and B150 for (G61450*Halberd).

Experimental design

All seeds were pre-germinated prior to sowing. The preliminary experiment of the parents was sown at a spacing of 5cm x 4cm with five seeds of each parent and a total of 40 seeds per tray. The genotypes Federation, Chinese Spring and CS(KF4A) were included with the above five genotypes, but results for only the five parental genotypes are reported. The experiment consisted of two replicates. The F_2 seeds were sown at a spacing of 5 cm x 5 cm and 25 F_2 seeds and five seeds of each parent were sown per tray. A total of five trays, or 125 F_2 seeds, were sown for each parental combination. The F_3 seeds were sown at a spacing of 5 cm x 3.5 cm and 35 F_3 seeds and five seeds of each parent were sown per tray. Three F_3 families were included in each tray, therefore

each family consisted of either 11 or 12 plants. As the major objective of testing the F_3 generation was to detect segregation within families and so determine whether the F_2 parent was homozygous or heterozygous, the plants of each family were grouped to minimize environmental variation within families. The number of plants grown at the F_2 and F_3 generations was determined according to theoretical segregation patterns, as outlined below.

For the initial experiment of the parental genotypes, the height of plants to the tip of the YEB and the development of symptoms of B toxicity, expressed as a percentage of the total leaf length for all fully expanded leaves, were measured three, four, five and six weeks after sowing.

The vigour and expression of B toxicity symptoms of progeny were compared with the parental standards within each tray approximately five or six weeks after sowing. The actual time varied slightly between seasons and was less during the warmer months. After scoring for response to B, F_2 plants were transplanted to normal potting mix to enable them to recover and produce sufficient seed for progeny testing. The F_2's of the two crosses (G61450*Halberd) and (G61450*Warigal) were screened during the months of November and December. The long daylength and high temperatures at this time of the year restricted the production of tillers subsequent to transplanting and many plants of these crosses produced insufficient seed for progeny testing. F_3 seed for these crosses was therefore derived from unselected F_2 plants grown in normal potting mix.

The results obtained for segregation at the F_2 and F_3 generations were compared by chi-square analysis with the expected frequencies for segregation at one and two loci. At both the F_2 and F_3 generations, plants were assigned to one of two categories, namely sensitive and intermediate - tolerant. When the ratio of sensitive : intermediate - tolerant plants was consistent with monogenic segregation, the ratio of the additional categories sensitive : segregating : tolerant was tested for goodness of fit to a 1 : 2 : 1 segregation ratio at the F_3 generation.

Selection of population sizes

The above population sizes were chosen on the basis of theoretical considerations related to expected frequencies for alternative hypotheses. When scoring plants as being either as sensitive as the sensitive parent or more tolerant than the sensitive parent, the expected frequency of sensitive types for segregation at one, two and three loci are 1/4, 1/16 and 1/64, respectively. The minimum family size to distinguish between the alternative hypotheses can be derived from the formula

$$\sqrt{n} = t\left[(a_1a_2)^{1/2} + (b_1b_2)^{1/2} / \; b_1 - a_1\right]$$

where a_1 and a_2 are the expected class frequencies for one hypothesis and b_1 and b_2 are the expected class frequencies for the alternative hypothesis and ($a_1 + a_2 = 1$ and $b_1 + b_2 = 1$) (Hanson, 1959). To distinguish between the frequencies of 1/4 and 1/16, approximately 50 F_2 plants or F_3 families would be required at $P < 0.05$ or 86 at $P < 0.01$, while to distinguish between the frequencies of 1/16 and 1/64 would require 234 F_2 plants or F_3 families at $P < 0.05$ and 404 at $P < 0.01$. In these series of tests, the number of plants grown was sufficient to distinguish between segregation at one and two loci, even allowing for the possible loss of plants for reasons other than B toxicity. However, often there were insufficient plants to distinguish between segregation at two and three loci.

The number of F_3 progeny needed to determine the genotype of an F_2 plant can also be calculated. The basis of scoring plants was that they were either sensitive or intermediate - tolerant, therefore the hypothesis that an F_2 plant was homozygous sensitive would be accepted only if all F_3 progeny were sensitive. For a single gene, the probability of obtaining a homozygous sensitive (aa) from a heterozygous (Aa) F_2 plant is 1/4 and the probability of all (n) progeny of an F_2 heterozygote being sensitive is $(1/4)^n$. For $n = 4$, $P < 0.01$ and $n = 5$, $P < 0.0001$. The probability of an F_3 family of all sensitive types being derived from a heterozygous F_2 plant is therefore very low when n = 11 or 12.

As an alternative, it would be possible to establish whether the F_2 plants in the intermediate - tolerant category were heterozygous (Aa) or homozygous tolerant (AA). For control at a single locus, 3/4 of the F_2 plants would be of these types. The progeny of heterozygous plants would include sensitive types at a frequency of 1/4 but the homozygotes would produce none. The critical test for heterozygotes would therefore be presence of sensitive types in segregating F_3 families. The probability of an individual offspring of a heterozygote being either homozygous tolerant or heterozygous (i.e not sensitive) is 3/4, therefore, for a family of size n, the probability of no sensitive types would be $(3/4)^n$. For n = 11, P = 0.042 and n = 12, P = 0.032. The number of F_3 progeny grown was therefore sufficient to determine whether the F_2 plants were homozygous sensitive, heterozygous or homozygous tolerant when parents differed at a single gene, with respect to response to B.

For a comparison between genotypes which differ at two loci (A and B), 9/16 of the F_2 plants would be able to produce sensitive (aabb) offspring. The genotype AaBb would produce sensitive types at a frequency of 1/16, therefore the probability of all progeny being sensitive would be $(1/16)^n$ and for n = 4, $P < 0.0001$. The probability of all F_3 progeny being sensitive and therefore misclassification of this particular genotype is very low. The genotypes Aabb and aaBb would segregate in the same manner as for control at a single locus as described above and the probability of all sensitive progeny resulting from such genotypes would be $(1/4)^n$ ($n = 4$, $P < 0.01$).

Therefore, sufficient F_3 progeny were grown to enable the identification of homozygous sensitive families for segregation at two loci. In view of the number of intermediate genotypes and the interaction between genotype and threshold B concentrations, it is unlikely that the genotypes of other F_2 types could be determined by progeny testing when two or more loci are segregating for response to B.

6.5.3 Results

Response of parents to high concentrations of B

The five genotypes could be grouped into three categories on the basis of seedling height, namely (1) G61450 and Halberd, (2) Warigal and (Wl*MMC) and (3) Kenya

Farmer (Fig. 6.7). Although the genotypes were not compared in the absence of applied B, the previous result for the pot experiment of Section 6.2 found no difference between the genotypes at the B0 control (Fig. 6.3). The ranking of genotypes in the presence of high concentrations of B was the same for both the pot experiment and for plants grown at high density in trays.

The ranking of genotypes for symptom expression (results for week six are given in Fig. 6.8) was consistent with the results for both dry matter yield and concentration of B in shoots for the pot experiment. The degree of discrimination between genotypes for symptom expression differed from that for seedling height. (Wl*MMC) developed more severe symptoms than Warigal (Fig. 6.8; Plate 8) and in this respect is consistent with the results of Section 4.3 (Table 4.1). Symptom expression by G61450 was consistently less severe than that by Halberd, although the absolute difference was small compared with the differences which resulted between the other genotypes. The degree of symptom expression by leaves one and two was more consistent with the expected response of genotypes (Table 6.1, Fig. 6.2) than symptom expression by leaf three. For example, symptom expression by leaves one and two was always more severe for (Wl*MMC) than Warigal but there was no difference between the two genotypes for symptom development of the third leaf at the B75 and B100 treatments. This could be attributed to the delayed emergence of the third leaf of (Wl*MMC) and therefore B accumulation for a shorter period.

The results for the parents indicate that the response to high concentrations of B, when grown at a high plant density corresponds with the response in a pot experiment. The degree of symptom expression enabled discrimination between Warigal, (Wl*MMC) and Kenya Farmer while assessment on the basis of vigour and symptom expression was required to distinguish between Halberd and Warigal. There was little difference between G61450 and Halberd for the three B treatments used in this experiment and a higher level of applied B may be required to distinguish between them.

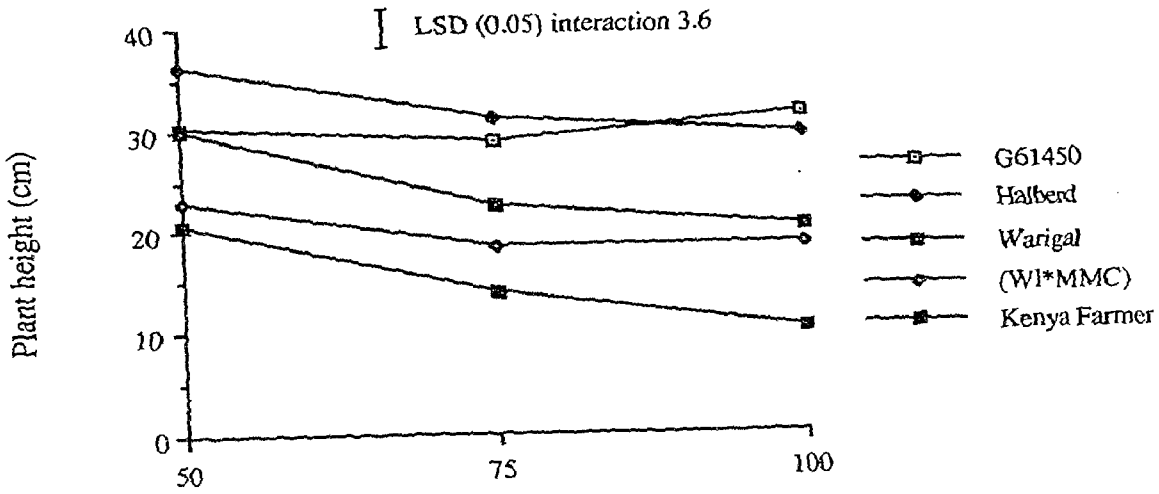
Figure 6.7 Plant height (cm) to the tip of the youngest emerged leaf blade for the five parental genotypes grown at the B50, B75 and B100 treatments.

(a) four and

(b) six weeks after sowing.

The vertical bars represent the LSD (0.05) for the genotype x treatment interactions.

(a) Week 4



(b) Week 6

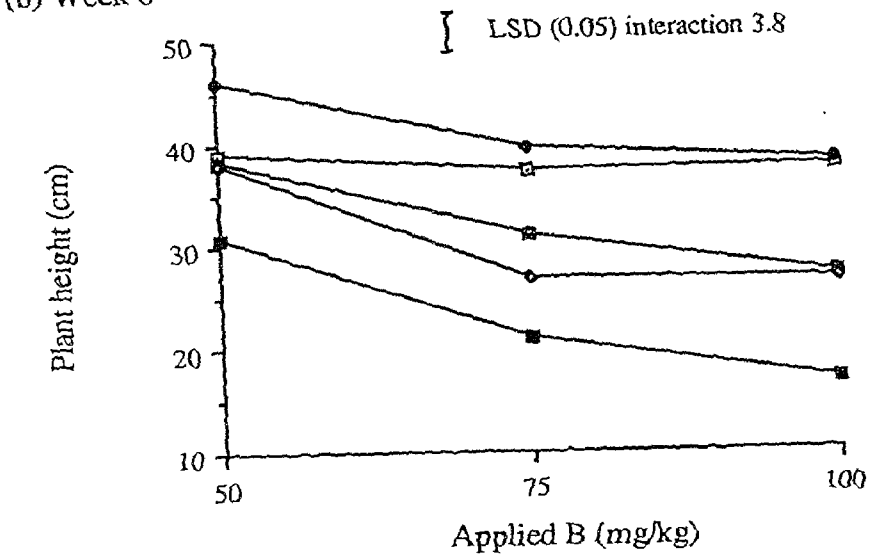


Figure 6.8 Expression of symptoms of B toxicity (necrotic region as a percentage of the total leaf length) for the first three leaves (leaf one is the first leaf formed) of five genotypes grown at three B treatments.

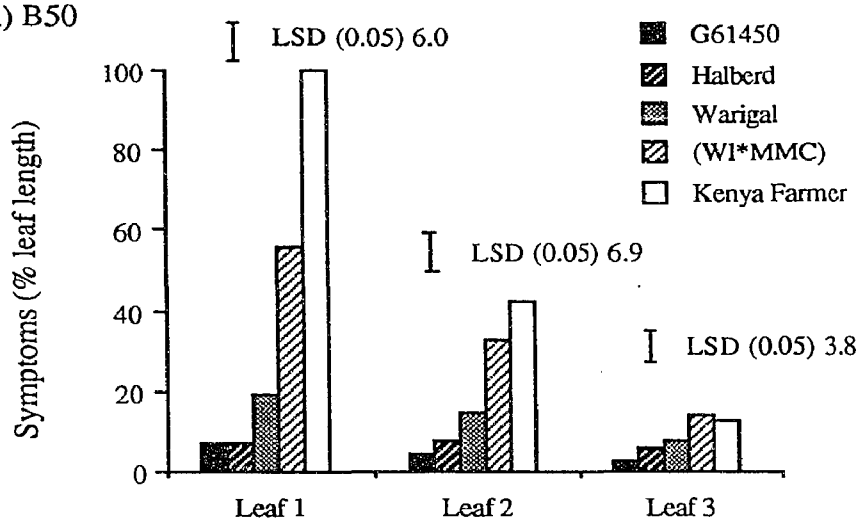
(a) B50

(b) B75

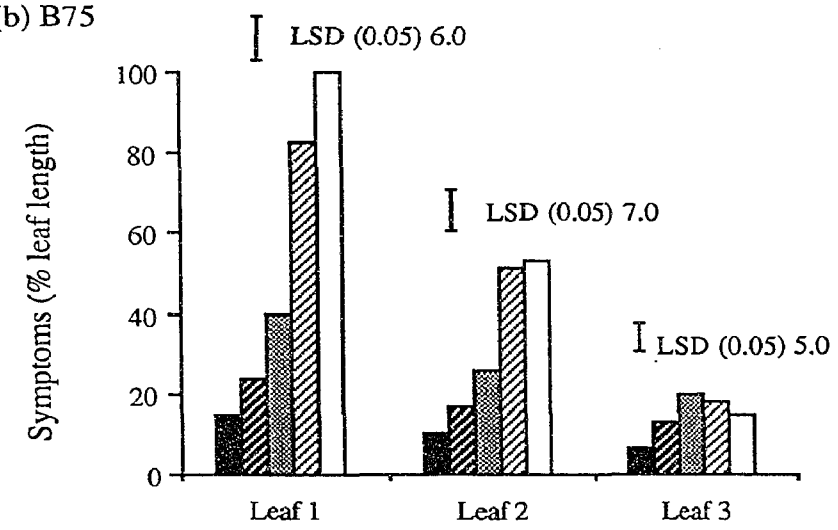
(c) B100

The vertical bars represent the LSD (0.05) between genotypes.

(a) B50



(b) B75



(c) B100

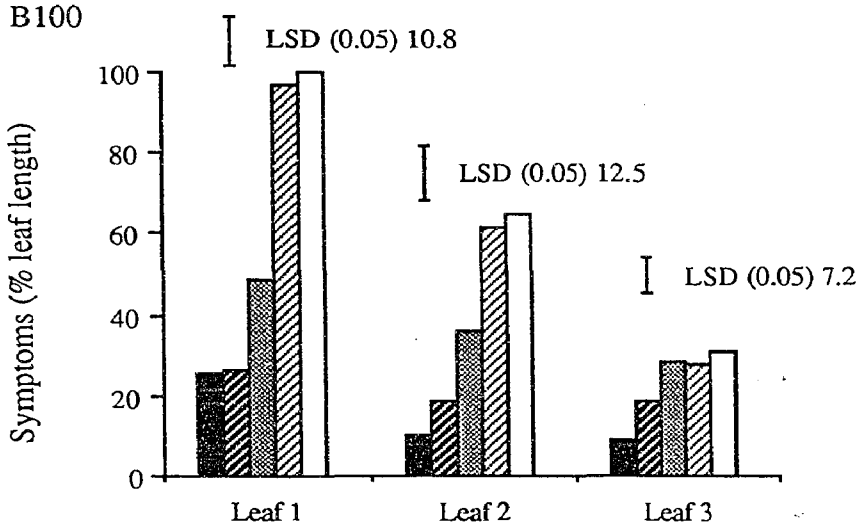


Plate 8. Comparison between (Wl*MMC) and Warigal for symptom expression during early seedling development. The first leaf of (Wl*MMC) is dead while the first leaf of Warigal is approximately 25% necrotic. The third leaf of (Wl*MMC) shows the "mid-leaf necrosis" symptom.


(WI*MMC)/W1/10
Warigal

Response of F_2 s to high concentrations of B

The F_2 plants for crosses with Kenya Farmer or (Wl*MMC) as the more sensitive parent were scored on the basis of symptom expression while the F_2s for (Halberd* Warigal), (G61450*Warigal) and (G61450*Halberd) were scored for vigour, as determined by plant height.

Chi-square analysis of the results for crosses with Kenya Farmer and (Wl*MMC) as the more sensitive parent are presented in Table 6.5. The results are consistent with segregation at a single locus for ((Wl*MMC)*Kenya Farmer) and for (Warigal*(Wl*MMC)). The number of sensitive plants in the F_2 generation of (Warigal*Kenya Farmer), (Halberd * Kenya Farmer), (Halberd * (Wl*MMC)) and (G61540 * (Wl*MMC)) were generally consistent with segregation at either two or three loci, but limitations resulting from the population size precludes testing to distinguish between control at two and three loci. As there was a single gene difference for control of response to B between Kenya Farmer and (Wl*MMC) and between (Wl*MMC) and Warigal, it is probable that Kenya Farmer and Warigal differ at two loci.

The height of F_2 plants of (G61450*Warigal) and (Halberd*Warigal) at B100 lie within the range of the parental standards (Fig. 6.9). It was not possible to assign F_2 plants to discrete categories but the high proportion of "Warigal types" among the F_2 of (G61450*Warigal) indicates segregation at relatively few loci. The result for (Halberd*Warigal) was inconclusive, principally due to the overlap in response between the parental standards. The range in heights of F_2 plants of (G61450*Halberd) exceeded the range of the parental standards and thus suggested transgressive segregation, or a different complement of genes controlling response to B between G61450 and Halberd. The tallest and shortest F_2 plants from each tray were selected to develop F_4 lines to test further whether there was in fact transgressive segregation in response to B with a procedure described later.

Table 6.5 Segregation in response to high concentrations of B for the F_2 populations with either Kenya Farmer or (Wl*MMC) as one of the parents. Individual plants were classified as either as sensitive as the more sensitive parent (sensitive) or more tolerant than the sensitive parent (tolerant - intermediate). Observed segregation ratios were compared with expected ratios for segregation at one and two loci.

Hybridisation		Observed and expected frequencies			χ^2_1
		Model	Tol - inter	Sensitive	
(Wl*MMC)*K F	Obs		91	29	
	Exp	3 : 1	90	30	0.01
	Exp	15 : 1	112.5	7.5	62.72
Warigal*K F	Obs		115	9	
	Exp	3 : 1	93	31	19.88
	Exp	15 : 1	116.25	7.75	0.08
Halberd*K F	Obs		119	5	
	Exp	3 : 1	93	31	27.97
	Exp	15 : 1	116.25	7.75	0.70
Warigal*(Wl*MMC)	Obs		95	29	
	Exp	3 : 1	93	31	0.10
	Exp	15 : 1	116.25	7.75	59.26
Halberd*(Wl*MMC)	Obs		115	10	
	Exp	3 : 1	93.75	31.25	18.37
	Exp	15 : 1	117.2	7.8	0.40
G61450*(Wl*MMC)	Obs		119	6	
	Exp	3 : 1	93.75	31.25	26.13
	Exp	15 : 1	117.2	7.8	0.23

P	0.01	0.05	0.20	0.50
χ^2_1	6.64	3.84	1.64	0.46

Response of F3s to high concentrations of B

The results for the screening of F3 families at high concentrations of B are presented in Table 6.6. As for the screening of plants of the F2 generation, the F3 plants were rated visually for symptom development for crosses with Kenya Farmer and (Wl*MMC) as the more sensitive parents and for vigour for other combinations. Initially, comparisons were made on the basis of families with all sensitive progeny compared with families which were either segregating or all tolerant and observed results were tested by chi-square analysis for goodness of fit to segregation ratios consistent with segregation at one and two loci. For crosses which had segregation ratios consistent with a single gene difference between parents, the F3 families were also assigned to three categories, namely all sensitive, segregating and all tolerant and tested for goodness of fit to a ratio of 1 : 2 : 1 (Table 6.6). The correspondence between plants rated as sensitive at the F2 and progeny rated as all sensitive at the F3 was also determined for all crosses which were progeny tested (Table 6.7).

The frequency of F3 families with all progeny sensitive to B was consistent with segregation at a single locus for ((Wl*MMC)*Kenya Farmer), (Warigal*(Wl*MMC)) and (Halberd*Warigal). The ratio of tolerant : segregating : sensitive families was also consistent with the monogenic segregation ratio of 1 : 2 : 1 for the three comparisons. (Examples of tolerant, segregating and sensitive F3 families for ((Wl*MMC)*Kenya Farmer are presented in Plate 9). The frequency of sensitive F3 families of (G61450*Warigal) was significantly less than expected for segregation at a single locus ($0.01<P<0.05$) but more than expected for segregation at two loci ($P<0.001$). Such deviations from both models may have resulted either by chance, through misclassification resulting from environmental variation or from segregation of a second gene of small effect. It is more probable that environmental variation would result in a sensitive family being classified as segregating than a segregating family being classified as sensitive because only one plant would need to be misclassified for the former but three quarters (assuming control at a single locus) for the latter type of error. For parents differing at two loci it is possible that families homozygous dominant at one locus and

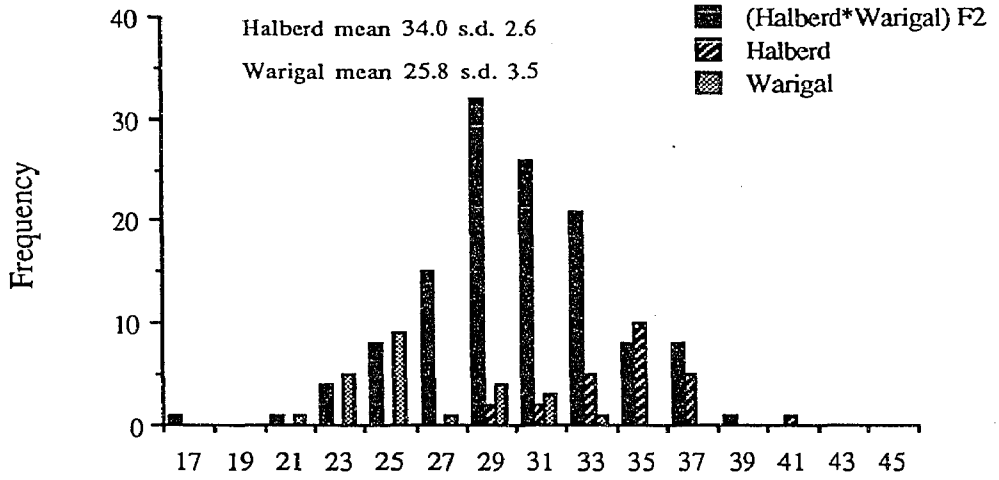
Figure 6.9 Height (cm) of F_2 plants and parents when grown at high concentrations of B.

(a) (Halberd*Warigal) at B100

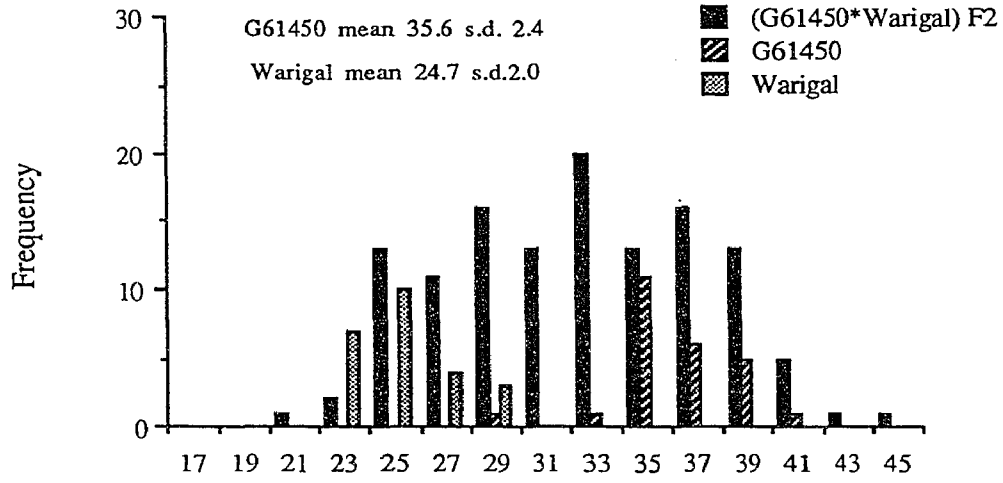
(b) (G61450*Warigal) at B100

(c) (G61450*Halberd) at B150

(a) (Halberd*Warigal)



(b) (G61450*Warigal)



(c) (G61450*Halberd)

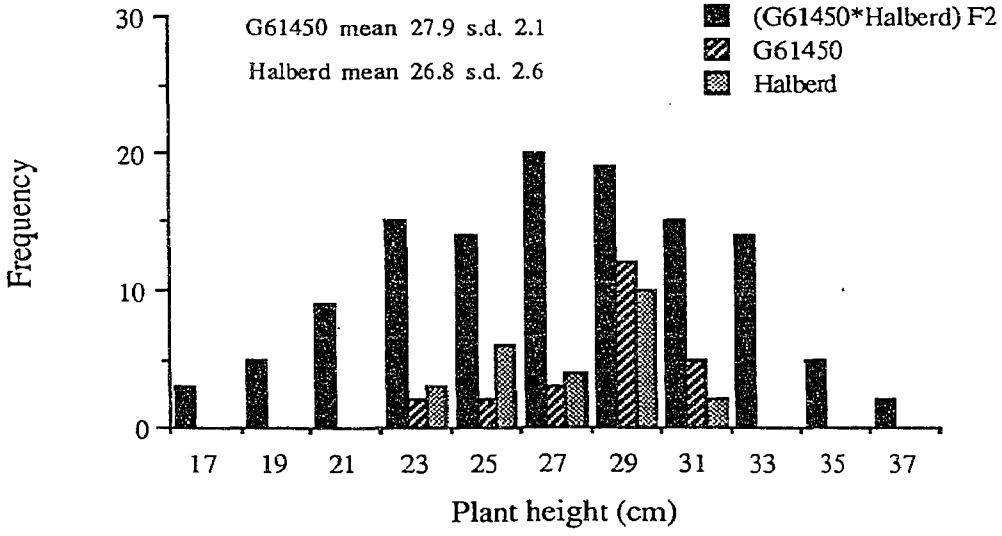


Table 6.6 Segregation in response to high concentrations of B for the F_3 families among five genotypes. Individual plants within families were classified as either as sensitive as the more sensitive parent (sensitive) or more tolerant than the sensitive parent (tolerant - intermediate) and families were classified as either homozygous sensitive or tolerant - segregating. Responses of families were compared with expected ratios for segregation at one and two loci. Combinations which were consistent with segregation at a single locus were retested for the family classifications tolerant, segregating and sensitive.

Hybridisation		Observed and expected frequencies			χ^2_1
		Model	Tol - Seg	Sensitive	
(Wl*MMC)*K F	Obs		95	22	
	Exp	3 : 1	87.75	29.25	3.20
	Exp	15 : 1	109.7	7.3	29.46
Warigal*KF	Obs		114	9	
	Exp	3 : 1	92.25	30.75	19.57
	Exp	15 : 1	115.3	7.7	0.06
Warigal*(Wl*MMC)	Obs		95	27	
	Exp	3 : 1	91.5	30.5	0.40
	Exp	15 : 1	114.4	7.6	50.12
Halberd*(Wl*MMC)	Obs		112	5	
	Exp	3 : 1	87.75	29.25	25.71
	Exp	15 : 1	109.7	7.3	0.47
G61450*(Wl*MMC)	Obs		113	6	
	Exp	3 : 1	89.25	29.75	24.23
	Exp	15 : 1	111.6	7.4	0.12
Halberd*Warigal	Obs		87	21	
	Exp	3 : 1	81	27	1.49
	Exp	15 : 1	101.2	6.8	29.45
G61450*Warigal	Obs		97	17	
	Exp	3 : 1	85.5	28.5	5.67
	Exp	15 : 1	106.9	7.1	13.16

Hybridisation		Model	Tol	Seg	Sens	χ^2_2
(Wl*MMC)*KF	Obs		38	57	22	
	Exp	1 : 2 : 1	29.25	58.5	29.25	4.46
Warigal*(Wl*MMC)	Obs		32	63	27	
	Exp	1 : 2 : 1	30.5	61	30.5	0.34
Halberd*Warigal	Obs		17	60	21	
	Exp	1 : 2 : 1	27	54	27	5.70
G61450*Warigal	Obs		27	70	17	
	Exp	1 : 2 : 1	28.5	57	28.5	7.68

P	0.01	0.05	0.20	0.50
χ^2_1	6.64	3.84	1.64	0.46
χ^2_2	9.21	5.99	3.22	1.39

Plate 9. Response of F3 families of ((W1*MMC)*Kenya Farmer) to the B50 treatment and comparison with the two parents.

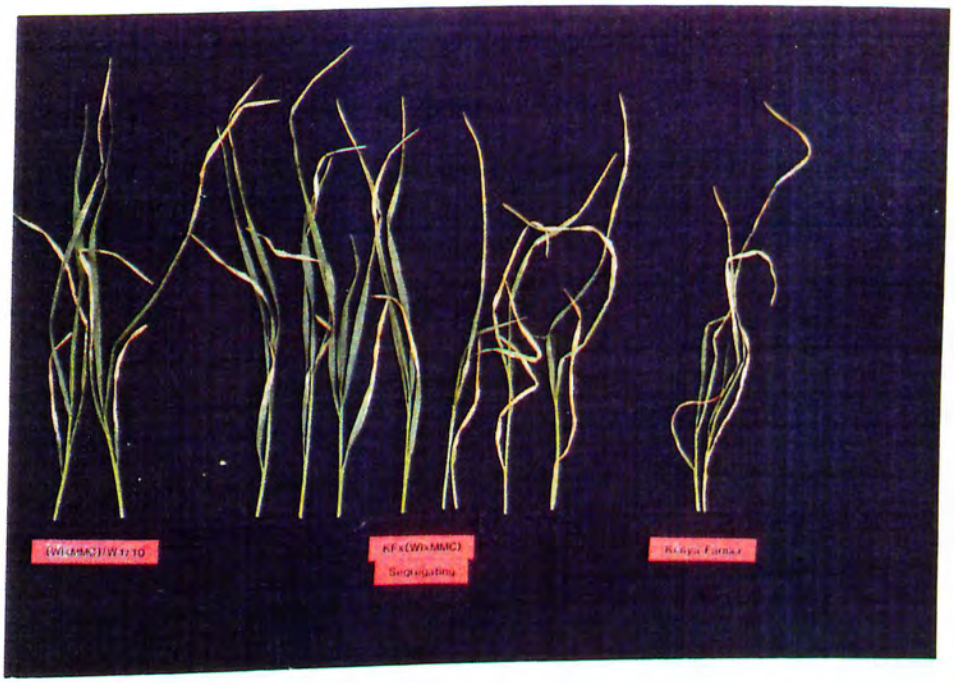
(a) a homozygous tolerant family,

(b) a segregating family and

(c) a homozygous sensitive family.

(a)

(b)



(c)

homozygous recessive at the other (e.g. AAbb), and therefore non-segregating, may be classified as double recessives (aabb).

For the combinations (Warigal*Kenya Farmer), (Halberd*Kenya Farmer), (Halberd*(Wl*MMC)) and (G61450*(Wl*MMC)) the observed frequencies of homozygous sensitive F_3 families were not significantly different from that expected for segregation at two loci..The hypothesis of segregation at three loci was not tested by chi-square analysis as the number in the expected category (125 x 1/64) was too low for statistical validity.

The rating of F_2 plants and their F_3 progeny was generally in close agreement (Table 6.7). For the exceptions where plants were scored as sensitive at the F_2, but not at the F_3, the F_3 families segregated for response to B and therefore the F_2 plants were actually heterozygotes. There were no instances of plants rated as sensitive at the F_2 producing all tolerant progeny. The greatest discrepancy between classification at the F_2 and the F_3 generations was for (Halberd*(Wl*MMC)) where seven families derived from putatively sensitive F_2 plants segregated in response to B. For five of the seven families, however, there were no F_3 progeny as tolerant as Halberd. This would indicate that while the F_2 plants were heterozygous, it was probably at only one of two loci with the other locus being homozygous sensitive (e.g. Aabb).

*Transgressive segregation for (G61450 * Halberd)*

A total of 53 F_3 families derived from unselected F_2 plants of (G61540 * Halberd) were tested at B150 and again there was evidence of transgressive segregation. The mean height over all plants within each family are compared with the mean heights of the parental standards within each tray (Fig 6.10a) and the heights of individual F_3 plants are compared with the two parents (Fig 6.10b). Among these, families with all progeny less vigorous than Halberd, or more vigorous than G61450 could be recognized and in particular there was a high proportion of very sensitive plants. A sensitive and a tolerant family are compared with the two parents in Plate 10).

Further evidence of transgressive segregation in response to B, between G61540 and Halberd, was obtained from F_4 lines derived from the most vigorous and least

Table 6.7 Comparison between the classification of F_2 plants and derived F_3 families to high concentrations of B.

Hybridisation	F_2 plants sensitive	F_3 families sensitive	Corresponding F_2 and F_3	F_2 sensitive F_3 segregating	F_2 sensitive F_3 missing	F_2 tol - inter F_3 sensitive
((Wl*MMC)*KF)	29	22	21	7	1	1
(Warigal*KF)	9	9	9	-	-	-
(Warigal*(Wl*MMC))	29	27	22	6	1	5
(Halberd*(Wl*MMC))	10	5	2	7[a]	1	3
(G61450*(Wl*MMC))	6	6	5	1	-	1

[a] 5 of the 7 families which segregated contained no tolerant types.

vigorous plants in each tray for the initial F_2 screening (Fig. 6.9c). A total of 10 F_2 plants (five tolerant and five sensitive) were selected and from each plant four unselected F_3 plants were grown. F_4 seed obtained from each of the F_3 plants was sown in 100 mm diameter pots containing B50 soil (four pre-germinated seeds per pot). The experiment consisted of two replicates of the F_3 lines and therefore eight replicates of each F_2 plant. Four pots each of G61540 and Halberd were included per replicate. Whole shoots were harvested four weeks after sowing and analyzed for B concentration.

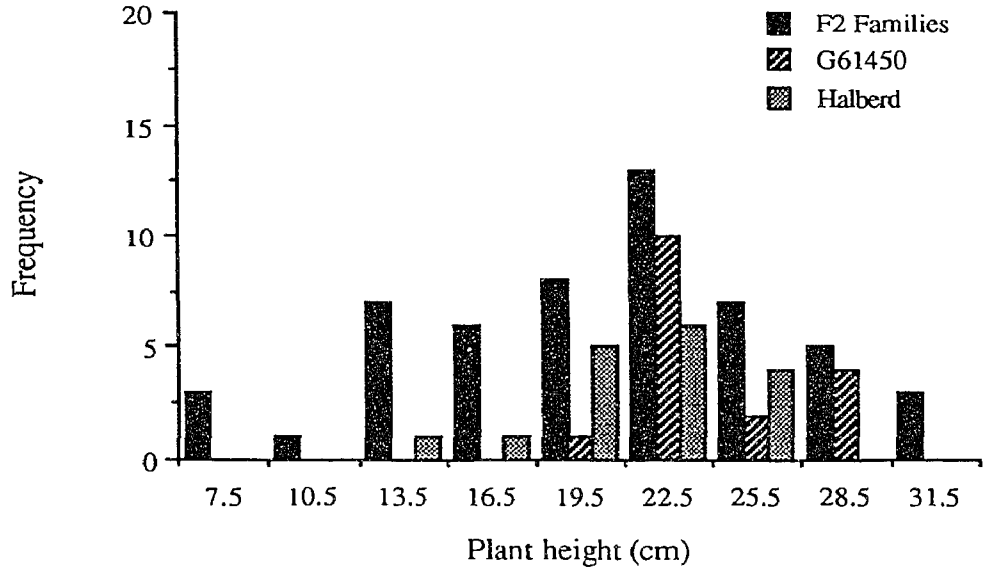
The B concentration in shoots of four lines derived from F_3 plants and two of the F_2 families was significantly greater than for Halberd (Table 6.8). The B concentration in shoots of three F_2 families was less than for the G61450 standards, although the differences were not significant at the 5% confidence level by the L.S.D. calculated from the Error Mean Square value of the analysis of variance. The magnitude of the error term of the analysis of variance is increased by variation within families due to heterozygosity of any of the F_2 plants and hence segregation at the F_3 and F_4 generations. Thus, the error term is a measure of the combined effect of environmental variation and genetic segregation within families. As an alternative means of analysis, the B concentrations of individual F_2 families were compared with the parental standards by an unpaired t-test. By this method the B concentrations in shoots of families 418-3 and 426-2 were significantly less than for G61450 and 414-2 and 442-1 were significantly greater than for Halberd. The families with low and high concentrations of B in shoots corresponded with the most vigorous and least vigorous F_2 plants, respectively.

Figure 6.10 Height (cm) of parents and F_3 plants of (G61450*Halberd) when grown at the B150 treatment.

(a) F_2 family height. Mean of 10 - 12 F_3 plants.

(b) Heights of individual F_3 plants over all families.

(a) F2 family means



(b) Individual F3 plants

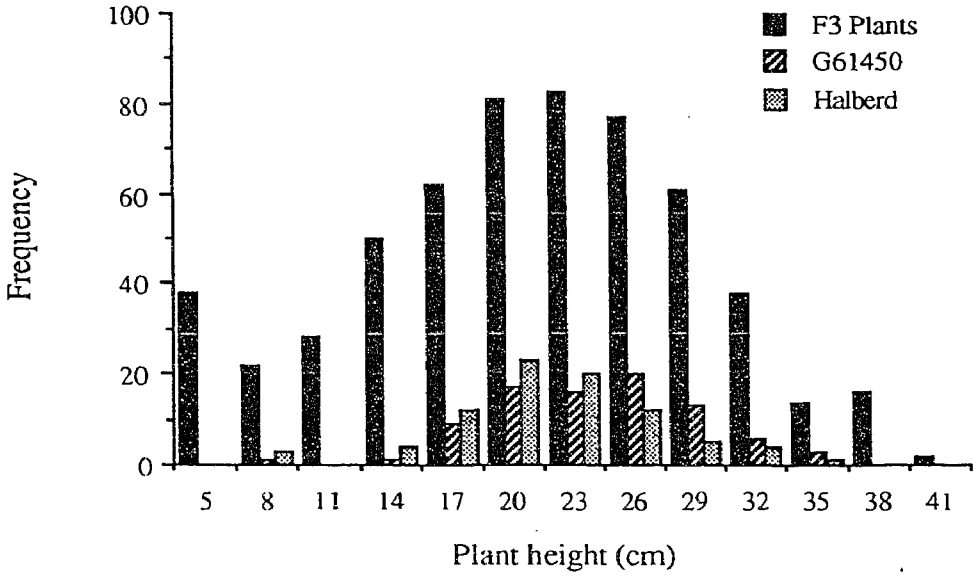


Plate 10. Response of sensitive and tolerant F3 families of (G61450*Halberd) and comparison with the two parents when grown at the B150 treatment.
Left to right : Halberd, a sensitive family, a tolerant family and G61450.

Table 6.8(a) Concentration of B (mg kg^{-1}) in whole shoots of F_4 plants, derived from individual F_2 selections of (G61450*Halberd) chosen for extremes of response to B, when grown at the B50 treatment. Four F_3 plants were selected per F_2 family. The value for each F_3 selection is the mean of two replicates and of four F_4 seeds per pot.

Family	F_2 height (cm)	B conc in shoots (mg kg^{-1})				
		F_3 selections				Family mean
414-2	16	244.3	182.8	278.2	227.1	233.1
407-1	17	214.3	193.8	207.6	220.8	209.1
425-1	17	178.4	188.3	210.8	253.1	207.7
442-1	22	224.9	290.2	184.6	292.5	248.1
443-1	22	128.9	178.8	189.5	197.5	173.7
400-1	33	135.2	119.3	108.3	147.5	127.6
418-3	35	105.8	101.1	91.2	127.8	106.5
426-2	35	110.1	102.5	101.6	103.8	104.5
410-1	36	124.6	118.1	119.8	113.1	118.9
436-3	36	142.6	105.2	112.6	126.5	121.7
G61450	28					127.8
Halberd	27					191.3

LSD (0.05) F_3 selections 58.3, F_2 family means 32.4

Table 6.8(b) Comparison by unpaired t - test for F_2 families of (G61450*Halberd) and the parents for concentration of B in shoots when grown at the B50 treatment. Tolerant families are compared with G61450 and sensitive families with Halberd.

Comparison with Halberd					
Family	414-2	407-1	425-1	442-1	443-1
t - test	2.17	1.40	0.97	2.25	1.15
P	P<0.05	n.s.	n.s.	P<0.05	n.s.
Comparison with G61450					
Family	400-1	418-3	426-2	410-1	436-3
t - test	0.03	2.39	2.83	1.35	0.54
P	n.s.	P<0.05	P<0.01	n.s.	n.s.

6.6 DISCUSSION

Tolerance in wheat to high concentrations of B was found to be under additive genetic control. Segregation of the F_2 generation derived from genotypes of extremes of response indicated multiple loci controlling tolerance to B, however, this could be partitioned into a series of major independent genes when the progeny from less extreme combinations were studied. The control was additive at both the intralocus level, in that the heterozygotes were intermediate to the two parents for response to B, and at the interlocus level with increasing levels of tolerance apparently resulting from an increase in gene dosage.

Tolerance to B was expressed as an incompletely dominant character and this resulted in the apparent tolerance of the heterozygote varying with parental combinations and the concentration of applied B. By the most definitive measure of response to B, namely the B concentration of shoots, response to B was clearly expressed as an incompletely dominant character, as was also the case for growth responses at the vegetative stage. The degree of sensitivity of the F_1 hybrid increased as successively more sensitive parents were combined with a tolerant genotype. The response at maturity was more equivocal and tolerance was expressed as a dominant character, for most genetic combinations, but as an incompletely dominant or recessive character for several combinations including the more sensitive genotypes. The discrepency between response at the vegetative stage and at maturity could be accounted for by compensatory growth as demonstrated in Chapter 4.

The altering response of heterozygotes could be explained theoretically by a series of responses representing different levels of gene dosage, or different levels of heterozygosity of the hybrids. As the threshold level of a particular genetic combination was exceeded, its response altered from being similar to the tolerant parent to intermediate to the two parents and tolerance varied from being expressed as a dominant character to an incompletely dominant character. The altering response of the F_1 hybrid, relative to the parents, is consistent with the theory of Knight (1973) on the behaviour of a hybrid of intermediate response to the parents, in varied environments. As a consequence of the

altering response of the heterozygotes, it was necessary to select specific levels of B stress for each parental combination when testing segregating generations.

The total range in response to B is under the control of a number of loci and the results obtained here indicate that these loci are independent. The evidence for independence of the loci arises from the series of single gene differences which could be recognized and from a low frequency of sensitive types in the progeny of crosses for genotypes two steps apart on the scale of tolerance. If the loci were linked, or there were other genetic interactions between loci, the frequency of sensitive types in the progeny of the more extreme crosses, such as (Warigal*Kenya Farmer) and (Halberd*(Wl*MMC)), would be greater than 1/16 as expected from digenic segregation. The presence of a number of loci is not unexpected for a hexaploid species, but any discussion regarding the probability of homoeologous relationships between the loci would be purely speculative at this stage as it is further explored in the following chapter.

It is probable that the degree of common ancestry among the chosen genotypes was a major factor contributing to the successful identification of major genes controlling response to B. As all genotypes, except G61450, were derived from an essentially similar gene pool and therefore had a degree of homogeneity of background genes, it is perhaps not surprising that segregation for response to B occurred at a limited number of loci. The choice of genotypes with some degree of homogeneity for background genes was more fortuitous than deliberate, with the exceptions of Warigal and (Wl*MMC) which were specifically chosen for this reason. Selection of contrasting phenotypes with common genetic background is an efficient method of simplifying genetic studies, particularly for quantitative studies.

G61450 was the genetically most distant genotype and transgressive segregation occurred when combined with Halberd. This indicates that G61450 and Halberd have different genetic sources of tolerance, although the physiological basis of tolerance appears to be the same and related to reduced accumulation of B in shoots. Transgressive segregation in response to salinity has been reported for rice (Moeljopawiro and Ikehashi, 1981) and vine (Sykes, 1985) and Yeo and Flowers (1986) proposed systematic pyramiding of genes for resistance to salinity (that is combining different mechanisms of

resistance) as it is unlikely that all possible mechanisms would be combined in the one genotype by chance. In the absence of evidence for different mechanisms of tolerance to B operating between genotypes, and given the high degree of tolerance to B already identified (Moody *et al.*, 1988), it is not likely that the need to utilize transgressive segregation to produce more tolerant varieties for the Australian cereal belt will arise. A knowledge of the genetic control among the most tolerant genotypes being used as sources of tolerance in the wheat breeding program at the Waite Agricultural Research Institute would be useful as it would indicate the genotypes most compatible with the current Australian varieties. The efficiency of incorporation of B tolerance would be greatest when the variation between the donor tolerant genotype and the recipient local variety results from allelic differences at the minimum number of loci.

The biochemical basis of tolerance is not known, however recent studies with root tip and callus culture have found that tolerance is expressed at the cellular level (Huang, Agronomy Dept. W.A.R.I., unpublished) and therefore probably arises from a property of cellular membranes. The means of uptake of B and transport across cell membranes is a contentious subject and opinions range from passive uptake and diffusion to predominantly active transport (see review Raven, 1980). Oliver and Barber (1966) determined that the majority, but not all, of the B uptake by soybeans could be accounted for by mass flow in the transpiratory water, while Bowen (1972) also reported that the uptake of B was not stoichiometrically related to water uptake. These results indicate that the uptake of B is regulated to some degree. The degree of variation for concentration of B in shoots between genotypes in the experiments of this thesis are greater than would be expected if genetic differences in uptake of B were simply related to transpiration. Different degrees of regulation due to additive gene action upon the membranes would account for differences in B accumulation, between genotypes. It is not known whether restricted uptake of B results from the active expression or non-expression of a gene(s) and whether all genes affecting B uptake have a similar gene product or act at the same site.

Despite the unknown mechanism of regulation of B uptake, a model of the genetic relationships between the five genotypes studied, based on the results of segregation of

the F_2 and F_3 generations, can be formulated (Fig 6.11). The model assumes one mechanism controlling B uptake but the effect may be either direct or indirect and for simplicity assumes alternative alleles at each locus. The gene symbol *Bor* will be assigned for response to B and as tolerance is of major agricultural significance in south eastern Australia, the upper case form will refer to tolerance. Alternative loci are referred to by Arabic numerals and the locus at which Halberd and Warigal segregate is assigned *Bor*1 due to the historical importance of this locus in Australia and the initial recognition of genetic variation in tolerance to B in wheat. Kenya Farmer could be considered as having the recessive allele at all loci common to the set of genotypes studied. (Wl*MMC) has the dominant allele at one locus more than Kenya Farmer, Warigal at two, Halberd at three and G61450 at three or more loci and at least two of these are common to Halberd and Warigal.

The identification of major genes controlling response to B is consistent with the similar response of wheat varieties within identifiable families, as found in Chapter 5 and the incorporation of tolerance to B into current Australian wheats should be readily achievable by backcrossing. Isogenic lines for response to B could be developed and these could be used to identify regions where B tolerance has a yield advantage, to develop diagnostic tissue tests for B toxicity and for physiological studies on the mechansim(s) of tolerance to B

Figure 6.11 Theoretical relationship between and possible genotypes for the five genotypes used in the study of the genetic control of tolerance to high concentrations of B for wheat. The gene symbol for response to B is *Bor* and the dominant form refers to tolerance. Alternative loci are denoted by Arabic numerals.

Line	Response to B	Genetic differences	Genotype
Kenya Farmer	Very sensitive		*bor1 bor2 bor3 bor4*
(Wl*MMC)	Sensitive	Single gene	*bor1 Bor2 bor3 bor4*
Warigal	Moderately sensitive	Single gene	*bor1 Bor2 Bor3 bor4*
Halberd	Moderately tolerant	Single gene	*Bor1 Bor2 Bor3 bor4*
G61450	Tolerant	Alternative gene(s)	*bor1 Bor2 Bor3 Bor4*

Two genes

One or two genes ?

Two genes

Chapter 7.

CHROMOSOMAL LOCATION OF GENES CONTROLLING TOLERANCE TO HIGH CONCENTRATIONS OF BORON

7.1 CHROMOSOMAL LOCATION OF GENES BY ANEUPLOID TECHNIQUES

7.1.1 Introduction

The location of genetic factors controlling the tolerance to high concentrations of B to chromosomes is required prior to determining whether the B tolerance genes are linked to other marker genes which may be more efficiently used for screening for tolerance. *Triticum aestivum* L. is a hexaploid species (2n=6x=42). The 21 chromosomes of wheat belong to one of three genomes (A, B and D) representing the ancestral diploid species, and within each genome chromosomes are assigned to one of seven homoeologous groups, numbered 1-7. Chromosomes within each homoeologous group are able to compensate to varying degrees for the loss of the other members of the group. Thus each homoeologous group has a high level of functional identity. The ability to tolerate the loss, or gain, of chromosomal material has enabled the development of a number of types of aneuploid lines, principally for the variety Chinese Spring (Sears, 1953, 1954). These aneuploid lines include :

Line	Characteristics	Karyotype
nullisomics	lacking one chromosome pair	20"
monosomics	lacking one chromosome	20"+1'
ditelosomics	lacking both copies of one arm for one chromosome pair	20"+t"
trisomics	addition of one chromosome	20"+1"'
tetrasomic	addition of one chromosome pair	$20"+1^{1V}$
nullisomic-tetrasomic	lacking one chromosome pair with the addition of one chromosome pair, usually of the same homoeologous group	$19"+1^{1V}$

At the Seventh International Wheat Genetics Symposium, Cambridge, 1988, it was decided that chromosomes originally designated 4A and 4B be redesignated 4B and 4A, respectively (Miller and Koebner, 1988). The new chromosome designations are used in this thesis.

7.1.2 Aneuploid techniques

The aneuploid lines may be used to locate genes to chromosomes. A number of methods are available and may be either direct, such as identifying chromosomes through absence of expression of a gene(s) in nullisomics, nulli-tetrasomics and ditelosomics, or indirect whereby the aneuploids are hybridized with other varieties, either for the development of intervarietal substitution lines, or for monosomic analysis.

Intervarietal substitution lines

In developing intervarietal substitution lines, each chromosome of a recipient variety, for which a monosomic series exists, is individually replaced by the homologous chromosome from a donor or test variety. The recipient variety is essentially reconstituted for the background chromosomes by a series of backcrosses to the monosomics of the recipient variety. Thus, each substitution line consists of one chromosome pair from the donor variety and 20 pairs from the recipient. The procedure for developing substitution lines is described in detail by Sears (1953) and Law and Worland (1973).

Substitution lines have the euploid chromosome constitution and are therefore genetically stable. They are homozygous for the substituted chromosome and close to homozygosity for the background chromosomes of the recipient variety. The background chromosomes will also be nearly identical to the recipient variety, but the actual degree of similarity will depend upon the number of backcrosses performed in developing the lines. As the background recipient chromosomes are not fully reconstituted there is the chance of incorrectly assigning genetic factors to a particular chromosome. The probability of misclassification will depend upon the complexity of the genetic control of the character

under study and the number of backcrosses performed in developing the intervarietal substitution lines (Law and Worland, 1973).

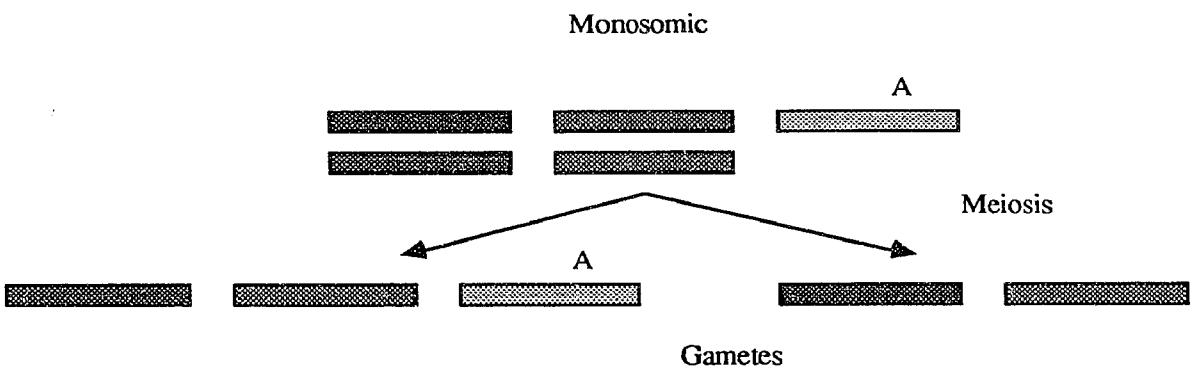
Substitution lines can be used for replicated experiments with a range of treatments. Comparisons between the individual substitution lines and the two parents may identify the particular chromosome(s) associated with a genetic trait, but the substitution lines do not give a direct measure of the number of genes for the trait located on the critical chromosome(s). Numerous examples of genes located by this method are listed in the "Catalogue of gene symbols for wheat" (McIntosh, 1988).

Monosomic analysis

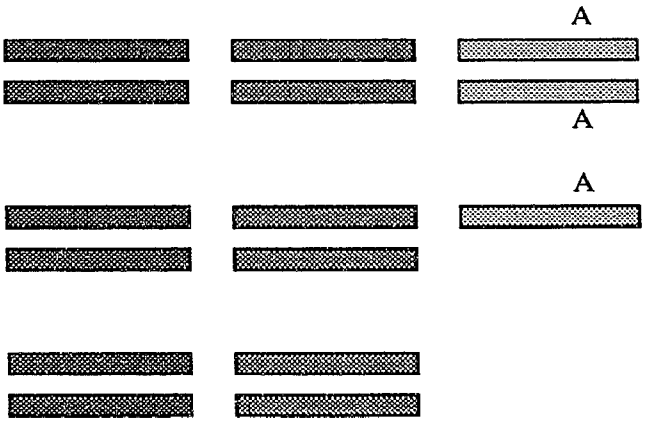
Monosomic analysis may be used for chromosomal location when a suitable set of substitution lines does not exist for the character under investigation. The utility of monosomics in genetic analysis is derived from the irregular behaviour and frequent loss of the unpaired monosome at reduction division of meiosis. Approximately 75% of female gametes have only 20 chromosomes while on the male side functioning gametes predominantly have 21 rather than 20 chromosomes due to the competitive advantage of 21 chromosome pollen. The progeny of a selfed monosome will consist of disomic, monosomic and nullisomic plants and typical frequencies are 24%, 73% and 3%, respectively (Sears, 1944, 1953). The behaviour of chromosomes of a monosomic line is depicted in Figure 7.1.

When a test variety is crossed to a monosomic, the chromosome constitution of the F_1 hybrid will be either 20"+1' or 21". The former situation will arise when a 20 chromosome female gamete is fertilized and occurs with an average frequency of 75%. The monosomic chromosome will be derived from the donor variety. The 21" F_1 hybrid occurs when a 21 chromosome female gamete is fertilized. The F_2 populations derived from the monosomic F_1 hybrids will be a mixture of disomics, monosomics and nullisomics. F_2 populations derived from monosomic F_1 hybrids for each of the 21 chromosomes are scored for segregation for the character in question. For a single dominant gene, where the gene is fully expressed at the hemizygous level, 20 of the 21

Figure 7.1 Behaviour of the chromosomes and possible progeny of a monosomic line. Three chromosomes are used for the example, one of which is present as a monosome.

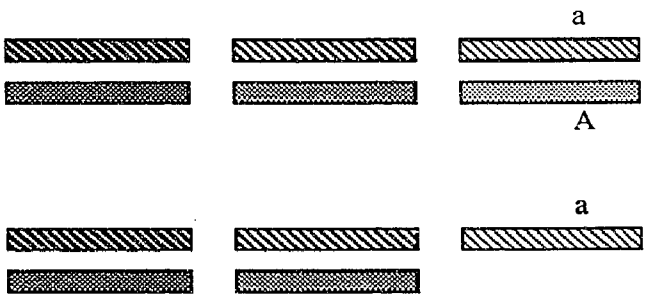


Progeny of a monosomic when selfed



Euploid	25%
Monosomic	72%
Nullisomic	3%

F1 progeny when monosomic crossed as female parent with alternative euploid variety with allele 'a'



Euploid	25%
Monosomic	75%

families will segregate 75% dominant to 25% recessive phenotypes. In the case of the critical chromosome, all disomic and monosomic plants will carry the dominant allele derived from the test variety and only nullisomic plants, or about 3% of the population, will respond as recessives. The critical chromosome can therefore be identified by deviation from the usual 3:1 segregation ratio (Sears, 1953). Monosomic analysis may also detect critical chromosomes for two or more genes and for different types of gene action and interactions (Sears, 1953; Kuspira and Unrau, 1959).

As an alternative to comparing the response of the F_2 populations derived from monosomic F_1 hybrid plants with theoretically derived expected segregation ratios, the F_2's of monosomic F_1 hybrids may be compared with the euploid F_2. The segregation pattern for the F_2 derived from the critical monosomic will be significantly different from the pattern of the euploid F_2. This method should identify critical chromosomes regardless of the number of gene pairs involved or gene action and interactions (Kuspira and Unrau, 1959).

If the F_2 population is not readily scored due either to non-discrete classes arising from partial dominance, or environmental influences upon the phenotype, it may be preferable to study F_3 progenies derived from disomic F_2 individuals. The chromosome originally monosomic in the recipient variety will now be replaced by the homologous chromosome of the test variety and, as this is disomic, characters under the control of the critical chromosome will not segregate. Location of the critical chromosome(s) can be achieved by observing segregation patterns of the F_3 progenies. Producing a number of F_3 lines for each chromosome will reduce the risk of misclassification due to background effects. The probability of obtaining n F_3 homozygous lines in the non-critical lines, by chance, is $(1/4)^n$ and so by testing four F_3 lines for each chromosome the probability of misclassification is reduced to 1/256 (Sears, 1953).

Two other methods of monosomic analysis have been described, namely reciprocal monosomic analysis (McKewan and Kaltsikes, 1970; Law *et al.*, 1981) and backcross reciprocal monosomic analysis (Snape and Law, 1980; Law *et al.*, 1981). Reciprocal monosomic analysis may be employed when two suitable monosomic series exist. The homologous monosomics are crossed and the resulting F_1 hybrid monosomics

will have different hemizygous chromosomes but identical backgrounds. Monosomic F_1 hybrids are identified cytologically and compared for the character under study. Differences among the hybrids may then be attributed to genetic effects of the hemizygous chromosomes. While this method was originally devised for comparisons at the F_1 generation (McKewan and Kaltsikes, 1970) it can be extended to later generations (Law *et al.*, 1981). The backcross reciprocal monosomic method may be used where there is only one monosomic series. The complete set of monosomics, as the female parent, are hybridized with a contrasting euploid genotype. Monosomic hybrids are selected from the progeny and used as both male and female parents in backcrosses to the original monosomics. This will produce reciprocal families with equivalent genetic background but different monosomic chromosomes. As this method requires a large amount of hybridization, it is probably best employed to compare a range of varieties for allelic variation in a single chromosome, as used by Snape and Law (1980). This implies that the critical chromosome is known.

Interspecific addition lines

An alternative means of identifying critical chromosomes is through the utilization of interspecific addition or substitution lines. *T. aestivum* is able to hybridize with other Triticeae species and this has allowed the production of amphiploids and chromosome addition and substitution lines between wheat and related species (see Shepherd and Islam (1988) for a compendium of wheat - alien chromosome lines). A high degree of homoeology between *T. aestivum* and related species has been revealed through the study of isozyme (Hart, 1987) and DNA (Sharp *et al.*, 1989) markers for wheat - alien addition and substitution lines. Alien addition and substitution lines may therefore be used to identify chromosomes controlling response to a particular character for the related species and so allow inferences to be made as to the probable chromosomal location of genes controlling characters within *T. aestivum*. Where sufficient genetic variation for a character is not present within *T. aestivum*, knowledge of the chromosomal location in a related species would facilitate introgression of the desired gene(s) into wheat.

Summary

The methods adopted for studying the chromosomal location of genes controlling tolerance to high concentrations of B were dependent upon the tolerance of the parent varieties used to produce the available aneuploid lines. Aneuploid lines of wheat have predominantly been developed for Chinese Spring which is intermediate in tolerance to B (Paull, 1985; Paull *et al.*, 1988). Intervarietal substitution lines and interspecific addition lines could be used where the donor variety or donor species is either more tolerant or more sensitive than Chinese Spring, the reciprocal monosomic method could be employed for two varieties of contrasting tolerance which have monosomic series, while for varieties without either a substitution or monosomic series, genes could be located by either testing the F_2 or F_3 from an F_1 hybrid monosomic or by the backcross reciprocal monosomic method.

7.2 RESPONSE OF INTERVARIETAL SUBSTITUTION LINE DONORS, MONOSOMIC VARIETIES AND INTERSPECIFIC AMPHIPLOIDS TO BORON

7.2.1 Introduction

Prior to commencing studies on the location of genetic factors controlling tolerance to high concentrations of B, it is necessary to evaluate the varieties which have been used in the development of intervarietal substitution lines and monosomic series. There is no formal publication listing all donor varieties for intervarietal substitution lines, and prior to 1988 (Worland, 1988) there was not such a list for the monosomic series, although a number are mentioned by Law *et al.* (1981). The varieties screened for response to B to enable choice of varieties for aneuploid studies were those suggested by Dr. C.N. Law, Plant Breeding Institute, Cambridge, U.K.

Several amphiploids between Chinese Spring and related species for which the interspecific addition lines exist were also screened for response to B.

7.2.2 Materials and methods

Varieties

Thirty varieties representing eight substitution donors into Chinese Spring and 22 monosomic series were compared for response to high concentrations of B. The B tolerant variety, Halberd, was included as a control grid. The seed of all varieties was obtained from the Australian Winter Cereals Collection. Four amphiploids between Chinese Spring and related species were compared separately with Chinese Spring and Halberd. Seed of the amphiploids was obtained from stocks maintained at the Waite Agricultural Research Institute by Dr. K.W. Shepherd.

Experimental design

All varieties were initially grown in large boxes containing B100 soil. The more sensitive of the monosomic varieties were omitted from a second evaluation at B150. The amphiploids were grown only at B150.

Five seeds of each variety or amphiploid were germinated in petri dishes and then sown in rows at a spacing of 3.5 cm within rows and 5cm between rows. Halberd was included as a control every fifth plot. All experiments consisted of two replicates.

Plants of the substitution and monosomic varieties were visually rated for response on a scale of one (very sensitive) to five (very tolerant). For the first experiment at B100, Halberd was rated as four, however as no varieties were more tolerant than Halberd, it was rated as five for the experiment at B150 to increase the number of categories to assign varieties to. Plants were harvested after seven weeks for the B100 experiment and nine weeks for the B150 experiment, oven dried and weighed. The height of the amphiploids, Chinese Spring and Halberd were measured seven weeks after sowing.

7.2.3 Results and discussion

At the B100 treatment several varietes were rated more tolerant than Chinese Spring and as tolerant as Halberd, but none were more tolerant than Halberd (Table 7.1). For the second screening at B150, only Federation and Saratovskaya 29 were more tolerant than Chinese Spring, but they were not as tolerant as Halberd. The intervarietal substitution line donors were generally similar to Chinese Spring in tolerance to B, although Kenya Farmer was considerably more sensitive than Chinese Spring at both treatments.

The Chinese Spring x *Agropyron elongatum* amphiploid was the most tolerant of the amphiploids and had similar vigour to Halberd (Table 7.1, Plate 11). *Ag. elongatum* has previously been described as tolerant to B (Schuman, 1969) and this tolerance appears to be expressed in a wheat background. All amphiploids, including Chinese Spring x *Ag elongatum* were observed to be less vigorous than Chinese Spring when grown for seed multiplication in nil applied B potting mix. The poor vigour of the amphiploids at the B150 treatment therefore might not have been entirely due to sensitivity to B.

The Chinese Spring/Kenya Farmer substitution lines, the Federation and Chinese Spring monosomic series and the Chinese Spring - *Ag elongatum* addition lines were

Plate 11. Response of amphiploids between Chinese Spring and related species to the B150 treatment and comparison with Chinese Spring and Halberd.
Left to right : Halberd, Chinese Spring, amphiploids between Chinese Spring and *Ag. elongatum*, *Ae. umbellulata*, Imperial rye and South Australian rye.

Table 7.1 Response to B for cultivars and amphiploids for which either intervarietal substitution lines, monosomic series or interspecific addition lines are available. Cultivars were visually compared with Halberd for vigour and symptom expression and rated on a scale of 1 - 5. Halberd rated 4 for B100 and 5 for B150. Values are the means of two replicates and five plants per replicate.

Cultivar	B100		B150	
	Dry wt ($g\ row^{-1}$)	Visual Rating	Dry wt ($g\ row^{-1}$)	Visual Rating
Substitution line donors				
Hope	0.54	2	0.12	2
Cappelle-Desprez	0.69	3	0.24	3
Timstein	0.53	2	0.12	2
Cheyenne	0.40	2	0.11	2
Thatcher	0.56	3	0.15	2
Lutescens 62	0.70	4	0.23	3
Ciano 67	1.13	4	0.24	3
Kenya Farmer	0.12	1	dead	1
Monosomic series				
Chinese Spring	0.66	3	0.25	3
Koga II	0.85	4	0.25	3
Bersee	0.47	3	0.27	3
Sava	0.75	3	0.35	3
Poros	0.37	2		
Chris	0.43	2		
Spica	0.79	3	0.39	3
Cheyenne	0.40	2		
Saratovskaja 29	0.65	3	0.56	4
Rannyaya	0.65	4	0.30	3
Hobbit	0.61	3	0.19	2
Bezostaya 1	1.15	4	0.16	2
Bezostaya 2	0.55	3	0.14	2
Aurora	0.96	4	0.20	2
Kavkaz	1.01	4	0.21	2
Mara	0.58	3	0.45	3
Kalyansona	0.57	3	0.48	3
Rescue	0.53	3	0.16	2
Redman	0.71	3	0.33	3
Wichita	0.55	2		
Federation	0.85	4	0.63	4
Gabo	0.51	2		
Halberd	1.15	4	1.35	5
Chinese Spring - alien amphiploids			Height (cm)	
Chinese Spring			25.2	
Halberd			35.1	
CS - *Ag. elongatum*			33.9	
CS - *Ae umbellulata*			15.8	
CS - Imperial Rye			16.6	
CS - S.A. Rye			14.8	


Hordeum
Cenosp. Spring
C. BrAg. elongatum
C. BrAe. umbellulata
AMPHIPLOIDS
BORON TOXICITY

selected to investigate the chromosomal location of genes controlling tolerance to B. In addition, as there were no very tolerant wheat varieties identified, it was decided to attempt to locate genes for the very tolerant genotype, G61450, by the monosomic method using Chinese Spring as the tester variety.

7.3 RESPONSE OF THE CHINESE SPRING / KENYA FARMER SUBSTITUTION LINES TO BORON

7.3.1 Introduction

The intervarietal substitution lines of Kenya Farmer into Chinese Spring were compared at a control and a high B treatment to identify chromosomes which have a significant effect upon response to B.

7.3.2 Materials and methods

The Chinese Spring/Kenya Farmer (syn. Chinese Spring/Kenya 833) substitution lines were developed by Snyder *et al.* (1963) and seed was kindly provided by Dr. R.A. McIntosh, Plant Breeding Institute, University of Sydney. The 21 substitution lines, Kenya Farmer and Chinese Spring were multiplied in pots in a glasshouse to produce sufficient seed of uniform size and age for conducting experiments.

Experimental design

B0 and B75 soil was weighed into 150 mm diameter pots (1.8 kg pot $^{-1}$) and B25 soil was weighed into 125 mm diameter pots (0.95 kg pot $^{-1}$). The B0 and B75 pots were arranged as a randomized complete block design of five replicates. The B25 pots were separated from the other treatments but were also arranged as a randomized complete block of five replicates. The experiment was conducted during the period 18th March - 14th May, 1987.

Three pre-germinated seeds were sown per pot and thinned to two plants after two weeks. The B25 plants were harvested at ground level four weeks after sowing. Shoots were rinsed in de-ionized water, oven dried and then analysed by ICP spectrometry. The plants of the B0 and B75 treatments were harvested eight weeks after sowing at which stage the B0 plants were at or near the boot stage. Shoots were oven dried and dry matter yield was determined.

7.3.3 Results

The data for dry matter production were subjected to a square root transformation before analysis of variance. The raw data, together with the transformed results are presented in Table 7.2. The yields of all lines were significantly reduced at the B75 treatment compared with the B0 control and the genotype x treatment interaction was significant indicating that there was variation between lines in their response to high concentrations of B. At the B0 treatment there were significant differences in yield between lines, therefore a comparison between the lines has also been presented as the yield at B75 relative to the B0 control (Figure 7.2).

At the B75 treatment Kenya Farmer yielded significantly less than Chinese Spring and all of the substitution lines. Several of the substitution lines also yielded significantly less than Chinese Spring and of these the 4A substitution was the most senstitive but it was not as sensitive as Kenya Farmer. The 4A substitution line was the only line, apart from Kenya Farmer to develop the "mid leaf necrosis" symptom characteristic of very sensitive genotypes.

The concentration of B in the shoots of Kenya Farmer, at the B25 treatment, was significantly greater than all other lines (Table 7.2). Although the B concentrations for several of the substitution lines were significantly higher than for Chinese Spring, none were significantly higher than all other lines. The B concentration in shoots was highest for the 4A substitution line and other substitution lines with a higher B concentration than Chinese Spring included, in descending order, 7B, 3B, 5D, 1D and 6B. Chromosome 4A of Kenya Farmer did not have any effect on the concentration of any other elements in the shoots, when compared with Chinese Spring (Table 7.3).

This experiment demonstrates an effect of chromosome 4A of Kenya Farmer upon the response to high concentrations of B, however this chromosome does not account for the total difference in response between Chinese Spring and Kenya Farmer. The results do not reveal whether the sensitivity of the 4A substitution line is under the control of one, or a number of genes, or whether the response is due to the presence of the Kenya Farmer chromosome or the absence of the Chinese Spring chromosome. The

Table 7.2 Dry matter yield and concentration of B in shoots for Chinese Spring, Kenya Farmer and the Chinese Spring - Kenya Farmer substitution lines grown at B0, B25 and B75 treatments. Square root transformed data are in brackets and significance levels refer to the transformed data.

Genotype	B0 Dry wt (g pot $^{-1}$)		B75 Dry wt (g pot $^{-1}$)		B25 B conc (mg kg^{-1})
Chinese Spring	6.04	(2.45)	2.13	(1.45)	311.9
Kenya Farmer	5.39	(2.31)	0.10	(0.33)	656.3
Substitution lines					
1A	5.71	(2.38)	1.47	(1.21)	320.7
1B	6.94	(2.63)	1.69	(1.29)	368.9
1D	6.49	(2.54)	1.91	(1.38)	388.4
2A	5.47	(2.33)	1.78	(1.33)	343.0
2B	5.93	(2.43)	1.75	(1.32)	321.4
2D	5.97	(2.43)	1.62	(1.28)	364.9
3A	5.59	(2.36)	1.72	(1.31)	344.8
3B	5.60	(2.36)	1.99	(1.41)	395.2
3D	5.11	(2.26)	1.88	(1.36)	362.2
4A	5.84	(2.42)	0.59	(0.76)	414.0
4B	5.58	(2.38)	1.54	(1.24)	339.3
4D	5.66	(2.38)	1.80	(1.34)	318.3
5A	5.62	(2.37)	2.31	(1.51)	328.8
5B	6.29	(2.51)	2.12	(1.46)	332.4
5D	5.92	(2.43)	1.54	(1.23)	390.4
6A	5.81	(2.41)	1.87	(1.35)	357.3
6B	5.95	(2.44)	1.75	(1.31)	384.2
6D	6.18	(2.47)	1.62	(1.27)	311.1
7A	5.67	(2.38)	1.44	(1.20)	352.3
7B	6.68	(2.58)	1.70	(1.30)	405.4
7D	5.81	(2.40)	1.96	(1.39)	343.0
LSD (0.05)	Genotypes (0.15),		Treatments (0.04),		Interaction (0.21) 59.2
(0.01)	(0.20)		(0.06)		(0.28) 70.7

Figure 7.2 Relative yield (yield at B75 / yield at B0) for the Chinese Spring / Kenya Farmer substitution lines.

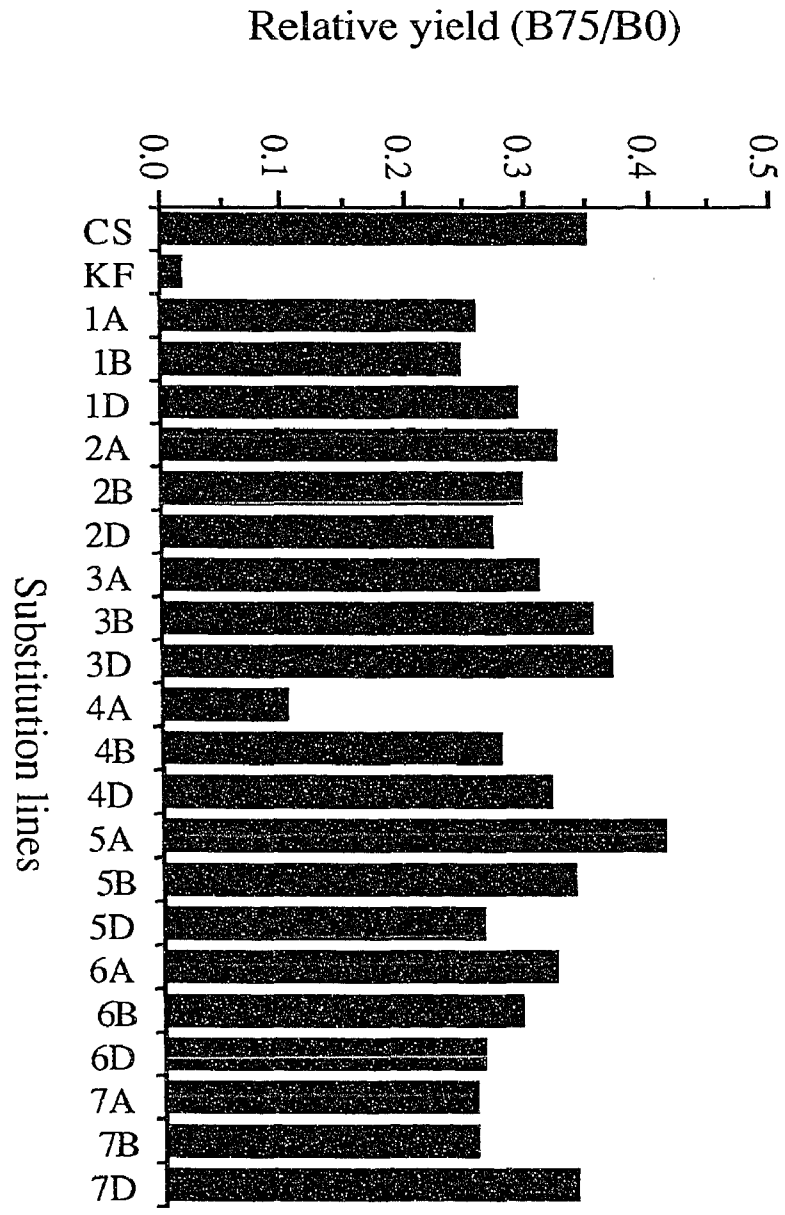

Relative yield (B75/B0)
0.0
0.1
0.2
0.3
0.4
0.5
CS
KF
1A
1B
1D
2A
2B
2D
3A
3B
3D
4A
4B
4D
5A
5B
5D
6A
6B
6D
7A
7B
7D
Substitution lines

Table 7.3 Concentration of 12 elements in the shoots of Chinese Spring, Kenya Farmer and the Chinese Spring - Kenya Farmer chromosome 4A substitution line when grown at the B25 treatment.

Element	Chinese Spring	Kenya Farmer	CS(KF4A)	LSD (0.05)[a]
P (%)	0.44	0.44	0.46	n.s.
K "	6.45	5.62	6.60	0.72
S "	0.34	0.27	0.36	0.04
Mg "	0.15	0.15	0.15	n.s.
Ca "	0.47	0.52	0.47	0.05
Cu (mg kg^{-1})	8.28	7.89	7.91	n.s.
Zn "	25.58	24.88	26.72	n.s.
Mn "	47.82	50.29	51.48	5.72
Al "	52.71	68.09	43.87	n.s.
B "	311.90	656.30	414.00	59.22
Fe "	78.42	85.21	75.61	n.s.
Na "	181.20	169.60	170.70	n.s.

[a] LSD calculated over all substitution lines and CS and KF

control of response to high concentrations of B by chromosome 4A is investigated in the following experiment.

7.4 EFFECT OF CHROMOSOME 4A UPON RESPONSE TO BORON

7.4.1 Introduction

The previous experiment demonstrated that the CS(KF4A) substitution line is significantly more sensitive than Chinese Spring to high concentrations of B. This may result from either the presence of the 4A chromosome of Kenya Farmer, or the absence of the 4A chromosome of Chinese Spring. The alternative hypotheses were tested in an experiment including the individual arms of chromosome 4A of Chinese Spring. If the response was due to the absence of the Chinese Spring chromosome, and if the gene(s) controlling tolerance is located on a single arm of chromosome 4A, one of the lines should be as sensitive as CS(KF4A). Alternatively, if the sensitivity of the substitution line results from the presence of the Kenya Farmer chromosome, the two lines of the individual chromosome arm should respond similarly to Chinese Spring, while if genes are located on both arms of Chinese Spring chromosome 4A both lines of individual arms of chromosome 4A would be intermediate in response to B.

The difference in response between Chinese Spring and Kenya Farmer was not fully accounted for by the chromosome 4A substitution line. To obtain an estimate of the number of loci at which Chinese Spring and Kenya Farmer differ for response to B, and the number of loci on chromosome 4A, the F_2 and F_3 generations for (Chinese Spring* Kenya Farmer) and (Chinese Spring*CS(KF4A)) were tested at high B treatments.

7.4.2 Materials and methods

Response of individual arms of chromosome 4A

Genotypes

Chinese Spring : CS

Chinese Spring / Kenya Farmer 4A substitution : CS(KF4A)

Kenya Farmer : KF

Chinese Spring ditelocentric 4AL : CSDT4AL

The ditelocentric for the short of chromosome 4A was not available, however a Chinese Spring translocation substitution line with ditelosomic 4AS translocated to the $1S^1$

chromosome of *Aegilops longissima* (Zeller, unpublished; described in Shepherd and Islam, 1988)) was available. This line lacks the long arm of chromosome 4A : Tr.4AS.1S^{l}.

Experimental design

The B treatments were B0, B25, B50, B75 and B100. Soil (1.70 kg pot^{-1}) was weighed into 150 mm diameter pots. The pots were arranged as a randomized complete block design with five replicates. The experiment was conducted during the period 3rd May - 20th June, 1988.

Three pre-germinated seeds were sown per pot and thinned to two plants after two weeks. Plants were regularly scored for development of tillers. The plants were harvested at ground level six weeks after sowing, rinsed with de-ionized water, oven dried and weighed. The B concentration in shoots was determined by ICP spectrometry for the B0, B25, B50 and B75 treatments.

Segregation in response to B

F_2 and F_3 seeds of (Chinese Spring*Kenya Farmer) and (Chinese Spring* CS(KF4A)) were grown at the B50 and B75 treatments, respectively, in plastic trays as described in Chapter 6. 125 F_2 seeds and 25 seeds of each parent were sown per cross.

As the most sensitive F_2 plants were considered unlikely to recover sufficiently, after transplanting to potting mix, to produce enough seed for progeny testing, unselected F_2 plants were grown in potting mix and F_2 derived F_3 families were screened for response to B. Between 10 and 12 plants were sown for each F_3 family and a total of 108 families for (Chinese Spring * Kenya Farmer) and 116 families for (Chinese Spring * CS(KF4A)) were tested. The F_2 plants from which the F_3 families for (Chinese Spring * CS(KF4A)) were derived were scored for the awn inhibiting hooded gene (*Hd*) which is located on the short arm of chromosome 4A and linked to the centromere with a recombination frequency of 7.7±3.7% (Rao, 1981). Chinese Spring has the genotype *HdHd* (Sears, 1954) and as CS(KF4A) is awned it is presumably *hdhd* .(Plate 12).

Plate 12. Spikes of Chinese Spring, the Chinese Spring (Kenya Farmer 4A) substitution line and Kenya Farmer. Chinese Spring has the awn inhibiting hooded gene (*HdHd*) located on chromosome 4A. As the substitution line develops awns it is presumably of the genotype *hdhd*.


Chinese Spring
CS(KF4A)
Kenya Farmer

7.4.3 Results

Response of the individual arms of chromosome 4A

Kenya Farmer was considerably more sensitive to B than all other lines. As this experiment was conducted primarily to compare the effect of the presence and absence of the two arms of chromosome 4A of Chinese Spring with the response of CS(KF4A), the results for Kenya Farmer have been omitted from statistical analyses as they would have influenced the statistical significance of genotype x treatment interactions.

The characteristic mid-leaf necrosis symptom developed for Kenya Farmer at all plus B treatments and for CS(KF4A) at B50, B75 and B100 treatments. Neither Chinese Spring nor the lines with the individual arms of chromosome 4A developed this symptom.

The development of tillers was recorded regularly and the time from sowing to commencement of tillering was determined (Table 7.4). At B0, CS(KF4A) tillered four days earlier than Chinese Spring while the ditelocentrics were intermediate in time to commencement of tillering. Increasing B treatments delayed tillering of all genotypes but to differing degrees. The early tillering of CS(KF4A) was not apparent at B50 and B75 and at the conclusion of the experiment only Chinese Spring and the Tr.4AS.1S^{l} translocation line had tillered at B100.

The yield data (Table 7.5) were subjected to a square root transformation and tested for analysis of variance. The effects for both B and genotypes were statistically significant ($F_{4,80} = 453.7$, $P<0.0001$ and $F_{3,80} = 2.95$, $P<0.01$, respectively), while the genotype x treatment interaction was also statistically significant ($F_{12,80} = 2.05$, $P<0.05$). The significant interaction appears to have arisen as much from the varied response between the B0, B25 and B50 treatments as at the higher treatments. Nevertheless, CS(KF4A) was the only line to yield significantly less than Chinese Spring at B100 and on this basis could be considered the most sensitive. The yields of CSDT4AL and Tr.4AS.1S^{l}, at B100, were not significantly different from either Chinese Spring or CS(KF4A), however Tr.4AS.1S^{l} was of lower vigour at the control.

Table 7.4 Days from sowing to commencement of tillering for Chinese Spring and aneuploids of chromosome 4A when grown at five B treatments. Values are the means of five replicates.

Genotype	Days to commencement of tillering				
	B0	B25	B50	B75	B100
Chinese Spring	19	21	24	31	42
CS(KF4A)	15	19	24	31	-
CSDT4AL	17	19	22	30	-
Tr.4AS.1S^{l}	17	18	23	30	42
Kenya Farmer	27	32	-	-	-

Table 7.5 Dry matter yield for Chinese Spring and aneuploids of chromosome 4A when grown at five B treatments. Significance values refer to the square root transformed data in brackets.

Genotype	Dry matter yield (g pot $^{-1}$)										
	B0		B25		B50		B75		B100		Mean
C S	1.76	(1.33)	1.56	(1.25)	1.51	(1.23)	0.66	(0.82)	0.39	(0.63)	(1.05)
CS(KF4A)	1.74	(1.32)	1.81	(1.35)	1.45	(1.20)	0.66	(0.82)	0.25	(0.50)	(1.04)
CSDT4AL	1.80	(1.34)	1.54	(1.24)	1.63	(1.28)	0.76	(0.87)	0.31	(0.56)	(1.06)
Tr.4AS.1S^{l}	1.57	(1.25)	1.58	(1.26)	1.34	(1.16)	0.62	(0.79)	0.32	(0.56)	(1.00)
K F	1.45		1.02		0.39		0.10		0.01		
Mean		(1.31)		(1.28)		(1.22)		(0.83)		(0.56)	

LSD (0.05) Genotypes (0.04), Treaments (0.04), Interaction (0.09)
(0.01) (0.05) (0.06) (0.12)

The concentration of B in shoots (Table 7.6) differed markedly between treatments and the data were subjected to a logarithmic transformation prior to statistical analysis. The genotype and B treatment effects were statistically significant ($F_{3,64}$ = 9.24, P<0.0001 and $F_{3,64}$ = 2068, P<0.0001, respectively) as was the genotype x treatment interaction ($F_{9,64}$ = 2.52, P<0.025).

The concentration of B in shoots at the B0 control was similar for all genotypes except CS for which the plants of two replicates had high concentrations of B and this was believed to be the result of contamination either of the soil or of the samples after harvest. At the B25 treatment there was no significant difference between CS, CS(KF4A) and CSDT4AL for concentration of B in shoots but the concentration for Tr.4AS.1S^{l} was significantly greater than for CS(KF4A). The results of the B50 and B75 treatments were more consistent with the yield results than for the lower B treatments and the concentration of B in shoots of CS(KF4A) was significantly greater than for CS at both treatments. The uptake of B was significantly affected by the short arm of chromosome 4A of CS and when absent, as for CSDT4AL, uptake was reduced.

Segregation in response to B

The segregating generations were rated in the same manner as for the experiments of Chapter 6. Plants were rated as either as sensitive as the sensitive parent, or more tolerant than the sensitive parent. Plants of intermediate response were not divided into categories as these would have been quite arbitrary and not necessarily related to distinct genotypes. For the progeny of (Chinese Spring*Kenya Farmer), in particular, several types could be recognized in the intermediate group. The progeny of (Chinese Spring* CS(KF4A)) were rated as sensitive if they developed the "mid leaf necrosis" symptom which all of the CS(KF4A) standards developed. The Chinese Spring standards did not develop this symptom. F_3 families were rated as sensitive when all plants were sensitive.

The results for the F_2 and F_3 generations and comparisons with expected ratios for one and two genes segregating are presented in Table 7.7. Ratios for three genes segregating were not tested as the expected frequency of homozygous sensitive types (n = 1/64), was too small for testing by chi squared analysis.

Table 7.6 Concentration of B in shoots for Chinese Spring and aneuploids of chromosome 4A when grown at four B treatments.Significance values refer to the logarithmically transformed data in brackets.

Genotype	B conc in shoots (mg kg^{-1})				
	B0	B25	B50	B75	Mean
C S	14.5 (1.11)	132.1 (2.12)	378.9 (2.56)	866.3 (2.94)	(2.18)
CS(KF4A)	11.2 (1.04)	121.8 (2.08)	454.3 (2.66)	1087.4 (3.04)	(2.21)
CSDT4AL	10.0 (1.00)	125.7 (2.10)	338.1 (2.53)	582.3 (2.76)	(2.10)
Tr.4AS.1S^{l}	12.6 (1.01)	156.4 (2.19)	405.7 (2.60)	951.4 (2.98)	(2.20)
KF	10.3	238.0	805.0		
Mean	(1.04)	(2.12)	(2.59)	(2.93)	
LSD (0.05)	Genotypes (0.05),	Treatments (0.05),	Interaction (0.10)		
(0.01)	(0.07)	(0.07)	(0.13)		

Table 7.7 Segregation of F_2 and F_3 generations of (Chinese Spring * Kenya Farmer) and (Chinese Spring * Chinese Spring (Kenya Farmer 4A)) in response to B. The tolerant and intermediate plants (F_2) or tolerant and segregating families (F_3) are pooled and their frequencies compared with the frequency of the sensitive category.

	Model	Tol/Inter	Sens	$\chi 2_1$	Probability
(CS*KF) F_2					
Observe		117	7		
Expect	3:1	93	31	23.75	P<0.001
Expect	15:1	116.25	7.75	0.01	P>0.90
(CS*KF) F_3 (F_2 families)					
Observe		103	5		
Expect	3:1	81	27	22.83	P<0.001
Expect	15:1	101.25	6.75	0.25	0.50<P<0.70
(CS*CS(KF4A) F_2					
Observe		96	28		
Expect	3:1	93	31	0.27	0.50<P<0.70
Expect	15:1	116.25	7.75	53.69	P<0.001
(CS*CS(KF4A) F_3 (F_2 families)					
Observe		91	25		
Expect	3:1	87	29	0.56	0.70<P<0.90
Expect	15:1	108.75	7.25	41.13	P<0.001

The results for (Chinese Spring*CS(KF4A)) are consistent with the ratios expected for segregation at a single locus and (Chinese Spring*Kenya Farmer) was consistent with segregation at two loci. It is not possible, however, to reject the hypothesis of segregation at three loci for (Chinese Spring*Kenya Farmer) as the population was too small to allow testing.

The response to B for F_3 families of (Chinese Spring*CS(KF4A)) and the awn characteristics of their F_2 parents were independent (Table 7.8).

7.4.4 Discussion

The simplest hypothesis to explain the difference in response to B between Chinese Spring and CS(KF4A)) would be that there is a single locus located on the short arm of chromosome 4A and allelic variation at this locus either directly or indirectly regulates B uptake. When absent, as with CSDT4AL, and possibly for a null allele as for a tolerant variety, B uptake is at a minimum. Chinese Spring and Kenya Farmer have alternative alleles and the Kenya Farmer type results in greater uptake of B.

The comparison of the response to high concentration of B for Chinese Spring, the CS(KF4A) substitution line and lines for the individual arms of chromosome 4A of Chinese Spring were inconclusive with respect to yield. The previously observed sensitivity of CS(KF4A) was not repeated. This was probably due to seasonal influences. The first experiment comparing the set of Chinese Spring / Kenya Farmer substitution lines with Chinese Spring was conducted during the period 18th March - 14th May, a period of quite high levels of evaporative stress in the glasshouse while the second experiment was conducted between 3rd May and 20th June, a time of lower growth rates and lower evapotranspiration. This may have minimized growth differences in response to B. The B concentrations in the shoots at the B25 treatment were considerably lower for the second than for the first experiment and this is also consistent with differences in transpiration demands under which the two experiments were conducted.

Chromosome 4A did not account for the total difference in growth response, and B accumulation, between Chinese Spring and Kenya Farmer, as determined by evaluation of the intervarietal substitution lines of Kenya Farmer into Chinese Spring. The

Table 7.8 Test for independence of response to B and the gene for hooded awns (*Hd*) located on the short arm of chromosome 4A. Awn characteristics were determined for F_2 plants of (Chinese Spring * Chinese Spring (Kenya Farmer 4A)) and the F_3 progeny were tested for response to B.

F_2 plants	Response of F_3 families to B		
	Tol / Seg	Sens	Total
Hooded	69	21	90
Non-hooded	22	4	26
Total	91	25	116

Test for independence of Hooded gene and response to B : $\chi^2 = 0.36$, $0.50<P<0.70$

segregation ratios for the F_2 and F_3 generations of (Chinese Spring*Kenya Farmer) demonstrate the parents differ for at least two independent loci, while the segregation ratios for (Chinese Spring*CS(KF4A)) were consistent with a single locus on chromosome 4A and this locus controls the expression of the "mid-leaf necrosis" symptom. Neither of the chromosome 4A ditelocentrics developed the "mid-leaf necrosis" symptom in response to high concentrations of B and this indicates that expression of the symptom requires the presence of the allele on chromosome 4A of Kenya Farmer.

The gene on chromosome 4A is independent of the hooded gene which is located on the short arm with a recombination frequency of 7.7± 3.7% with the centromere (Rao, 1981). If the gene controlling response to B is in fact located of the short arm, and this could be confirmed by telocentric mapping, it would also be independent of the centromere.

Reasons for not identifying other chromosomes carrying genes controlling response to B for the Chinese Spring / Kenya Farmer substitution lines might include :

(1) either insufficient B treatments or replicates to differentiate between substitution lines more tolerant than CS(KF4A) but more sensitive than the other non-critical substitution lines;

(2) one major gene on chromosome 4A and several or more modifying genes, distributed among other chromosomes of Kenya Farmer, which act in an additive manner but when present alone, as for substitution lines, have small effect;

(3) misclassification either during or subsequent to production of the substitution lines. When developing intervarietal substitution lines it is possible that the univalent chromosome may either "shift" or "switch" during backcrossing and result in all or part of the supposed substituted chromosome actually being the recipient type. The probability of such occurrences increases if the substitution lines are developed without genetical or cytological markers for each chromosome (Law and Worland, 1973). The awnedness of CS(KF4A) is evidence that this line is correct.

7.5 RESPONSE OF THE MONOSOMICS OF CHINESE SPRING TO BORON

7.5.1 Introduction

The expected segregation ratios for a critical chromosome, in monosomic analysis, depend upon the number and expression of genes controlling the trait. For the simplest situation of a single dominant gene which is fully expressed in the hemizygous condition, segregation in the F_2 generation for a non-critical chromosome will result in a ratio of 3 dominant : 1 recessive, while the critical chromosome will segregate approximately 97 : 3, with the 3% recessives being nullisomes (Sears, 1953).

Previous experiments have demonstrated that tolerance to B is inherited as a partially dominant character and therefore tolerance is unlikely to be fully expressed when in the hemizygous condition. If this were the situation it would have a significant effect upon expected ratios for monosomic analysis. For a character under the control of a single gene and complete dominance of expression, the segregation ratio for a non-critical chromosome would be 75 tolerant : 25 sensitive and for a critical chromosome would be 97 : 3. On the other hand, with incomplete dominance of expression, expected segregation ratios for a non-critical chromosome would be 25 tolerant : 50 intermediate : 25 sensitive and for the critical chromosome would be 24 tolerant : 73 intermediate : 3 sensitive. The 73% of intermediate response would be monosomes while nullisomes would again comprise the sensitive 3%. Although the frequency of sensitive types is the same for both completely and incompletely dominant gene expression, the presence of an intermediate class for a hemizygous ineffective gene would exacerbate any environmental influences upon expression of response and reduce the probability of identifying a critical chromosome.

An experiment was undertaken to compare the response of the progeny of the 21 individual monosomics of Chinese Spring to high concentrations of B to determine whether response is modified when genes are present in the hemizygous condition and, if so, to possibly identify chromosomes involved in the control of response to B. This was

initially conducted at only a high B treatment but was then repeated with a control and a high B treatment.

7.5.2 Materials and methods

Experiment 1

Seed derived from each of the 21 monosomic lines of Chinese Spring were grown in plastic trays containing B75 soil. One line was sown per tray with 25 seeds of the monosomic line and 10 seeds of Chinese Spring included as a standard. Seeds were arranged at a spacing of 5cm x 5cm. Observations on plant growth were made and four weeks after sowing plants were scored for the presence or absence of tillers. Plants of selected lines were transplanted to low B potting mix and pollen mother cells were examined at meiosis for chromosome number to determine whether there was an association between the development of tillers under high B conditions and chromosome complement.

Experiment 2

The experimental design was as for Experiment 1 but also included a B0 control treatment. Plants were scored regularly for presence of tillers. The experiment was conducted for four weeks, at which time plants were individually harvested, oven dried and weighed. The dry weights of plants derived from the monosomic lines were compared with the Chinese Spring standards within each tray

7.5.3 Results and discussion

At the conclusion of the first experiment a total of two out of the 210 Chinese Spring standards had developed tillers at the B75 treatment. For the monosomic families, however, chromosomes 4A, 4B and 4D had 24, 16 and 18 plants, respectively, which had tillered. No other lines had more than three plants which tillered and a total of only 16 plants for all the other18 lines developed tillers.

All plants of the 4A, 4B and 4D monosomic progeny were transplanted to low B potting mix and a random selection of each were examined at meiosis to determine

whether the presence of tillers was associated with the monosomic condition (Table 7.9a). As too few observations were made to allow statistical analysis of individual lines, the results for the group four chromosomes were pooled and tested for independence of tillering at a high B treatment and the monosomic condition. The contingency test (Table 7.9b) was highly significant ($\chi^2 = 12.96$, $P<0.001$). Plants monosomic for one of the chromosomes of group four therefore have an enhanced ability to tiller, compared with euploid plants, under high B conditions.

This result was further tested by comparing tillering at low and high B treatments to determine whether enhanced tillering of the monosomics of homoeologous group four also occurred at a low B treatment, or if it was a consequence of the monosomes being more tolerant to high concentrations of B, than Chinese Spring, and tolerance was expressed by the ability to tiller as described in earlier chapters.

When repeated with a control and high B treatments the development of tillers was recorded regularly, specifically to determine whether plants derived from monosomics of homoeologous group four produced more tillers than Chinese Spring and the other monosomic lines at the B0 treatment as well as at B75. The number of plants per line which had produced tillers at the B0 treatment 12, 14 and 16 days after sowing is presented in Figure 7.3. At day 12 only five out of the 210 Chinese Spring plants had developed tillers while at day 16 more than 98% had tillered. There was considerable variation between lines in commencement of tillering. The progeny of the monosomics of chromosomes 5A, 4A and 4B commenced tillering about two days earlier than other monosomic lines and Chinese Spring, while the 4D monosomics tillered at about the same time as Chinese Spring. At day 14, seven lines had tillered to the same extent as the progeny of monosomic 4B. The very low degree of tillering for the progeny of monosomics 6D and 7D could be related to very shrivelled grain and consequently plants of poor vigour.

At the B75 treatment more than half the progeny had produced at least one tiller 16, 25 and 21 days after sowing for the 4A, 4B and 4D lines, respectively. In no other case had more than half the plants tillered by the completion of the experiment at day 28,

Table 7.9 (a) Presence or absence of tillers 28 days after sowing and karyotype for the individual progenies of monosomics 4A, 4B and 4D when grown at B75.

Monosomic parent	Karyotype	+ tillers	- tillers
4A	21"	1	1
	20"+1'	8	-
4B	21"	-	4
	20"+1'	5	1
4D	21"	1	3
	20"+1'	7	1

Table 7.9 (b) Test for independence of development of tillers and the monosomic condition for the combined progeny of monosomics 4A, 4B and 4D when grown at B75.

Monosomic parent	Karyotype	+ tillers	- tillers	Total
4A 4B 4D	21"	2	8	10
	20"+1'	20	2	22
	Total	22	10	32

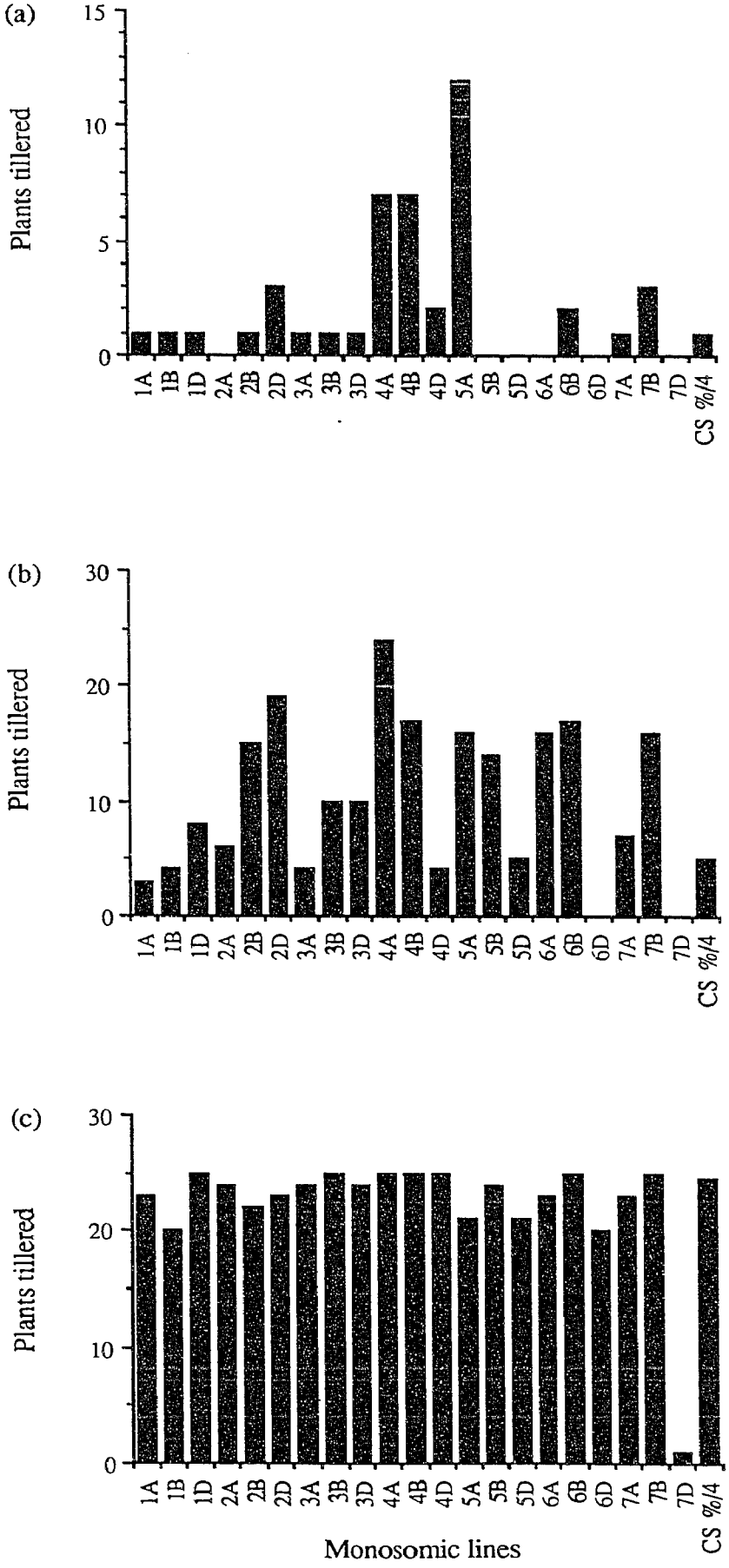
Test for independence of tiller development and monosomics $\chi^2 = 12.96$, $P < 0.001$

Figure 7.3 Number of plants which had produced tillers in the progeny of the monosomics of Chinese Spring grown at the B0 control treatment. 25 plants were sown per line. Tiller production of the Chinese Spring standards is expressed as percentage / 4.

(a) 12 days,

(b) 14 days and

(c) 16 days after sowing.


(a)
Plants tillered
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 CS %/4
(b)
Plants tillered
30
20
10
0
1A 1B 1D 2A 2B 2D 3A 3B 3D 4A 4B 4D 5A 5B 5D 6A 6B 6D 7A 7B 7D CS %/4
(c)
Plants tillered
30
20
10
0
1A 1B 1D 2A 2B 2D 3A 3B 3D 4A 4B 4D 5A 5B 5D 6A 6B 6D 7A 7B 7D CS %/4
Monosomic lines

while less than 30% of the Chinese Spring standards had tillered at that stage. Not only did the progeny of monosomics 4A, 4B and 4D initiate tillers earlier at B75 but they also developed more tillers per plant. This effect also occurred at the control for 4A and to a much lesser extent for 4B and 4D (Table 7.10).

There was a highly significant interaction between genotype and the ability to tiller at high B concentrations. For the monosomics of chromosome 4A the ability to develop tillers at a high B treatment can at least in part be attributed to a greater level of tillering at the low B control, however this was not necessarily the situation for the monosomics of chromosomes 4B and 4D. In a study of the nullisomics of Chinese Spring, Sears (1944, 1954) recognised an inhibitor of tillering located on chromosome 4A . The experiments reported here with monosomic 4A of Chinese Spring demonstrate that the inhibitor gene is hemizygous ineffective and so is only fully expressed when present in two doses. It is possible that homoeoloci for inhibition of tillering, but of lower activity, are also present on chromosomes 4B and 4D, and the generally inhibitory effect of high concentrations of B on tillering accentuate the differences in tillering between the monosomics of 4B and 4D and the euploid Chinese Spring plants.

Alternatively, the ability of the monosomes of 4B and 4D, and possibly also 4A, to tiller at higher concentrations of B may result from them being more tolerant than Chinese Spring to B. Uptake and tolerance to high concentrations of B is under additive gene control and a previous experiment has shown that when the short arm of chromosome 4A is absent B uptake is reduced. A similar situation may arise when a gene controlling response to B is present in the hemizygous condition; that is the monosomic may be more tolerant than the euploid.

Irrespective of the reason for the enhanced tillering of the monosomics of the group four chromosomes at a high B treatment, it is significant that it is able to be expressed at a B treatment which is otherwise inhibitory to tiller initiation for euploid Chinese Spring plants.

The dry weights of the progeny of the monosomics were compared with the Chinese Spring standards. The dry weights of Chinese Spring were significantly different between trays, therefore each line was compared with the Chinese Spring standards

Table 7.10 Number of tillers per plant 28 days after sowing for the progeny of the monosomics of Chinese Spring when grown at B0 and B75 treatments. Plants with only a main shoot are classified as 0 tillers.

Monosomic Progeny	B0				B75			
	Tillers / plant				Tillers / plant			
	0	1	2	3	0	1	2	3
1A	2	15	8	-	20	5	-	-
1B	3	18	3	1	24	1	-	-
1D	-	22	3	-	23	2	-	-
2A	-	23	2	-	18	6	1	-
2B	2	13	10	-	21	3	1	-
2D	2	16	6	1	19	6	-	-
3A	1	19	5	-	19	6	-	-
3B	-	21	4	-	14	8	3	-
3D	1	18	6	-	14	11	-	-
4A	-	2	15	8	1	4	14	5
4B	-	13	11	1	7	10	8	-
4D	-	13	10	2	4	8	13	-
5A	3	6	13	3	24	-	-	-
5B	1	12	12	-	24	1	-	-
5D	2	15	7	-	24	1	-	-
6A	2	13	6	3	15	9	-	-
6B	-	18	6	1	18	6	-	-
6D	1	14	10	-	21	4	-	-
7A	-	19	5	-	23	2	-	-
7B	-	21	4	-	24	1	-	-
7D	16	8	-	-	25	-	-	-
Chinese Spring	1	134	40	5	133	58	4	-

within the same tray by an unpaired t-test. As the progeny of monosomics consist of euploid, monosomic and nullisomic plants, deviations from the Chinese Spring standards would be due to the effect of either the vigour, or the tolerance / sensitivity to B, of the monosomics and nullisomics. Significant differences in dry matter were found between the progeny of eight of the monosomics and Chinese Spring at the B0 and B75 treamtents (Table 7.11). The extremely low yields for chromosomes 6D and 7D could be attributed to small grain.

The progeny of monosomic 4B yielded significantly more than Chinese Spring, at B75 and the progeny of monosomic 4D yielded significantly less than Chinese Spring at B0 but the same at B75. This suggests that the monosomics of chromosomes 4B and 4D are more tolerant than Chinese Spring to high concentrations of B. On the other hand, the progeny of monosomics 2A, 3A and 7B were more sensitive than Chinese Spring. The dry weights of the progeny of monosomic 7A were greater than Chinese Spring at both the B0 and B75 treatments. In view of the number of comparisons it would perhaps be more appropriate to use the 1 % confidence level for determining significant results, in which case only the response for chromosome 2A would be considered significant.

This experiment has shown that the response to B is modified when genes are in the hemizygous condition and tillering, in particular, is influenced. When rating the progeny of a monosomic plant for response to B, the criterion of ability to tiller, which has previously been found to be very useful for euploid plants, should be used with caution, particularly for the group four chromosomes as at least with chromosome 4A the inhibitory factor for tillering is not fully expressed in the hemizygous condition.

Table 7.11 Comparison between Chinese Spring (CS) and the progeny of the monosomics of Chinese Spring for dry matter yield when grown at B0 and B75. The yield of Chinese Spring is the mean of 10 plants and the monosomics the mean of 25 plants. Yields are compared by unpaired t - tests.

Monosomic Progeny	B0			B75		
	Dry wt (g plant $^{-1}$)			Dry wt (g plant $^{-1}$)		
	CS	Mono's	t-test	CS	Mono's	t-test
1A	0.216	0.208	0.465	0.170	0.171	0.081
1B	0.282	0.267	0.757	0.157	0.151	0.579
1D	0.276	0.279	0.228	0.146	0.155	1.030
2A	0.227	0.277	3.317**	0.169	0.175	0.540
2B	0.273	0.283	0.539	0.140	0.148	0.513
2D	0.256	0.276	1.161	0.163	0.171	0.678
3A	0.261	0.274	0.971	0.176	0.150	2.160*
3B	0.228	0.223	0.291	0.165	0.152	1.133
3D	0.255	0.244	0.622	0.169	0.156	1.500
4A	0.237	0.231	0.262	0.172	0.149	1.627
4B	0.262	0.264	0.179	0.148	0.180	2.388*
4D	0.264	0.236	1.784*	0.183	0.188	0.351
5A	0.269	0.253	0.560	0.173	0.160	1.046
5B	0.258	0.254	0.270	0.145	0.128	1.474
5D	0.232	0.208	1.327	0.150	0.137	1.170
6A	0.257	0.260	0.850	0.135	0.142	0.441
6B	0.250	0.249	0.050	0.153	0.143	1.204
6D	0.308	0.202	5.917***	0.170	0.123	6.055***
7A	0.244	0.285	2.221*	0.129	0.152	2.941**
7B	0.232	0.272	2.316*	0.140	0.153	1.010
7D	0.390	0.203	10.874***	0.190	0.084	8.742***

*, ** and *** significant at 5%, 1% and 0.1% levels, respectively.

7.6 RECIPROCAL MONOSOMIC ANALYSIS OF FEDERATION AND CHINESE SPRING FOR TOLERANCE TO BORON

7.6.1 Introduction

Federation was found to be more tolerant than Chinese Spring to high concentrations of B (Chapter 7.2). As monosomic series exist for both varieties the reciprocal monosomic method may be adpoted for the identification of chromosomes having a major effect on control of tolerance to B.

7.6.2 Materials and methods

Seed

Seed derived from the monosomics of Chinese Spring and Federation were sown in standard potting mix and monosomic plants for the complete set of Chinese Spring monosomics and 19 of the 21 monosomics of Federation (monosomics 2B and 2D missing) were identified by examination of pollen mother cells at Metaphase I and were used as the female parents for hybridization with the alternative cultivar. F_1 hybrid plants were grown in standard potting mix, examined for chromosome complement at Metaphase I of meiosis and monosomic plants were selected. The selected plants were allowed to self pollinate and F_2 seed was obtained from individual F_1 hybrids. F_2 seed was also produced for the euploid hybridization between Chinese Spring and Federation.

Experimental design

The F_2s derived from individual monosomic F_1 hybrid plants for all available lines were compared with the euploid F_2 for response to B. B100 soil was weighed into plastic trays and two trays were assigned to the F_2 of each monosomic line while a total of six trays were sown to the euploid F_2 seed. Seeds were sown at a spacing of 5cm x 3.5cm and a total of 35 F_2 seeds and five seeds of each euploid parent were included per tray.

Trays were arranged at random within each monosomic series and three trays of the euploid F_2 were included per monosomic series. The experiment required three

adjacent benches in a glasshouse and the middle bench was shared by the two monosomic series. The trays were re-randomized weekly.

Scoring of the experiment commenced five weeks after sowing. Plants were harvested at ground level and the length of symptoms of B toxicity was measured for the three oldest leaves of individual plants. The plants of the Chinese Spring monosomic crosses were harvested first and as the harvest required four days the plants of the Federation monosomics were subjected to B stress for longer than the Chinese Spring monosomics.

Evaluation of response

The parental cultivars were included to provide a measure of environmental variation, between trays, rather than for comparisons with the F_2 plants. It proved to be impossible to assign individual F_2 plants to discrete categories, therefore each monosomic F_2 line was treated as a population and compared with the euploid F_2 for mean length of symptom expression of the first three leaves. The response of a critical chromosome would vary between reciprocal combinations. For the (Chinese Spring monosomics*Federation), the Chinese Spring monosome is replaced by the homologous chromosome of the more tolerant Federation and all plants, except nullisomics, would carry the genes of the Federation chromosome in either the homozygous or hemizygous condition. If the chromosome is critical with respect to tolerance to B there would be a low frequency of sensitive plants among the F_2 generation and this would result in a low mean symptom expression. The converse would apply for the (Federation monosomics* Chinese Spring) and the F_2 generation of critical chromosomes would have a low frequency of tolerant plants and a high mean symptom expression.

(In the case of a single gene difference for B tolerance, between Federation and Chinese Spring, replacement of the critical chromosome of Federation would result in all sensitive plants)

7.6.3 Results and Discussion

Parental standards

The mean symptom expression for the two parents together with the F ratios and levels of significance for comparisons between trays for the standards are shown in Table 7.12. The symptoms of B toxicity were less severe on leaves one and two of Federation,

than Chinese Spring, but symptoms were more severe on leaf three of Federation. This apparent anomaly can be accounted for by the fact that the third leaf of Federation emerged before the third leaf of Chinese Spring and therefore B accumulated over a longer period. This response is consistent with the results of Chapter 6. Many plants of Federation had poor vigour and this was related to damaged seed rather than B toxicity. Consequently, the symptom expression between trays was more variable for Federation than Chinese Spring and this was most pronounced within the block of (Chinese Spring monosomics*Federation).

The symptom expression on the first leaf of Chinese Spring was uniform between trays within both monosomic blocks while the significance of the symptom expression of the second leaf varied between blocks. Overall, the symptom expression by Chinese Spring was considered to be sufficiently uniform within monosomic series to pool the results of the two trays for each monosomic cross and make direct comparisons between the monosomic F_2s of individual lines and the euploid F_2 within each monosomic series.

Symptoms of B toxicity for the standards were more severe within the block of Federation monosomics and this could be attributed to the fact that the Federation monosomics were harvested second and up to three days later than the Chinese Spring monosomics. The two monosomic series were therefore analysed separately.

Monosomic F_2s

The significance of the difference in symptom expression among the monosomic F_2s and the euploid F_2 of each monosomic block was tested by analysis of variance for the symptom expression of the first three leaves. Highly significant differences occurred between monosomic lines within each monosomic series and this indicates that there were significant differences between chromosomes in their influence upon expression of symptoms of B toxicity. The mean length of symptom expression of the individual lines and F ratios for comparison between lines for the first three leaves of the euploid and monosomic F_2's are presented in Figure 7.4. The mean length of symptoms of all leaves was less than for the euploid F_2 for several lines of the (Chinese

Table 7.12 Expression of symptoms of B toxicity (cm / leaf) and F value for the analysis of variance when measuring the uniformity of symptom expression of the parental standards, between trays, within each monosomic series for the screening of the F_2s for the reciprocal monosomics between Chinese Spring and Federation grown at B100.

Leaf	Parent	Symptoms (cm)	F value		P
C S monos * Fdn					
L1	C S	3.34	$F_{(44,157)}$	1.42	0.05<P<0.10
	Fdn	1.76	$F_{(44,137)}$	2.31	P<0.01
L2	CS	6.57	$F_{(44,146)}$	1.52	0.025<P<0.05
	Fdn	5.05	$F_{(44,136)}$	2.23	P<0.01
L3	CS	6.47	$F_{(44,146)}$	1.35	0.05<P<0.10
	Fdn	8.27	$F_{(44,126)}$	1.17	P>0.10
Fdn momos * CS					
L1	CS	3.88	$F_{(40,145)}$	1.04	P>0.25
	Fdn	2.23	$F_{(40,133)}$	1.47	0.05<P<0.10
L2	CS	7.02	$F_{(40,145)}$	1.07	P>0.25
	Fdn	5.55	$F_{(40,133)}$	1.47	0.05<P<0.10
L3	CS	6.68	$F_{(40,133)}$	1.83	P<0.01
	Fdn	8.60	$F_{(40,125)}$	1.05	P>0.25

Spring monosomics * Federation) and greater than the euploid F_2 for several lines of the (Federation monosomics * Chinese Spring). These lines are consistent with the response expected for critical chromosomes. The difference in response between reciprocal combinations (Fig. 7.5) was greatest for chromosome 7B followed by chromosomes 3A, 3B and 5B. Federation monosomic 2B was not available so reciprocal comparisons could not be made but the mean symptom expression for the F_2 of (Chinese Spring monosomic 2B * Federation) was low therefore chromosome 2B must also be considered as a possible location of genes controlling tolerance to B.

The results for mean symptom expression of B toxicity of individual lines for the reciprocal monosomic analysis of Chinese Spring and Federation do not unequivocally demonstrate the chromosomal location of genes controlling tolerance to B. However, the lines of several chromosomes consistently responded in the manner expected of a critical chromosome. Of these, 7B appears to be the most probable chromosome associated with tolerance to B and other chromosomes which were also implicated include 3A, 3B, 5B and possibly 2B.

At the time of harvest, plants had generally not commenced tillering and the only plants to have developed tillers were the F_2's of (Federation monosomic 4A*Chinese Spring) and (Federation monosomic 4B*Chinese Spring). Within these families a total of five out of 59 and three out of 48 plants had tillered, respectively. This result is consistent with tillering being under the control of hemizygous ineffective inhibitory genes located on the chromosomes of homoeologous group four.

A single albino plant was observed among the F2 of (Federation monosomic 7B* Chinese Spring). McIntosh and Baker (1968) reported that albinism of wheat was associated with nullisomy of chromosome 7B and the gene was localised to the long arm. The albino plant observed in the F2 population was presumably nullisomic for chromosome 7B and this character lends support to the correct classification of the 7B monosomic of Federation.

Figure 7.4 Symptom expression (cm) for the F_2 progeny of reciprocal monosomics between Chinese Spring and Federation grown at the B100 treatment and comparison with the euploid F_2. Symptoms for individual lines are the means of approximately 60 plants.

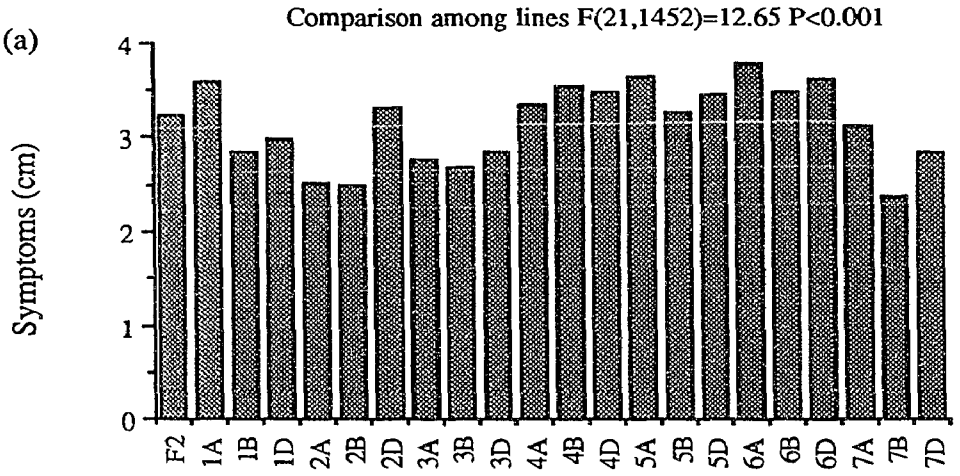
F_2 progeny of (Chinese Spring monosomics * Federation)

(a) Leaf 1

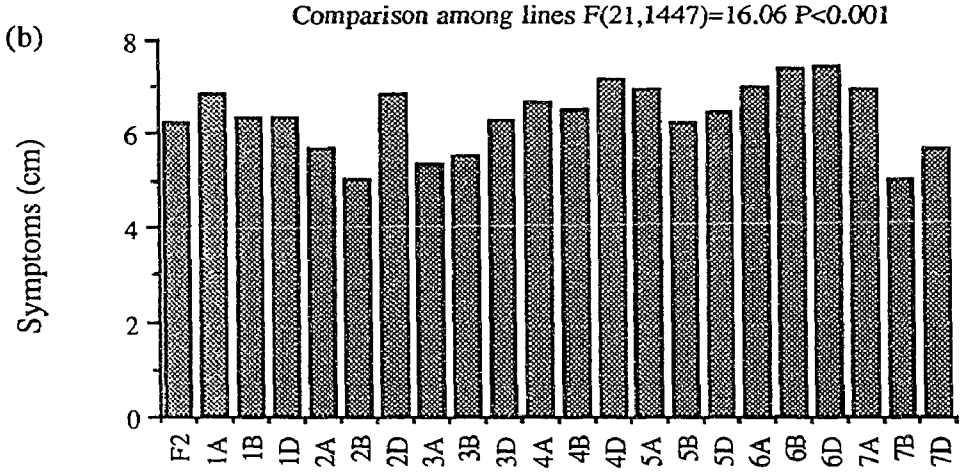
(b) Leaf 2

(c) Leaf 3

(a)


Comparison among lines F(21,1452)=12.65 P<0.001
Symptoms (cm)
0
1
2
3
4
F2
1A
1B
1D
2A
2B
2D
3A
3B
3D
4A
4B
4D
5A
5B
5D
6A
6B
6D
7A
7B
7D

(b)


Comparison among lines F(21,1447)=16.06 P<0.001
Symptoms (cm)
0
2
4
6
8
F2
1A
1B
1D
2A
2B
2D
3A
3B
3D
4A
4B
4D
5A
5B
5D
6A
6B
6D
7A
7B
7D

(c)

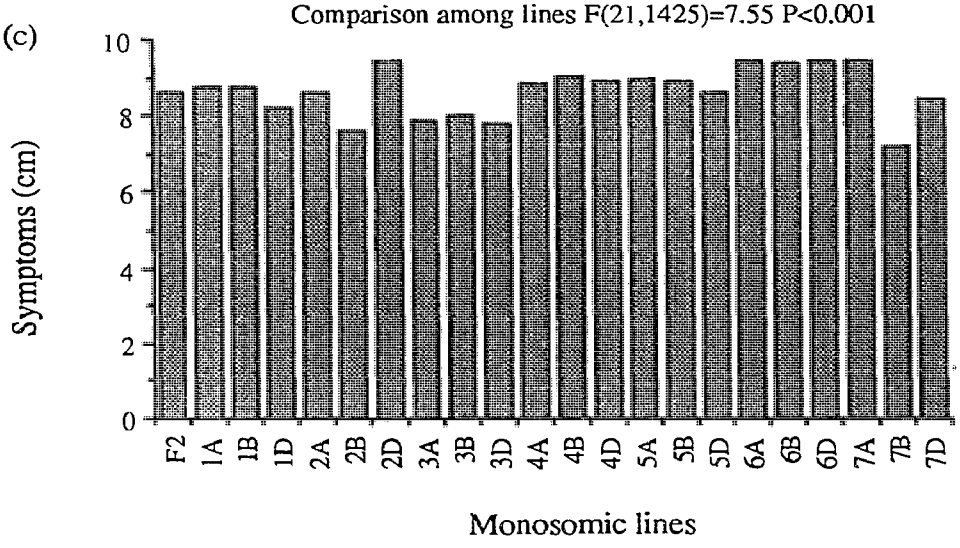

Comparison among lines F(21,1425)=7.55 P<0.001
Symptoms (cm)
0
2
4
6
8
10
F2
1A
1B
1D
2A
2B
2D
3A
3B
3D
4A
4B
4D
5A
5B
5D
6A
6B
6D
7A
7B
7D
Monosomic lines

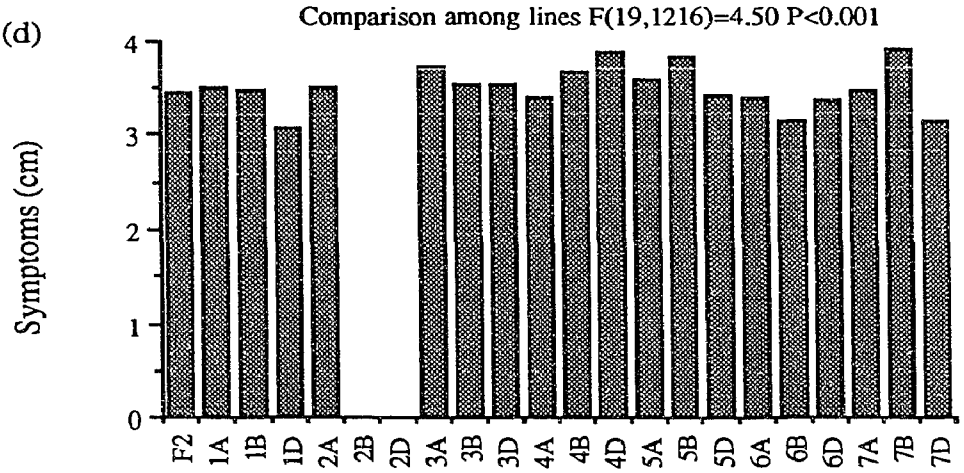
Figure 7.4 contd. F_2 progeny of (Federation monosomics * Chinese Spring)

(d) Leaf 1,

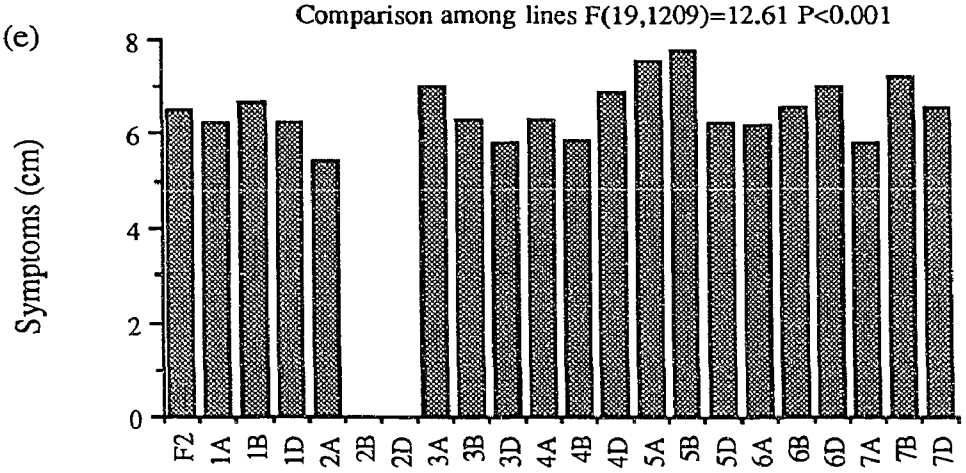
(e) Leaf 2 and

(f) Leaf 3.

(d)


Comparison among lines F(19,1216)=4.50 P<0.001
Symptoms (cm)
0
1
2
3
4
F2
1A
1B
1D
2A
2B
2D
3A
3B
3D
4A
4B
4D
5A
5B
5D
6A
6B
6D
7A
7B
7D

(e)


Comparison among lines F(19,1209)=12.61 P<0.001
Symptoms (cm)
0
2
4
6
8
F2
1A
1B
1D
2A
2B
2D
3A
3B
3D
4A
4B
4D
5A
5B
5D
6A
6B
6D
7A
7B
7D

(f)

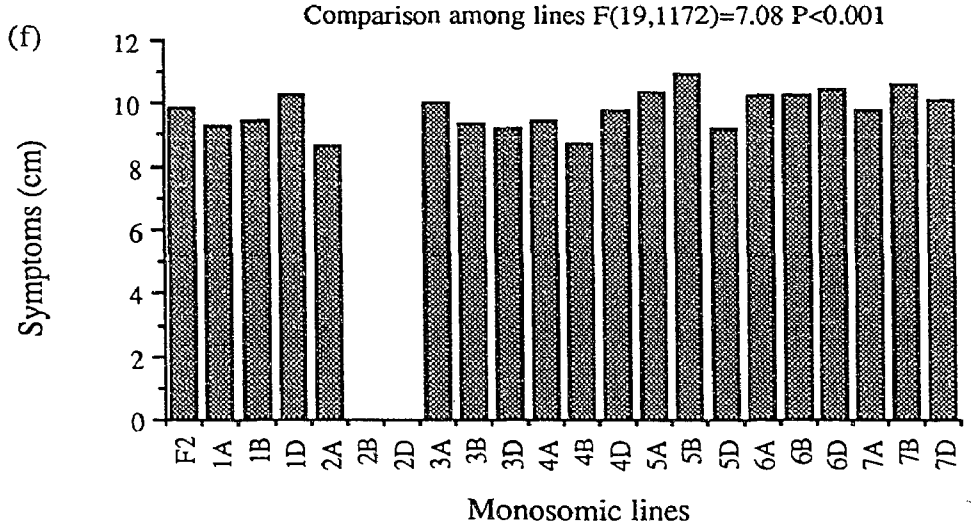
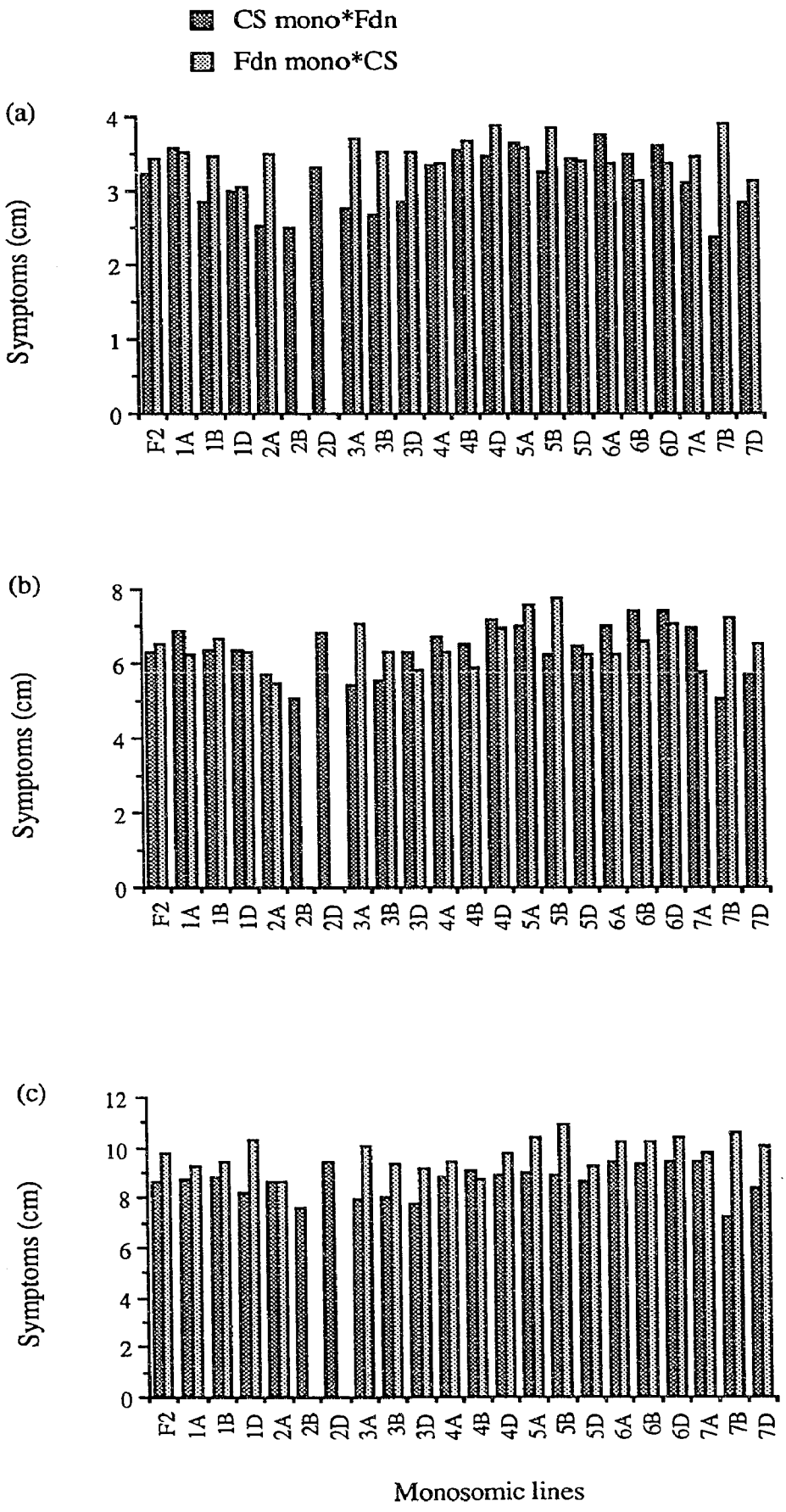

Comparison among lines F(19,1172)=7.08 P<0.001
Symptoms (cm)
0
2
4
6
8
10
12
F2
1A
1B
1D
2A
2B
2D
3A
3B
3D
4A
4B
4D
5A
5B
5D
6A
6B
6D
7A
7B
7D
Monosomic lines

Figure 7.5 Comparison between the F_2 progeny of (Chinese Spring monosomics * Federation) and (Federation monosomics * Chinese Spring) for mean symptom expression (cm).

(a) Leaf 1,

(b) Leaf 2 and

(c) Leaf 3.


CS mono*Fdn
Fdn mono*CS
(a)
Symptoms (cm)
0
1
2
3
4
(b)
Symptoms (cm)
0
2
4
6
8
(c)
Symptoms (cm)
0
2
4
6
8
10
12
F2
1A
1B
1D
2A
2B
2D
3A
3B
3D
4A
4B
4D
5A
5B
5D
6A
6B
6D
7A
7B
7D
Monosomic lines

7.7 MONOSOMIC ANALYSIS OF CHINESE SPRING AND G61450 FOR TOLERANCE TO BORON

7.7.1 Introduction

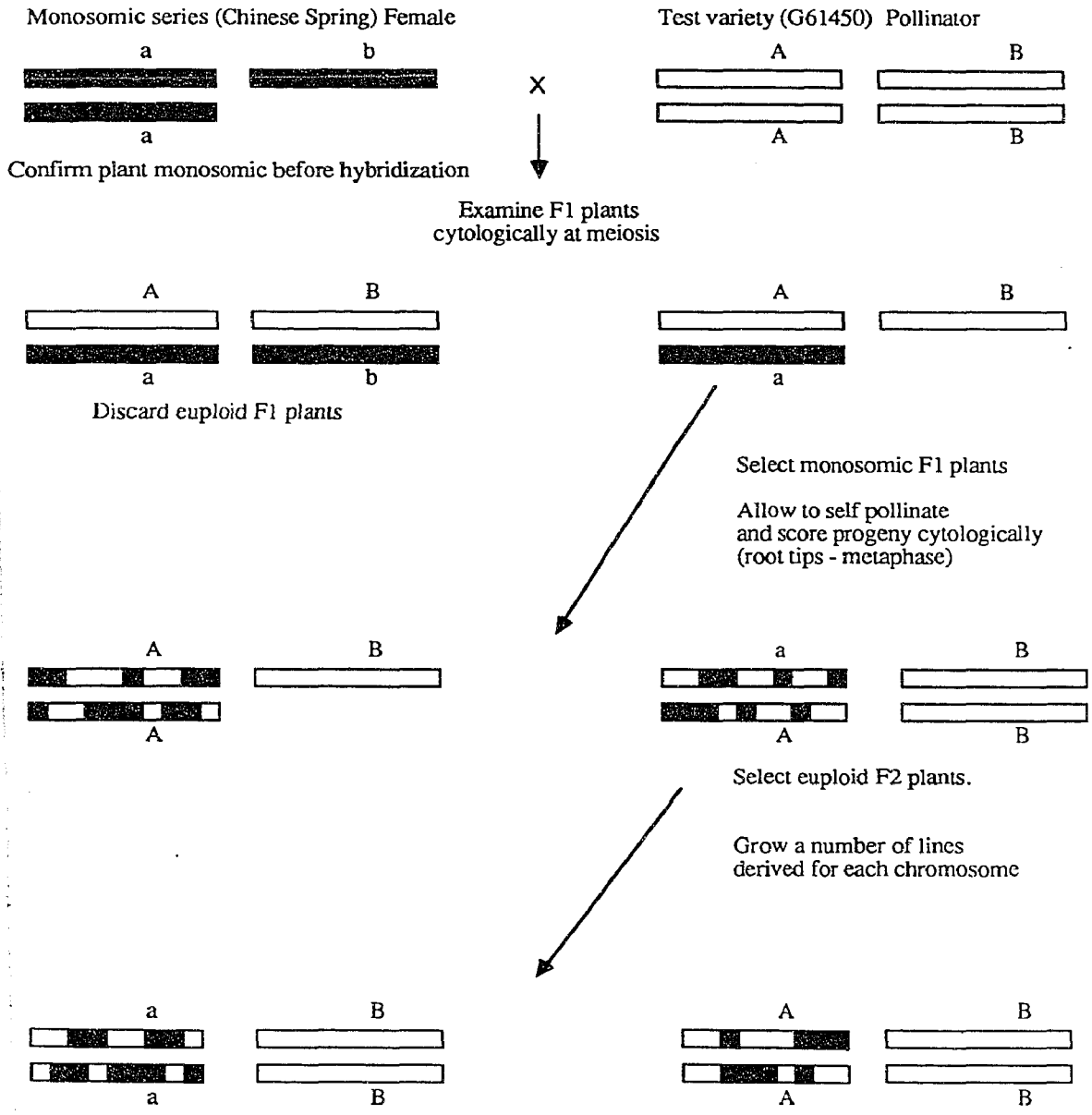
The initial screening of donor varieties of intervarietal substitution lines and monosomic series failed to identify any very tolerant varieties. Chromosomal location of genes for a tolerant variety, and in this case G61450 requires monosomic analysis. As the reciprocal monosomic method could not be used, a set of F_3 lines were developed in which each chromosome of Chinese Spring was replaced, or substituted, by its G61450 homologue.

Development of F_3 "substitution lines"

The procedure used to produce the F_3 substitution lines is outlined in Figure 7.6. Initially the Chinese Spring monosomics as the female parents were pollinated by G61450. The pollen mother cells of F_1 hybrid plants were examined at metaphase I and monosomic plants were selected. Hence the monosomic chromosome was derived from G61450. F_2 seed from the selected plants was germinated and chromosome counts made of root tip squashes. Euploid, or 42 chromosome plants were selected and planted in normal B potting mix for seed multiplication. For these plants the chromosome originally monosomic in Chinese Spring had been replaced by the homologous pair from G61450. There would therefore be no assortment, or recombination, for characters under the control of the substituted chromosome. All background chromosomes were heterogeneous for the two parental types.

At least four F_3 lines were produced for each of the 21 chromosomes and each line was derived from a single euploid F_2 plant. The probability of a gene controlling tolerance to B being homozygous tolerant on a non-critical (i.e. non-substituted) chromosome would be 1/4. With four lines for each chromosome the probability of all lines being homozygous tolerant for a non-critical chromosome would therefore be $(1/4)^4$, or 1/256. Two generations of selfing together with the four lines for each chromosome were also considered sufficient to minimize any background effects which

Figure 7.6 Procedure for development of "F3 substitution lines" between the monosomics of Chinese Spring and the B tolerant genotype, G61450.



F3 lines with original monosomic chromosome substituted by the homologue of the test variety

Homozygous for genes on the substituted chromosome but heterogenous for all background chromosomes

are normally accounted for by reciprocal hybridizations in the reciprocal monosomic method.

This procedure would be equally applicable to one or several genes controlling tolerance to B. For a single gene difference between parents, all plants for the four lines of the critical substituted chromosome would be as tolerant as G61450. For a difference between parents of two or more additive genes, no plants for the lines of the critical chromosome would be as sensitive as Chinese Spring as the locus on the substituted chromosome would always be homozygous tolerant. Depending upon segregation at other loci, the plants of lines for the critical chromosome may vary from intermediate to the two parents to as tolerant as G61450.

7.7.2 Materials and methods

The F_3 "substitution lines" were evaluated for response to high concentration of B in two stages. The initial testing included only two or three lines for each of the 21 chromosome families. The most sensitive families were rejected at this first stage and this allowed more intensive testing of all lines of the remaining families.

Experiment 1

Two or three lines for each chromosome family, except 5A for which only one line was initially available, were grown in plastic trays containing B100 soil. A total of 35 F3 seeds and five seeds of each of the parents were sown per tray at a spacing of 3.5 cm x 5 cm. There were three randomly assigned lines per tray, two with 12 plants and one with 11.

Plants were harvested four weeks after sowing and visually rated for symptom expression on the first and second leaves and assigned to one of three categories, (1) as tolerant as G61450, (2) intermediate to the two parents and (3) as sensitive as Chinese Spring.

Experiment 2

All F3 lines for seven substituted chromosomes were grown in plastic trays containing B100 soil. Two lines were randomly assigned per tray and therefore one line had 17 plants and the other 18 plants. Five plants of each parent were included as a check grid. Plants were harvested six weeks after sowing at which time most plants were at the four leaf stage. The necrotic symptoms of the first three leaves was measured and plants were rated along with the two parents on the basis of symptom expression.

7.7.3 Results and discussion

Experiment 1

The visual ratings at harvest are presented in Table 7.13. None of the chromosome families had all tolerant plants for all lines, therefore the difference between Chinese Spring and G61450 for tolerance to B was under the control of more than one gene. The chromosome families selected for the second stage of testing were those with at least one line having no plants as sensitive as Chinese Spring and other lines having only a low frequency of Chinese Spring types. The families with a low frequency of Chinese Spring types were retained for further testing to allow for the possibility of misclassification due to environmental variation. Chromosome 4A was also retained, despite all lines having at least one sensitive plant, as it had proved to be a critical chromosome for the Chinese Spring / Kenya Farmer substitution lines. At the time of selecting families for retesting the Chinese Spring / Federation monosomics had not been evaluated.

Experiment 2

The chromosome families retested were 1A, 3A, 4A, 5A, 5B, 6D and 7D. The uniformity of symptom expression for the Chinese Spring and G61450 standards, between trays, was tested by analysis of variance. The results for the each of the first three leaves, and for the sum of symptoms for the first three leaves, were not significantly different between trays. The results for all trays were therefore pooled and compared directly. The variation in symptom expression within and between trays, as determined

Table 7.13 Symptom rating of the F_3 progeny of (Chinese Spring monosomics * G61450) when grown at B100. Plants were compared with G61450 and Chinese Spring standards within each box. Each family derived from a single disomic F_2 plant.

Monosomic	Family 1			Family 2			Family 3		
	T[a]	I	S	T	I	S	T	I	S
1A	1	8	2	6	5	0			
1B	0	4	8	0	6	5			
1D	1	9	1	0	2	10	0	4	8
2A	0	8	4	4	6	2	1	8	2
2B	3	6	2	0	3	9			
2D	0	9	3	2	8	2	1	8	3
3A	0	8	3	3	9	0	0	12	0
3B	0	6	6	0	8	4			
3D	1	6	5	0	10	1	0	5	5
4A	5	6	0	1	9	2	3	8	1
4B	0	11	0	2	6	4	3	7	2
4D	0	11	0	4	8	0	0	1	9
5A	5	7	0						
5B	2	9	0	3	9	0	5	5	1
5D	0	3	9	3	8	0	1	10	0
6A	1	8	3	2	5	4	5	4	3
6B	2	9	1	4	8	0	0	4	7
6D	-	9	2	3	7	1	4	8	0
7A	1	10	1	0	7	5	0	5	7
7B	0	10	2	2	7	3	0	1	11
7D	0	12	0	2	9	0			

[a] Rating of symptom expression ; T = tolerant (G61450), I = intermediate to the two parents, S = sensitive (Chinese Spring).

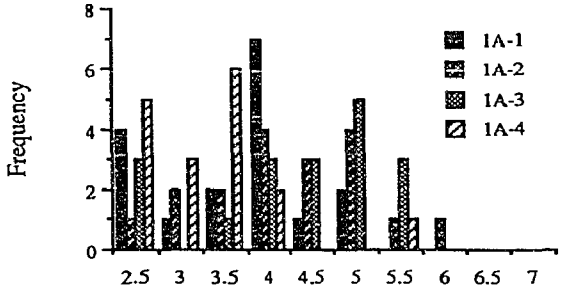
by the coefficient of variation, differed between leaves. For the Chinese Spring standards the coefficients of variation were : Leaf 1 (L1) 47%, Leaf 2 (L2) 21.6%, Leaf 3 (L3) 14.6% and for the sum of the three leaves (L1+2+3) 19.2%. The expression of symptoms by the third leaf and the sum of the symptoms of the first three leaves were chosen for comparing the F3 chromosome substitution lines with Chinese Spring as these were least affected by environmental variation. The results for symptom expression are presented in Figures 7.7 (L3) and 7.8 (L1+2+3).

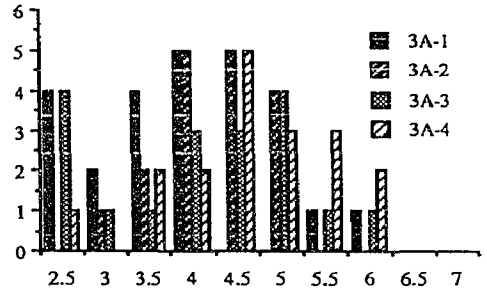
The initial experiment including the 21 chromosome families indicated that there is a difference of more than one gene between Chinese Spring and G61450 for control of tolerance to B, therefore the critical chromosome families may include plants intermediate to the two parents but none as sensitive as Chinese Spring. As there was some overlap in the degree of symptom expression between the parents for L3, and near overlap for L1+2+3 (Figures 7.7 & 7.8), an intermediate class would show considerable overlap with Chinese Spring. Selection of a threshold level of symptom expression to differentiate between Chinese Spring and plants intermediate to the two parents would therefore be quite arbitrary.

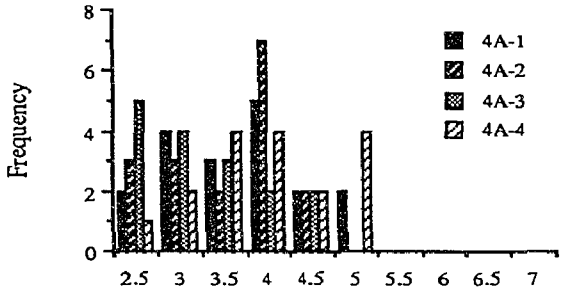
It would be possible to calculate a threshold for distinguishing between Chinese Spring types and an intermediate class based on certain assumptions about the performance of the intermediate category relative to the parents. For example, the simplest model would be for intermediate types to be exactly mid-way between the parents with a similar distribution about the mean. An alternative approach, which would not require any assumptions to be made about the expression of symptoms for an intermediate class, would be to compare the frequency of plants within the categories of more severe symptom development. The more tolerant lines would have a lower frequency, or absence, of plants in these categories, as would also be the expectation for a mathematically derived model based on the performance of the parents.

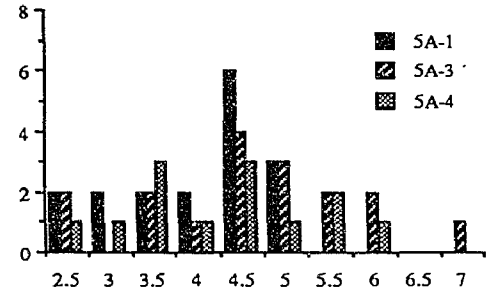
Chromosomes 7D and 4A are the most probable locations of genes controlling response to B for this particular parental combination. The mean length of symptoms for L3 of Chinese Spring was 5.3 cm. Only four plants of the five 7D lines had symptoms of 5 cm or greater and all of these were less than 6cm. For the sum of the symptoms of the

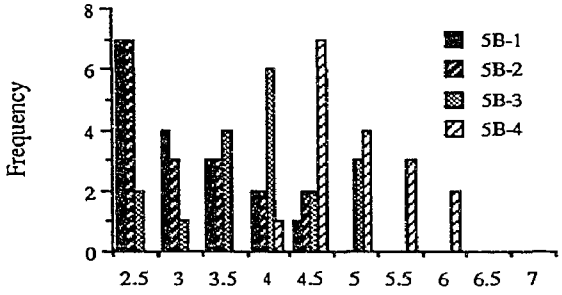
Figure 7.7 Frequency distribution for symptom expression (cm) of Leaf 3 of individual plants for the "F_3 substitution lines" between the monosomics of Chinese Spring and G61450 and comparison with the two parents when grown at the B100 treatment.

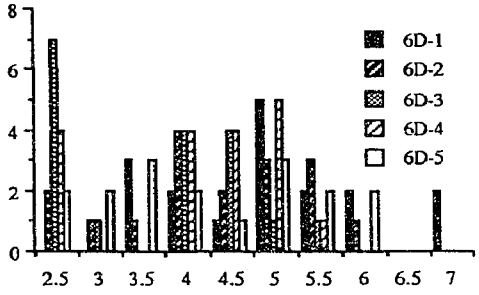

Frequency
1A-1
1A-2
1A-3
1A-4
2.5 3 3.5 4 4.5 5 5.5 6 6.5 7

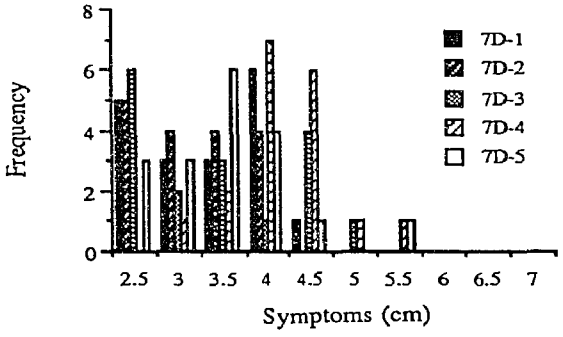

3A-1
3A-2
3A-3
3A-4
2.5 3 3.5 4 4.5 5 5.5 6 6.5 7


Frequency
4A-1
4A-2
4A-3
4A-4
2.5 3 3.5 4 4.5 5 5.5 6 6.5 7


5A-1
5A-3
5A-4
2.5 3 3.5 4 4.5 5 5.5 6 6.5 7


Frequency
5B-1
5B-2
5B-3
5B-4
2.5 3 3.5 4 4.5 5 5.5 6 6.5 7


6D-1
6D-2
6D-3
6D-4
6D-5
2.5 3 3.5 4 4.5 5 5.5 6 6.5 7


Frequency
7D-1
7D-2
7D-3
7D-4
7D-5
2.5 3 3.5 4 4.5 5 5.5 6 6.5 7
Symptoms (cm)

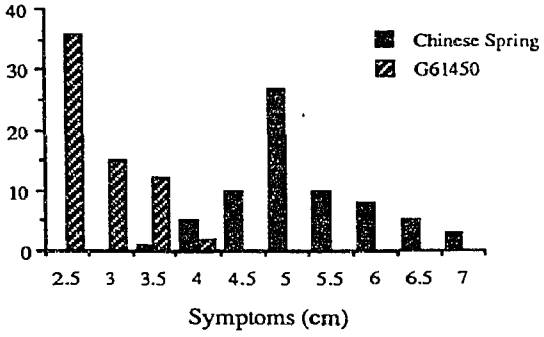
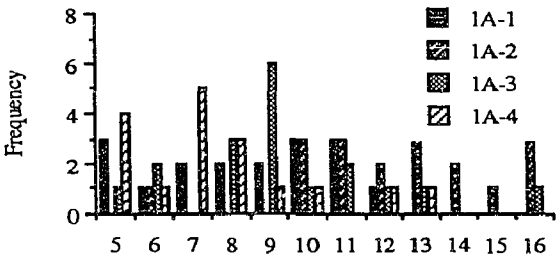
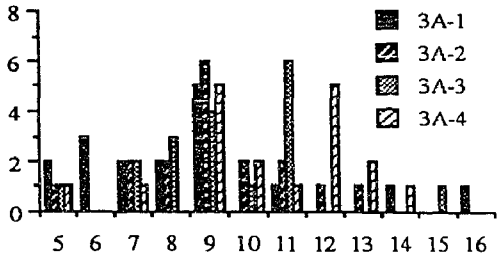
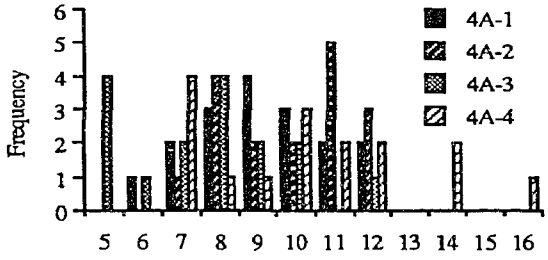
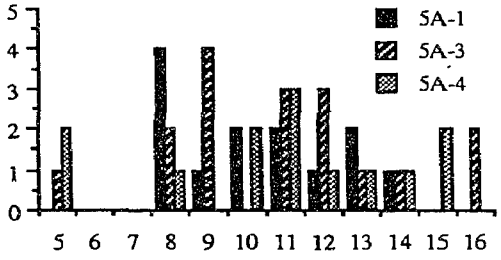

Chinese Spring
G61450
2.5 3 3.5 4 4.5 5 5.5 6 6.5 7
Symptoms (cm)

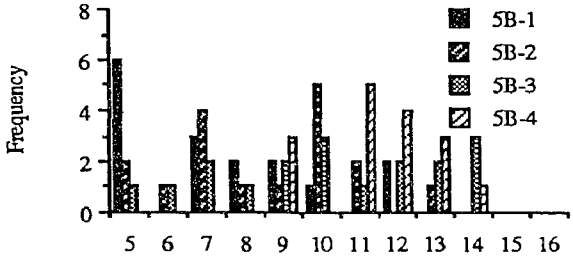
Figure 7.8 Frequency distribution for symptom expression (cm) of Leaves 1+2+3 of individual plants for the "F_3 substitution lines" between the monosomics of Chinese Spring and G61450 and comparison with the two parents when grown at the B100 treatment.

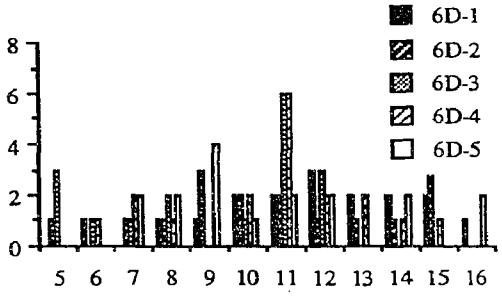

Frequency
1A-1
1A-2
1A-3
1A-4
0 2 4 6 8
5 6 7 8 9 10 11 12 13 14 15 16

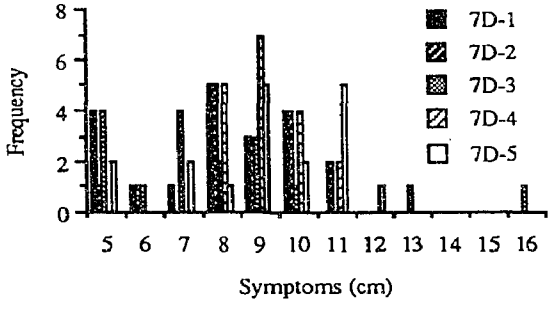

3A-1
3A-2
3A-3
3A-4
0 2 4 6 8
5 6 7 8 9 10 11 12 13 14 15 16

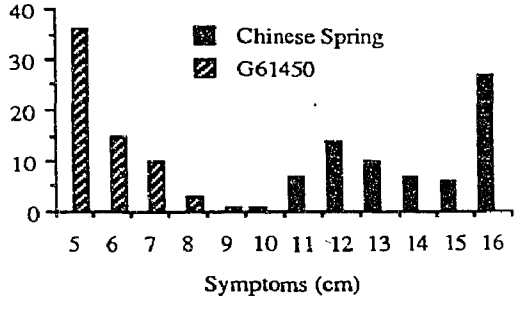

Frequency
4A-1
4A-2
4A-3
4A-4
0 1 2 3 4 5 6
5 6 7 8 9 10 11 12 13 14 15 16


5A-1
5A-3
5A-4
0 1 2 3 4 5
5 6 7 8 9 10 11 12 13 14 15 16


Frequency
5B-1
5B-2
5B-3
5B-4
0 2 4 6 8
5 6 7 8 9 10 11 12 13 14 15 16


6D-1
6D-2
6D-3
6D-4
6D-5
0 2 4 6 8
5 6 7 8 9 10 11 12 13 14 15 16


Frequency
7D-1
7D-2
7D-3
7D-4
7D-5
0 2 4 6 8
5 6 7 8 9 10 11 12 13 14 15 16
Symptoms (cm)


Chinese Spring
G61450
0 10 20 30 40
5 6 7 8 9 10 11 12 13 14 15 16
Symptoms (cm)

first three leaves, the mean length of symptoms for Chinese Spring was 14.6 cm. Only three plants for the five 7D lines had a total length of symptoms of 12 cm or more, while only three plants for the four 4A lines had total symptoms of greater than 12 cm.

The other chromosome families, identified as having one or more lines with no sensitive plants in the initial experiment, all proved to have sensitive plants among their progeny when a greater number of lines were screened. This highlights the need for sufficient lines to be tested to distiguish between tolerance due to a gene located on the substituted chromosome and the chance occurrence of homozygosity for a gene on a background chromosome.

It is not possible to determine unequivocally which are the critical chromosomes controlling response to B on the basis of this experiment as it was not possible to ascertain whether the more severe symptoms for the few plants of the lines of chromosomes 7D and 4A resulted from environmental effects or if they were sensitive segregants. Had the plants not been harvested at ground level prior to measuring the symptoms, the more sensitive plants could have been progeny tested to determine their genotype. Nevertheless, the results indicate that the most probable location of genes controlling response to B are chromosomes 7D and 4A.

7.8 RESPONSE OF THE CHINESE SPRING X *AGROPYRON ELONGATUM* AMPHIPLOID AND ADDITION LINES TO BORON.

7.8.1 Introduction

The initial screening of the amphiploids between Chinese Spring and related species indicated that the Chinese Spring x *Ag. elongatum* amphiploid was more tolerant than Chinese Spring to high concentrations of B. This conclusion, reached on the basis of vigour at a single B treatment, requires testing at a range of B treatments to confirm that the differences in vigour were in fact related to differences in tolerance to B. If differences in response to B were to be confirmed the Chinese Spring - *Ag. elongatum* addition lines could be tested to identify chromosomes of *Ag. elongatum* which have a significant effect in the control of tolerance to B.

7.8.2 Materials and methods

Chinese Spring x *Ag. elongatum* amphiploid

Seed

Seed of the *T. aestivum* (var. Chinese Spring) x *Ag. elongatum* amphiploid ($2n = 8x = 56$) was derived from stocks held at the Waite Agricultural Research Institute and originally obtained from Dr. J. Dvorak (University of California, Davis). All seed used in the experiment was obtained from a single plant which was cytologically examined at Metaphase I of meiosis to ensure the correct chromosome complement. The response of the amphiploid was compared with Chinese Spring and Halberd. Seed of Chinese Spring and Halberd was obtained from stocks held at the Waite Agricultural Research Institute.

Experimental design

B0, B25, B50, B75 and B100 soil was weighed into 150 mm diameter pots (1.8 kg pot $^{-1}$). The five B treatments and three genotypes were combined factorially and pots were arranged as a randomized complete block design with three replicates. Three pre-germinated seeds were sown per pot and thinned to two plants after two weeks. The experiment was completed seven weeks after sowing. Shoots were harvested at ground

level, rinsed in de-ionized water, oven dried and weighed. The concentration of B in shoots was determined by ICP spectrometry for all genotypes and treatments.

Chinese Spring - *Ag. elongatum* addition lines

Seed

The seed used for this experiment was obtained from Dr. J. Dvorak and the production of the addition lines is described by Dvorak and Knott (1974). Hart and Tuleen (1983) demonstrated, by the use of isozyme markers, that several of the addition chromosomes are in fact translocated and they have subsequently developed disomic addition lines to complement the original set of whole chromosome addition lines. Limited time precluded obtaining the correct addition lines and multiplying them under quarantine conditions prior to testing for response to B. Rather, the addition lines already available at the Waite Agricultural Research Institute were used although there was a possibility that results could be confounded by the presence of translocations.

The response of the addition lines designated I, II, III, IV, VS, VL and VI (Dvorak and Knott, 1974) were compared with Chinese Spring and the Chinese Spring x *Ag. elongatum* amphiploid at three B treatments.

Experimental design

B0, B50 and B100 soil was weighed into plastic trays. Within each tray there were nine rows of five plants and plants were arranged at a spacing of 5 cm x 3.5 cm. One row of each genotype was sown per tray and there were three replicates of each treatment. Plants were harvested at ground level six weeks after sowing and the five plants per row were bulked. The shoots from the B50 treatment were rinsed in de-ionized water. Samples were oven dried and then weighed. The concentration of B in shoots of the B50 treatment was determined by ICP spectrometry.

7.8.3 Results and discussion

Chinese Spring x *Ag. elongatum* amphiploid

The yields of Chinese Spring, the amphiploid and Halberd were greatest at the B25 treatment and yields decreased at successively higher levels of applied B (Fig 7.9a). The amphiploid and Halberd yielded significantly more than Chinese Spring at the B75 and B100 treatments. The concentration of B in shoots was similar for the amphiploid and Halberd at all treatments and significantly less than for Chinese Spring at the B50, B75 and B100 treatments (Fig 7.9b)

This result confirms the earlier observation of the Chinese Spring x *Ag. elongatum* amphiploid being more tolerant than Chinese Spring. Tolerance is associated with reduced accumulation of B in shoots and in this respect is the same as the variation in response to B found within *T. aestivum* . The amphiploid displayed a similar level of tolerance to B as did Halberd. As a number of wheat genotypes more tolerant than Halberd to high concentrations of B have been identified (Moody *et al.*, 1988), the tolerance of *Ag. elongatum* is not required for introgression into wheat.

Chinese Spring - *Ag. elongatum* addition lines

Significant differences in yield occurred between genotypes at all B treatments (Table 7.14). At the B0 treatment the amphiploid and addition VL were of very low vigour while all other addition lines were of similar vigour to Chinese Spring. The yield over all genotypes was significantly greater at B50 than B0 and this was most pronounced for the amphiploid and addition II. At B100 the amphiploid yielded significantly more than all addition lines, while addition II was the highest yielding of the addition lines. The amphiploid and addition II were also the only genotypes to produce tillers at B100.

The B concentration in shoots at the B50 treatment differed significantly between genotypes (Table 7.14). The concentration for the amphiploid was less than for Chinese Spring and all addition lines except addition II while the B concentration of addition II was significantly less than for Chinese Spring and all addition lines except addition IV.

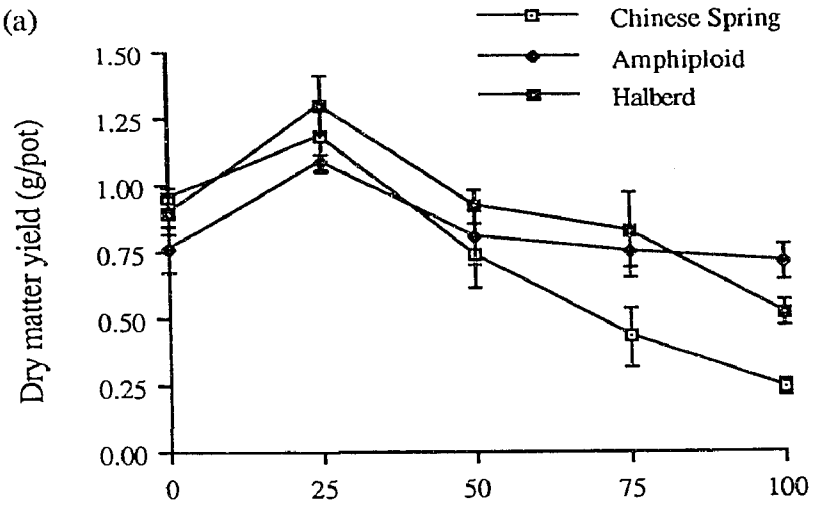
Figure 7.9 Response of Chinese Spring, the Chinese Spring x *Ag. elongatum* amphiploid and Halberd when grown at five B treatments.

(a) Dry matter yield (g pot^{-1}) and

(b) B concentration of shoots (mg kg^{-1}).

Vertical bars represent the standard deviations of the means.

(a)


Chinese Spring
Amphiploid
Halberd
Dry matter yield (g/pot)
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0
25
50
75
100

(b)

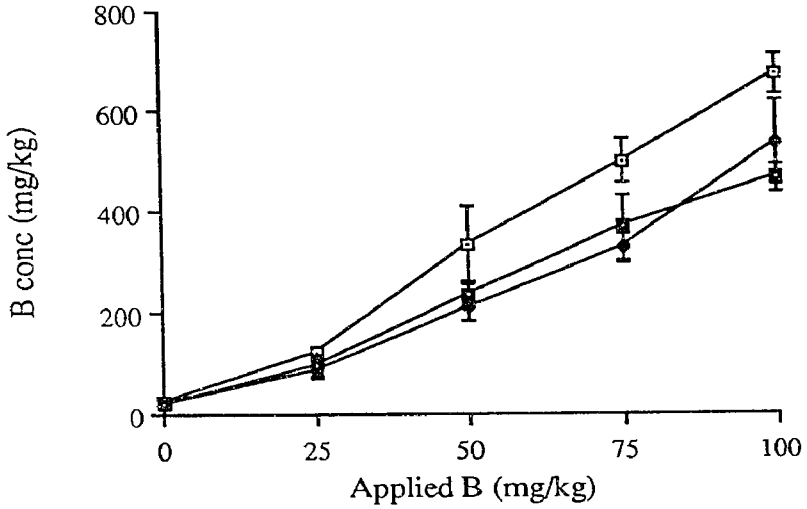

B conc (mg/kg)
800
600
400
200
0
0
25
50
75
100
Applied B (mg/kg)

Table 7.14 Dry matter yield (g / 5 plant row) and relative yield of Chinese Spring, Chinese Spring x *Ag. elongatum* amphiploid and addition lines when grown at B0, B50 and B100. Concentration of B in shoots for the B50 treatment.

Genotype		Dry matter yield (g)				B conc B50 (mg kg^{-1})
		B0	B50	B100	Mean	
Chinese Spring		0.88	0.96	0.47	0.77	370
Amphiploid		0.55	0.90	0.84	0.76	230
Addition lines						
I[a]	1E[b]	0.82	0.77	0.40	0.66	487
II	7E	0.90	1.22	0.63	0.91	284
III	4E	0.94	1.10	0.39	0.81	352
IV	3ES/7EL	0.80	0.95	0.52	0.76	342
VS	3ES	0.91	0.96	0.50	0.79	496
VL	2E#	0.55	0.66	0.28	0.50	414
VI	5ES/2E*	0.74	0.76	0.46	0.65	356
Mean		0.79	0.92	0.50		

LSD (0.05) genotypes 0.09, treatments 0.05, interaction 0.16 LSD (0.05) 65

[a] addition line designations according to Dvorak and Knott (1974)

[b] addition line designations according to Hart and Tuleen (1983)

#,* VL and VIL are opposite arms of chromosome 2E (Hart and Tuleen, 1983).

The B concentrations of additions I and VS were significantly greater than for Chinese Spring.

The results for the concentration of B in shoots indicate that the addition of a chromosome of *Ag. elongatum* may have the effect of either reducing, or increasing B uptake, relative to Chinese Spring. The addition of chromosome II, or 7E, resulted in the most significant reduction in B uptake and this addition was also the most tolerant with respect to yield. Chromosomes which were associated with increased uptake of B were I (1E) and VS (3ES).

Chromosome IV is a translocated chromosome where IVS = 3ES and IVL = 7EL (Hart and Tuleen, 1983). The B concentration in shoots of addition IV (3ES/7EL) was significantly less than for addition VS (3ES), but not significantly greater than for addition II (7E) which indicates that the control of reduced B uptake for chromosome 7E is located on the long arm.

None of the addition lines was as tolerant as the amphiploid, therefore the genes of *Ag. elongatum* which control tolerance to high concentrations of B are located on more than one chromosome.

7.9 DISCUSSION

The chromosomal location of genes controlling tolerance to B was undertaken by three general methods, namely intervarietal substitution lines, monosomic analysis and interspecific addition lines and the wheat variety Chinese Spring was common to all comparisons. The difference in tolerance to B, between genotypes, could not be attributed to the effect of a single chromosome for any of the comparisons although several chromosomes were consistently implicated as having a major effect.

The majority of donor varieties for intervarietal substitution lines into Chinese Spring were of similar tolerance and none more tolerant than Chinese Spring while Kenya Farmer was very sensitive to B. Intervarietal substitution lines were therefore of limited application in determining location of genes controlling tolerance to B. Only the Chinese Spring / Kenya Farmer substitution lines were tested and chromosome 4A was found to have a significant effect upon response to B.

Location of genes for genotypes more tolerant than Chinese Spring was undertaken by the F_2 reciprocal monosomic method for comparing between Chinese Spring and Federation and with F_3 lines derived from monosomic F_1 hybrids for comparing between Chinese Spring and G61450. Chromosomes which were most strongly implicated in the control of tolerance to high concentrations of B were 4A, 7B and 7D while several other chromosomes were also identified as the possible location of genes for B tolerance. Both comparisons identified the most probable location of genes for tolerance to B and in neither case could the difference in response, between parents, be attributed to a single chromosome. Several factors which lead to the uncertainty of the results can be proposed including:

(1) parents differed at more than one locus with respect to control of tolerance to B. The segregation on background chromosomes resulted in the progeny of the critical chromosome not responding as the more tolerant of the two parents.

(2) tolerance to high concentrations of B is expressed as an incompletely dominant character and so a monosomic plant does not fully express the tolerance exhibited by a euploid plant.

(3) there may be minor or modifying genes on many chromosomes.

These three factors resulted in continuous variation within the test populations of the monosomic crosses and so plants could not be assigned to discrete categories and allow the experiments to be analysed by the conventional chi-squared method.

The addition lines of *Ag. elongatum* into Chinese Spring were also tested for tolerance to high concentrations of B. As there is a high degree of homoeology between *T. aestivum* and related species, inferences could be drawn regarding the probable location of genes of *T. aestivum* from the location of genes in *Ag. elongatum* . The results of this experiment did not account for the total tolerance of the Chinese Spring x *Ag. elongatum* amphiploid and this could be accounted for, in part, by the incomplete set and the presence of translocated chromosomes in the addition lines tested. Nevertheless, chromosome 7E proved to have a significant effect upon tolerance to B and comparisons between a ditelosomic and a translocated addition line indicated that the control is located on the long arm.

Consistent trends for the chromosomal location of genes controlling response to high concentrations of B resulted over all experiments. The chromosomes implicated for individual experiments were :

(1) Chinese Spring / Kenya Farmer substitution lines : Chromosome 4A had a major effect and an experiment with the individual arms of chromosome 4A of Chinese Spring indicated that genes controlling uptake of B are located on the short arm. Allelic variation for response to B was identified for chromosome 4A. Other substitution lines with high concentrations of B in shoots, in descending order, were 7B, 3B, 5D, 1D and 6B.

(2) Monosomics of Chinese Spring : Monosomes of homoeologous group 4 tillered at high B treatments. Progeny of monosomics 4B and 4D yielded significantly more and 2A, 3A and 7B significantly less than Chinese Spring at a high B treatment compared with a low B treatment.

(3) Reciprocal monosomic analysis for Chinese Spring and Federation : Greatest difference in symptom expression, between reciprocal crosses, resulted for

chromosome 7B. Other chromosomes for which differences in symptom expression between reciprocal crosses resulted included 2B, 3B and 5B.

(4) Monosomic analysis for Chinese Spring and G61450 : No sensitive plants in F_3 lines developed from the monosomes of 7D and 4A.

(5) Chinese Spring - *Ag. elongatum* addition lines : Concentration of B in shoots of addition 7E significantly less and additions 1E and 3ES significantly more than for Chinese Spring.

Chromosomes of all homoeologous groups were implicated to some extent, however the chromosome identified as having the major effect in all comparisons belonged to either group seven or four. Chromosome 4A was the most significant of the group four chromosomes while chromosomes 7B, 7D and 7E all had a major effect in individual experiments. Chromosome 4A is a complex chromosome and includes two translocations (Naranjo *et al.*, 1988). A segment of 7BS is translocated to 4AL. 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 also hybridized with 4BL (Chao *et al.*, 1989). In addition, a segment of 4AL carrying *β-Amy-B1* has been translocated to 5AL (Sharp *et al.*, 1989).

The homoeology between chromosome 4A and the group seven chromosomes raises the possibility of the 4A gene(s) being located on the translocated segment, however the balance of evidence is contrary to this hypothesis. B uptake by CSDT4AL was less than for Chinese Spring which suggests control of uptake is located on 4AS, while results for the Chinese Spring - *Ag. elongatum* addition lines indicate that control of B uptake is located on the long arm of 7E. The significant effect of chromosome 7B for the reciprocal monosomics between Chinese Spring and Federation is also contrary to this hypothesis if the 7BS/4AL translocation is universal for *T. aestivum* .

Transgressive segregation in response to high concentrations of B was described between G61450 and Halberd in the previous chapter. As Halberd and Federation are closely related it is probable that they have common genes controlling tolerance to B and therefore Halberd would have a gene located on chromosome 7B. On the other hand, chromosome 7D, but not 7B, appeared to have a significant effect upon the tolerance of

G16450 to B. This contrast in location of genes, between G61450 and Federation, is consistent with the previously observed transgressive segregation.

Further evidence of the location of genes controlling tolerance to B would be obtained by demonstration of linkage between B response and other marker genes. Linkage maps using DNA markers, or restriction fragment length polymorphisms, have recently 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 B to determine whether any markers are linked to tolerance to high concentrations of B.

The probability of a number of non-homoeologous loci controlling response to B in hexaploid wheat is not unexpected when the range in tolerance to B in wheat is compared with other species. Experiments conducted with the diploid species *Hordeum vulgare* (Nable, 1988), *Pisum sativum* (Materne, 1989) and some Medicago spp. (A. Lake and A, Kourmozis, pers. comm.) have identified a comparable range in tolerance as found in wheat and a similar mechanism appears to be operating in all species. Such variation could arise from either one highly polymorphic locus or a number of additive genes. The variation in wheat appears to be principally under the control of independent additive loci and similar control in diploid species would almost certainly include genes on more than one chromosome.

Chapter 8.

CORRELATION BETWEEN RESPONSE TO BORON AND OTHER QUANTITATIVE CHARACTERS, WITH PARTICULAR REFERENCE TO GRAIN YIELD AND NUTRIENT UPTAKE, FOR FIELD GROWN WHEAT.

8.1 INTRODUCTION

The previous chapters have demonstrated considerable variation among wheat genotypes for response to B. Response is highly correlated between the glasshouse and naturally occurring high B conditions in the field and is under the control of major genes. Incorporation of B tolerance into intolerant local varieties should therefore be readily achieved through backcrossing. The success of backcrossing, with respect to release of adapted varieties will be determined by linkage of deleterious genes to the donor gene for B tolerance and pleiotropic effects of the gene for B tolerance on other characters.

Evidence of genetic association between B tolerance and another character can be provided by producing hybrids between two genotypes of contrasting B tolerance differing for other character(s) and observing a significant co-variance, or correlation coefficient, between B tolerance and the other variable(s) in a segregating generation.

Varieties are not suitable for testing for co-variance between characters as significant results may reflect the background genotype of the chosen varieties rather than genetic interaction (Snape, 1987). The probability of such a situation resulting between B tolerance and other characters for Australian wheat varieties could be high given the close ancestral relationship between B tolerant varieties. Syme and Thompson (1977) studied 43 historically important Australian wheat varieties for morphological attributes and when hierarchically classified all varieties identified as being tolerant to B in Chapter 4, with the exception of Currawa, were in the same category. Alternatively, a significant correlation between B tolerance and another trait may also arise for varieties of diverse geographical origin with adaptation to other factors common to environments where high concentrations of B occur but this does not necessarily imply a genetic interaction.

Two types of genetic material may be used for studying pleiotropic and linkage effects, namely isogenic lines and random lines arising from a single hybridization (Snape, 1987). Development of both types involves hybridization between genotypes with alternative alleles for the trait of interest followed by a study of segregation ratios or statistics. Isogenic lines are produced by a series of backcrosses between a recurrent parent (usually locally adapted but lacking for one trait) and a donor parent which has the desired trait. Single plants of the alternative genotypes are selected from the progeny of a heterozygous plant after a number of backcrosses (usually more than five) and lines developed from these plants are compared for co-variance between the trait under study and other characters. To use the isogenic line method it is necessary that the trait being transferred to the recurrent parent is under major gene control. Random lines may be developed when the genetic control is not known. Varieties of alternative genotypes are hybridized and lines are developed from random F_2 plants. The correlation between any number of characters and the character under study can then be tested. Both types of lines could also be used to test for interactions between environmental or agronomic variables and the response to B.

The random line approach was chosen to study the interaction between response to B and other factors. The reasons for selecting this method were firstly that lines could be generated in less time and so allow more extensive field evaluation and secondly the development of isogenic lines assumes major gene control and this fact was not known at the commencement of the project. (Now, as part of the total investigations of B toxicity at the Waite Institute, isogenic lines are being generated and studied.)

The concentration of B in plant tissues was measured by ICP-spectrometry by a method which enabled simultaneous determination of the concentrations of 14 elements. This therefore provided the opportunity to test whether the control of B uptake is independent of other nutrients.

The random lines were also used to determine the yield advantage of B tolerance at high B sites and the concentration of B in grain associated with yield responses. As this aspect requires testing over multiple sites and seasons, the results of the current study are preliminary although the lines developed can now be grown for further study in this area.

8.2 MATERIALS AND METHODS

8.2.1 Development of random lines

The genotypes chosen as parents for the development of random lines were Halberd and (W1*MMC)/W1/10. These genotypes represented the range in response to B known at the commencement of the project and subsequent studies have demonstrated that their contrasting response to B is controlled by two genes (Chapter 6).

The population consisted of 35 families, each derived from a single F_2 plant and within each family there were eight lines each derived from the bulked seed of a random F_3 plant. The F_2 and F_3 plants were grown at the Waite Agricultural Research Institute, without selection. The procedure for developing and evaluating the lines is outlined in Figure 8.1.

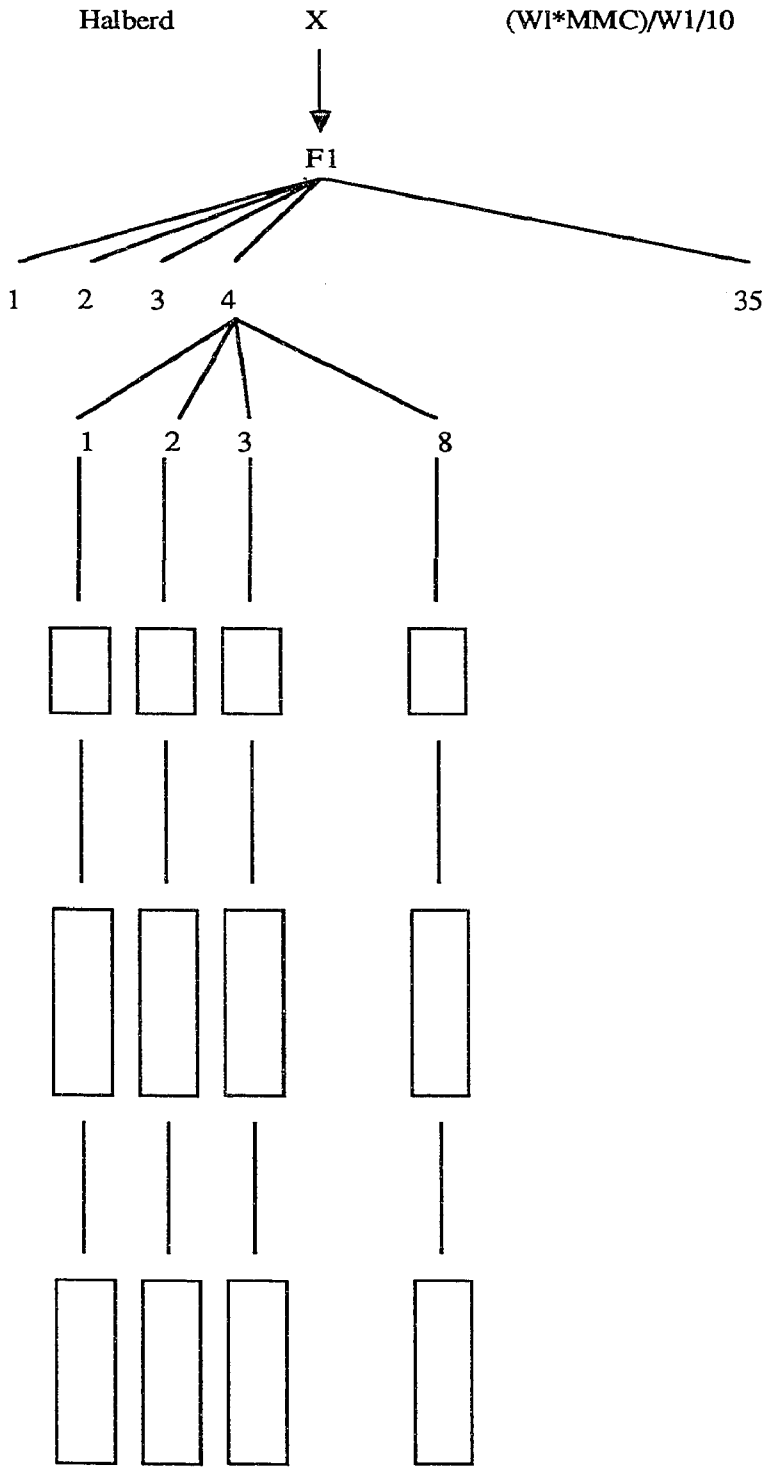
8.2.2 F_4 short plots

The seed obtained from each F_3 plant was sown as an individual F_4 short plot at Two Wells. Short plots consist of plots 1.2 m long and four drill rows wide. These plots are frequently used for early generation testing and seed multiplication when insufficient seed is available for full size yield plots. The 280 individual lines were sown at the rate of 8 g plot $^{-1}$ on 9th June 1987. The experiment consisted of a completely randomized block, without replication, with a grid of Halberd included every fourth plot for a total of 90 plots. Five plots of (W1*MMC) were included to provide a balanced layout of 15 bays x 25 columns and a further nine plots were also included among border plots for a total of 14 plots of (W1*MMC). Plots were harvested at maturity and the grain from individual plots was analyzed by ICP-spectrometry.

8.2.3 F_5 and F_6 yield plots

F_5 plots were sown at Two Wells and Rudall, in 1988, and F_6 plots at Two Wells, Rudall, Minnipa, Walpeup, Windsor and Waite in 1989, as standard yield plots sown over 6m and trimmed to 4.2 m x 4 drill rows, at a sowing rate of 30 g plot $^{-1}$. The sowing dates were :

Figure 8.1 Procedure for the development and evaluation of the random lines of (Halberd*(Wl*MMC)). The F_4 and later generation plots were based on individual F_2, F_3 derived lines.


Halberd
X
(Wl*MMC)/W1/10
F1
F1 - Waite
1
2
3
4
35
F2 Single plants no selection Waite
1
2
3
8
F3 Single plants no selection Waite
Seed of individual plants bulked
F4 Short plots Two Wells grain analysed
Seed of individual lines resown
F5 Yield plots Two Wells Rudall Shoots, grain analysed
Seed of individual lines resown
F6 Yield plots 6 sites

F_5 experiments 1988; Two Wells 23rd June, Rudall 15th June,

F_6 experiments 1989; Two Wells 31st May, Rudall 17th May, Minnipa 31st May, Windsor 26th May, Waite 15th May and Walpeup 17th May.

Seed for the F_5 experiments was obtained from the F_4 plots grown at Two Wells in 1987. Insufficient seed was available to sow all lines at both sites. Two Wells was sown preferentially and missing lines for Rudall were sown to plots of Warigal (total of 24 plots). Seed for the 1989 F_6 experiments was obtained from the F_5 experiment grown at Rudall, 1988, and as such 24 lines were missing. Experiments were arranged as completely randomized blocks, without replication and the parents, Halberd and (Wl*MMC), were included as a control grid. In the F_5 experiments, alternate parents were sown every third plot for a total of 75 plots of each, while the F_6 experiments included alternate parents every fourth plot for a total of 45 plots of each. Plots of Warigal were included to provide a balanced layout for the F_5 (20 plots of Warigal) (15 bays x 30 columns) and F_6 (five plots of Warigal) (15 bays x 25 columns) experiments. Plots of Warigal were also included to compensate for missing lines in the F_6 experiments (24 plots).

Selected F_5 plots at Two Wells were sampled to provide tissue for ICP analysis when plants were at or near the boot stage (9th September, 1988; 78 days after sowing). Families sampled were selected on the basis of concentration of B in the grain of the F_4 plots and included the four lowest and four highest families for B concentration and eight randomly selected families. All lines within these 16 families were sampled. Twenty plots of Halberd and (Wl*MMC) and 10 plots of Warigal were also sampled. Samples consisted of 10 whole shoots per plot. These were rinsed with de-ionised water, oven dried, ground in a stainless steel mill and analysed for concentration of B and other elements by ICP-spectrometry.

The stage of development of all plots at Two Wells was determined on 23rd September 1988 (92 days after sowing).

All experiments were harvested at maturity and grain yields determined. Grain of the families which were sampled at the vegetative stage and standards, from both Two Wells and Rudall in 1988, was analysed by ICP-spectrometry. The shoots and grain of

the F_6 lines in 1989 have not been chemically analyzed. Rather, the grain of five plots of each of the standards at each site was analyzed to give a measure of the B status of the site and variation between sites for the concentration of other elements.

8.3 RESULTS

8.3.1 Chemical analyses

Boron

The concentration of B in grain of the F_4 plots grown at Two Wells in 1987 differed significantly between families. The minimum, maximum, mean and standard deviations for B concentrations of the 35 families, together with results of the two parents are presented in Table 8.1. The magnitude of the standard deviation for B concentration varied considerably between families and this could be attributed to families derived from both heterozygous (large standard deviation) and homozygous (small standard deviation) F_2 plants. Families can be recognized in which the B concentrations of all lines are within the mean ± two standard deviations for one or other of the parents, while families which segregated to cover the range of both parents also occurred. The B concentration of all lines of one family was within the mean ±2 s.d. of the Halberd standards and five families were within the mean ±2 s.d. of (W1*MMC). For two genes segregating, 1/16 of the families derived from F_2 plants would be homozygous for each parental type and so for 35 F_2 derived families there should be approximately two families of each parental type. The greater than expected number of families of (W1*MMC) types may have arisen from either the smaller number of standards and therefore large standard deviation for (W1*MMC) or families derived from F_2 plants heterozygous at one of two loci (e.g. *Bor*1*bor*1*bor*2*bor*2).

The concentration of B in shoots and grain also differed between families for the F_5 plots at both sites in 1988 (Table 8.2). The concentration of B in grain was significantly correlated between sites and seasons while the concentration of B in shoots at Two Wells in 1988 was significantly correlated with grain B for the 1987 F_4 experiment at Two Wells and the 1988 F_5 experiment at Rudall (Fig. 8.2). When

Table 8.1 Boron concentrations (mg kg^{-1}) in grain from F4 plots of (Halberd* (W1*MMC)) grown at Two Wells in 1987. The minimum and maximum concentrations of lines within families and means and standard deviations of families are compared with the two parents, Halberd and (W1*MMC).

Family	B conc (mg kg^{-1})				Family	B conc (mg kg^{-1})			
	Min	Max	Mean	s.d.		Min	Max	Mean	s.d.
1	4.58	8.33	6.04	1.51	19	1.75	8.29	3.51	2.03
2	2.84	8.07	5.02	1.78	20	2.95	3.86	3.42	0.32
3	3.13	10.33	6.01	2.84	21	5.36	7.68	6.52	0.80
4	2.53	3.34	2.91	0.32	22	2.79	3.99	3.20	0.44
5	2.52	4.61	3.44	0.70	23	2.67	6.81	3.69	1.36
6	1.95	4.06	3.07	0.77	24	2.48	8.85	3.49	2.17
7	2.73	4.21	3.03	0.49	25	2.45	3.98	3.02	0.53
8	2.46	6.58	4.11	1.25	26	2.50	3.66	2.93	0.34
9	5.67	8.66	7.36	0.99	27	2.54	6.03	3.89	1.22
10	2.52	5.05	3.53	0.96	28	2.98	6.64	4.03	1.31
11	4.07	5.95	4.76	0.78	29	2.56	6.35	3.96	1.17
12	2.65	7.78	4.80	1.95	30	2.58	3.87	3.24	0.43
13	2.35	3.61	2.87	0.43	31	2.51	6.11	3.52	1.22
14	2.68	5.05	4.13	0.80	32	5.18	8.25	6.61	1.21
15	5.06	7.86	6.14	0.85	33	5.85	7.14	6.33	0.43
16	2.46	5.02	3.73	0.82	34	3.20	4.00	3.64	0.34
17	2.40	3.50	3.02	0.37	35	2.20	4.38	3.07	0.64
18	2.16	4.69	2.99	0.81					

LSD (0.05) family means 1.11

Parent	Number	Min	Max	Mean	Std dev
Halberd	90	1.84	3.88	2.65	0.36
(W1*MMC)	14	4.97	8.21	6.67	0.89

Table 8.2 B concentration (mg kg^{-1}) in shoots and grain for F_5 plots of (Halberd * (Wl*MMC)) and parental standards grown at Two Wells and Rudall in 1988. Values are the mean of eight lines per family. Values for the standards are the mean of 20 plots with the exception of shoots of Warigal which is the mean of 10 plots.

Family	B conc (mg kg^{-1})					
	TW shoots		TW grain		Rudall grain	
	Mean	s.d.	Mean	s.d.	Mean	s.d.
1	76.5	27.8	5.50	1.22	3.81	0.81
4	56.1	14.1	3.76	0.68	2.91	0.64
6	55.3	11.2	3.30	0.39	3.30	0.59
9	76.0	21.3	7.84	1.74	6.56	1.42
11	76.2	22.2	5.17	1.92	4.25	0.84
13	57.9	13.8	3.79	0.89	2.79	0.52
14	75.8	18.6	4.50	0.85	3.85	0.43
17	65.6	9.4	3.81	0.77	3.02	0.82
18	51.4	10.4	3.18	0.77	2.54	0.55
21	90.3	16.6	6.21	0.61	4.46	0.36
23	73.0	21.0	4.42	1.50	3.27	0.91
26	73.0	10.6	3.52	0.72	2.93	0.72
27	67.7	20.0	4.69	1.13	3.42	0.85
30	58.9	13.6	4.15	0.78	2.91	0.66
32	95.1	29.9	7.04	1.72	4.18	0.46
33	108.5	30.3	7.51	1.84	4.75	0.62
LSD (0.05)	19.2		0.84		0.74	
Halberd	53.8	11.6	3.36	0.88	2.96	0.55
Warigal	84.7	20.0	4.53	1.02	3.84	0.82
(Wl*MMC)	103.7	25.2	7.47	1.26	4.52	0.71

comparing between the concentration of B in shoots and grain, there was one family (Family 9) which was a consistent outlier and this family has been indicated in Figure 8.2.

Despite the statistically significant correlation between B concentration of grain at Two Wells and at Rudall, in 1988, a significant genotype x environment interaction resulted when the B concentration of grain was tested by analysis of variance. This is most simply demonstrated by the interaction for the three standards, Halberd, Warigal and (Wl*MMC) ($F_{(2,114)} = 24.0$, $P<0.001$). When the mean concentration of each genotype is plotted against the site mean for the standards (Fig 8.3a) it can be seen that the relative increase in the concentration of B in grain of Halberd and Warigal was similar from Rudall to Two Wells (14% and 18%, respectively) but the concentration of B in grain of (Wl*MMC) increased 65% from Rudall to Two Wells. A similar interaction occurred when the mean concentrations of B in grain of the individual (Halberd* (Wl*MMC)) families are compared between Rudall and Two Wells ($F_{(15,224)} = 2.41$, $P<0.01$) (Fig 8.3b). Family 9 is again indicated and it can be seen that its concentration of B in grain at Rudall was considerably higher than all other families although this was not the case at Two Wells. Although the genotype x environment interaction was significant, there was little change of rank of families. Rather, the effect was the result of a greater increase in the concentration of B in grain for the high B families when moving from a relatively high to a high B site.

In the F_6 experiments, significant differences resulted, between sites and genotypes, for the concentration of B in grain of the three standards (Table 8.3). The B concentration of the grain of the standards was greatest at Two Wells. High B concentrations also resulted at Rudall and Minnipa while concentrations were intermediate at Windsor and Walpeup and low at Waite. Again there was a statistically significant interaction for B concentration between sites and genotypes ($F_{(10,72)} = 5.47$, $P<0.001$). This could be attributed to little or no difference between genotypes at the sites of lowest B concentrations but increasing differences at higher B sites.

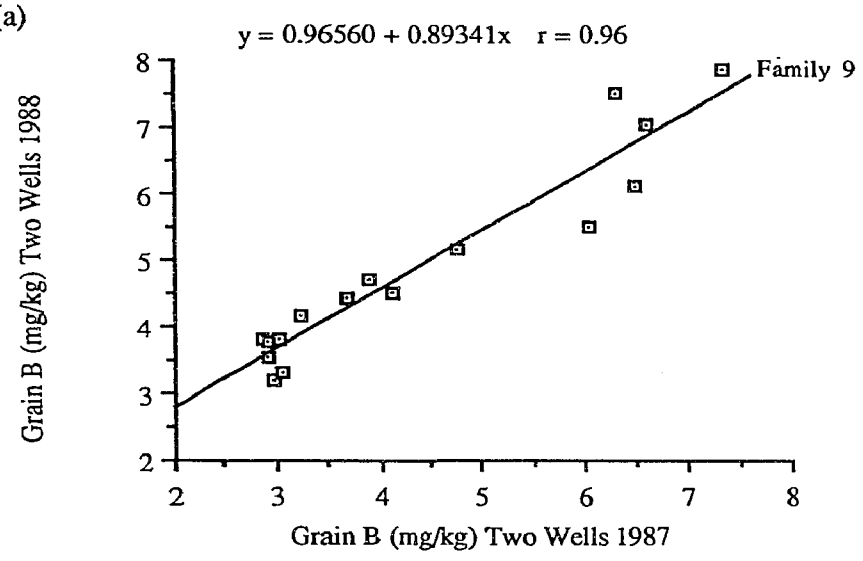
Figure 8.2 The correlation between concentration of B in shoots and grain between sites and seasons for the (Halberd*(Wl*MMC)) families. All data points are the family mean concentrations. Family 9, which was a consistent outlier, is indicated.

(a) concentration of B in grain at Two Wells in 1987 v concentration in grain at Two Wells in 1988.

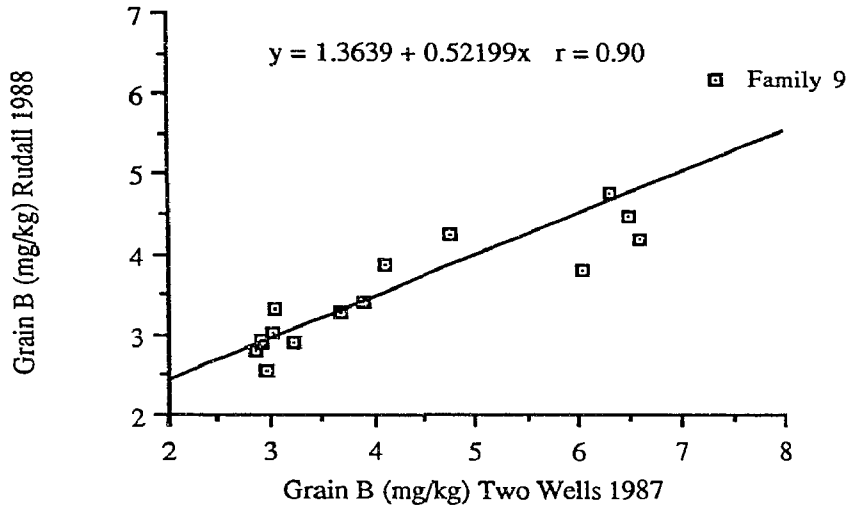
(b) concentration of B in grain at Two Wells in 1987 v concentration in grain at Rudall in 1988.

(c) concentration of B in grain at Two Wells in 1988 v concentration in grain at Rudall in 1988.

(a)


y = 0.96560 + 0.89341x r = 0.96
Family 9
Grain B (mg/kg) Two Wells 1988
Grain B (mg/kg) Two Wells 1987
2
3
4
5
6
7
8

(b)


y = 1.3639 + 0.52199x r = 0.90
Family 9
Grain B (mg/kg) Rudall 1988
Grain B (mg/kg) Two Wells 1987
2
3
4
5
6
7
8

(c)

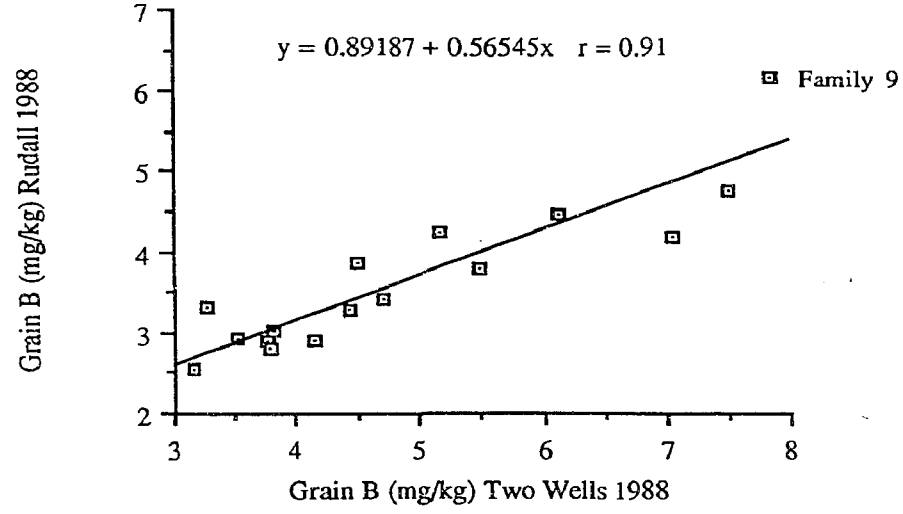

y = 0.89187 + 0.56545x r = 0.91
Family 9
Grain B (mg/kg) Rudall 1988
Grain B (mg/kg) Two Wells 1988
2
3
4
5
6
7
8

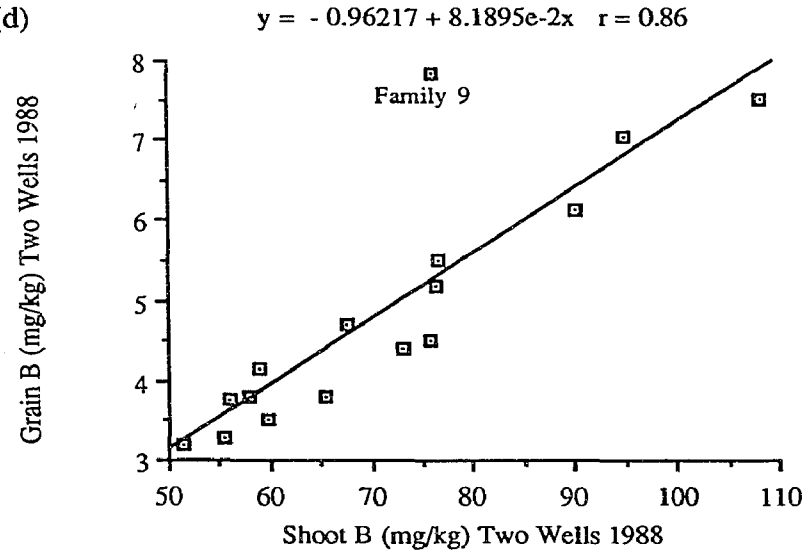
Figure 8.2 contd.

(d) concentration of B in shoots at Two Wells in 1988 v concentration in grain at Two Wells in 1989.

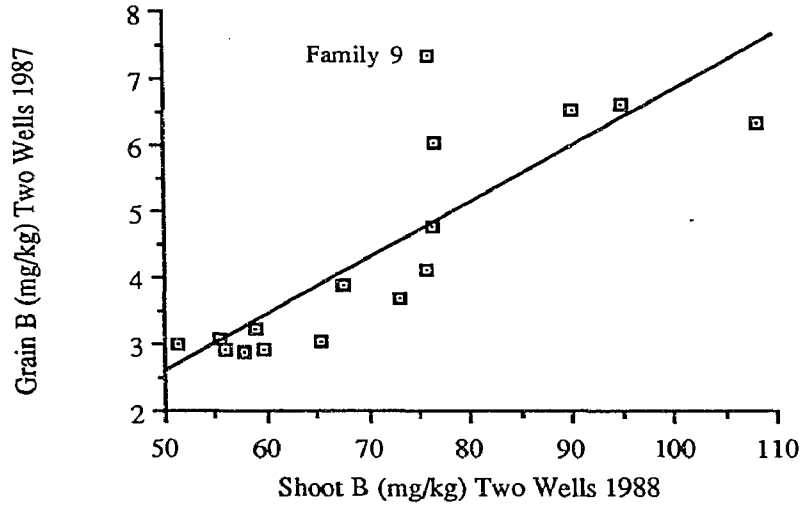
(e) concentration of B in shoots at Two Wells in 1988 v concentration in grain at Two Wells in 1987.

(f) concentration of B in shoots at Two Wells in 1988 v concentration in grain at Rudall in 1988.

(d)


y = - 0.96217 + 8.1895e-2x r = 0.86
Family 9
Grain B (mg/kg) Two Wells 1988
Shoot B (mg/kg) Two Wells 1988
8
7
6
5
4
3
50
60
70
80
90
100
110

(e)


y = - 1.6230 + 8.4188e-2x r = 0.83
Family 9
Grain B (mg/kg) Two Wells 1987
Shoot B (mg/kg) Two Wells 1988
8
7
6
5
4
3
2
50
60
70
80
90
100
110

(f)

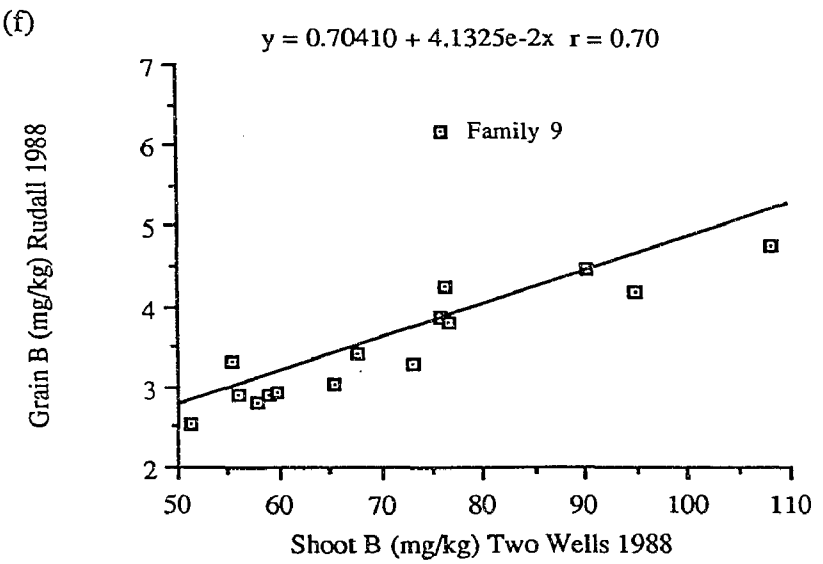
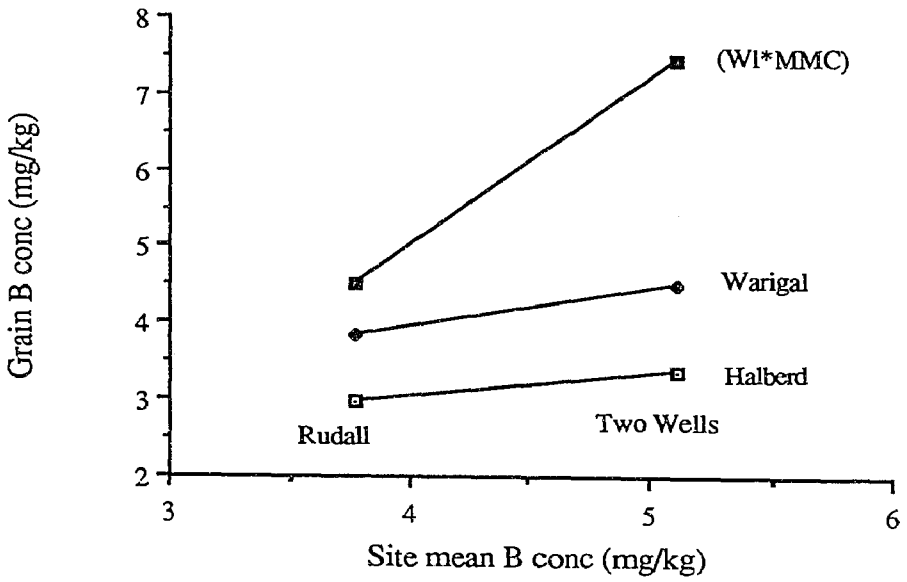

y = 0.70410 + 4.1325e-2x r = 0.70
Family 9
Grain B (mg/kg) Rudall 1988
Shoot B (mg/kg) Two Wells 1988
7
6
5
4
3
2
50
60
70
80
90
100
110

Figure 8.3 Genotype x environment interaction for concentration of B in grain. The mean concentration of B in grain of the three standards, Halberd, (Wl*MMC) (20 plots) and Warigal (10 plots) and the family means for 16 families of (Halberd*(Wl*MMC)) (mean of 8 lines/family) are plotted against the site means at Rudall and Two Wells in 1988. Family 9, which was an outlier in Figure 8.2 is indicated.

(a) interaction for the standard genotypes.

(b) interaction for the families of (Halberd*(Wl*MMC)).

(a)


Grain B conc (mg/kg)
8
7
6
5
4
3
2
(Wl*MMC)
Warigal
Halberd
Rudall
Two Wells
3
4
5
6
Site mean B conc (mg/kg)

(b)

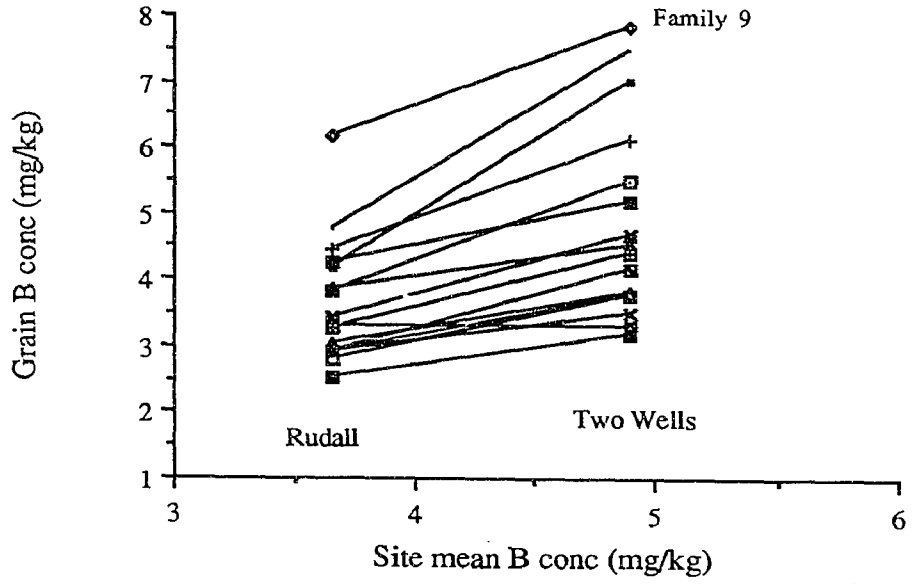

Grain B conc (mg/kg)
8
7
6
5
4
3
2
1
Family 9
Rudall
Two Wells
3
4
5
6
Site mean B conc (mg/kg)

Table 8.3 Boron concentration (mg kg^{-1}) in the grain of Halberd, Warigal and (Wl*MMC) which were included as standards in the F_6 experiments grown at six sites in 1989. Values are the means of five plots.

Site	B conc (mg kg^{-1})			
	Halberd	Warigal	(Wl*MMC)	Site mean
Two Wells	5.05	7.32	9.99	7.44
Rudall	3.17	3.69	8.14	5.00
Minnipa	2.92	4.40	6.95	4.76
Windsor	1.82	1.80	4.23	2.62
Walpeup	1.72	2.23	2.84	2.26
Waite	1.02	1.30	1.25	1.19
Genotype mean	3.13	4.15	6.68	

LSD (0.05) sites 0.78, genotypes 0.60, interaction 1.34

Other elements

ICP-spectrometry provides multi-element analysis and of the 14 elements which were determined, the concentrations of 10 elements (B, Ca, Cu, K, Mg, Mn, Na, P, S and Zn) are considered. The four elements not described are Co and Mo (concentrations below the detection limit), Al (of no biological significance) and Fe (subject to contamination). All elements are virtually completely extracted from shoots by nitric acid digestion, however the nitric acid digestion procedure does not extract all Na from grain (Zarcinas *et al.*, 1987). The results for Na may therefore not represent the total Na content of grain.

The significance of the differences among families for all elements, including B, is indicated by the F ratios from analysis of variance for concentrations of elements in grain at Two Wells, 1987 and 1988 and at Rudall, 1988 and for shoots at Two Wells, 1988 (Table 8.4). Significant differences occurred between families for the concentration of many elements in shoots at Two Wells in 1988 and in grain for both sites and seasons. The F ratios were greatest for B and this is to be expected as the parents were specifically chosen for contrasting response to B. The relative range in concentration of elements, between the minimum and maximum family means, was also greatest for B followed by Na for which there was a two fold difference among family means for concentration in shoots at Two Wells in 1988. The occurrence of significant differences among families for elements other than B allows two types of comparisons to be made. Firstly, the relationship between concentration in shoots and grain and for concentration in grain over sites and seasons can be determined for each element. Secondly, comparisons can be made between the concentration of B and other elements to determine whether there are pleiotropic interactions between uptake of B and other elements.

The significant differences between families for the concentration of elements in shoots and grain indicates that there was segregation at the F_2 generation for accumulation of all elements. The families were derived from both homozygous and heterozygous plants and the magnitude of the standard deviation of the individual families would provide a measure of the probable genotype of the F_2 plant, with respect to each element. The standard deviations would be lowest for families derived from homozygous

Table 8.4 The minimum and maximum family mean concentrations of elements in shoots and grain, mean concentrations over all families and F ratios for comparison among families for experiments grown at Two Wells in 1987 and 1988 and at Rudall in 1988. Mean concentrations of elements in the parental standards are also included for comparison.

Site and tissue	Element									
	P (%)	K	S	Mg	B	Ca	Cu	Mn	Na	Zn
					(mg kg^{-1})					
Two Wells 1987										
Grain										
Minimum	0.26	0.30	0.14	0.11	2.87	253	4.37	41.8	43.1	11.2
Maximum	0.34	0.38	0.18	0.14	7.36	358	5.58	57.5	87.9	16.4
Mean	0.30	0.34	0.15	0.12	4.14	296	4.98	49.1	61.3	13.3
$F_{(34,245)}$	2.78	2.45	6.61	4.13	10.28	5.42	4.13	3.72	1.39	3.85
Halberd	0.29	0.32	0.15	0.12	2.66	299	4.64	45.8	44.0	13.0
(WI*MMC)	0.29	0.39	0.15	0.13	6.67	325	4.82	46.6	91.7	14.0
Two Wells 1988										
Grain										
Minimum	0.18	0.33	0.13	0.10	3.18	173	3.86	36.9	85.5	9.9
Maximum	0.22	0.38	0.15	0.12	7.84	220	4.71	49.2	136.7	13.9
Mean	0.20	0.35	0.14	0.11	4.90	196	4.25	41.6	109.8	12.2
$F_{(15,112)}$	2.25	4.66	6.36	6.06	12.79	5.14	3.76	4.07	1.83	3.37
Halberd	0.19	0.33	0.14	0.10	3.36	200	4.14	38.0	83.2	11.5
(WI*MMC)	0.21	0.38	0.14	0.11	7.47	218	4.37	39.3	123.9	12.8
Shoots										
Minimum	0.15	1.94	0.21	0.14	51.4	1050	5.35	56.4	752	13.8
Maximum	0.20	2.33	0.25	0.18	108.5	1350	6.60	79.7	1437	16.8
Mean	0.17	2.15	0.23	0.16	71.5	1250	5.97	66.2	981	15.4
$F_{(15,112)}$	2.05	0.70	1.76	3.00	5.40	1.50	2.37	2.79	2.43	0.77
Halberd	0.18	2.32	0.24	0.15	53.8	1250	6.13	71.2	972	15.2
(WI*MMC	0.19	2.08	0.23	0.16	103.7	1300	5.98	61.6	1275	16.0
Rudall 1988										
Grain										
Minimum	0.26	0.30	0.15	0.12	2.49	195	4.41	24.3	47.7	7.3
Maximum	0.32	0.36	0.18	0.14	6.16	236	5.10	34.2	84.7	9.3
Mean	0.30	0.33	0.16	0.13	3.65	213	4.73	28.5	63.0	8.2
$F_{(15,112)}$	1.91	1.02	2.65	2.80	13.00	1.25	1.81	1.95	3.47	1.67
Halberd	0.28	0.32	0.16	0.12	2.96	219	4.56	26.8	52.6	8.3
(WI*MMC)	0.30	0.35	0.16	0.13	4.52	228	4.89	26.3	57.2	8.5

LSD (0.05) $F_{(34,245)}$ 1.45, $F_{(15,112)}$ 1.76

F_2 plants. The proportion of families with low standard deviations, and therefore the proportion of homozygous F_2 plants, would reflect the genetic control and the number of homozygous plants would be greatest when the uptake of an element is under the control of a single gene. The mean and standard deviation for the concentrations of all elements for each family and all sets of digests are given in Appendix E.

The means of the concentrations of elements in shoots and grain for each family were compared between sites and seasons (Table 8.5). The concentration of each element in the grain, except Na and Zn, was significantly correlated between seasons at Two Wells, however the grain concentrations of only B, Cu, Mg and S were significantly correlated between Two Wells and Rudall in 1988. B was the only element for which the concentrations in shoots and grain were significantly correlated in all instances. The concentration of Na in shoots at Two Wells, 1988 was significantly correlated with that in grain at Two Wells, 1987 and 1988 but was not significantly correlated with the concentration of Na in grain at Rudall, 1988.

Boron was unique among the elements in that the concentrations of B in shoots and grain were highly correlated. The absence of consistent significant correlations for all other elements may arise from differences in partitioning between shoots and grain, yield related dilution effects or differences in the distribution of the root system and associated changes in nutritional status between vegetative growth and post anthesis development.

The correlations between the uptake of B and other elements were compared for both shoots and grain (Table 8.6). All correlation coefficients between the concentrations of B and other elements in the shoots, at Two Wells, 1988, were non-significant. The genetic control of uptake of B and other elements, with respect to shoots, is therefore independent. When comparing between the concentration of B and other elements in grain, a significant correlation resulted for K at Two Wells, 1987 and 1988, but not at Rudall, while a significant correlation with Zn resulted at Two Wells in 1988 but not for Two Wells, 1987 or Rudall, 1988. The inconsistent results for grain suggest that the significant correlations between B and K and Zn are either a secondary effect, or if they are the result of a direct interaction with uptake of B, the interaction is subject to environmental influences. The non-significant correlations for shoots and inconsistent

Table 8.5 Correlation coefficients (r) for comparisons of the concentration of elements in grain, over sites and seasons, and the concentration in grain and shoots for the families of (Halberd*(W1*MMC)) grown at Two Wells in 1987 and 1988 and at Rudall in 1988. Comparisons are over 16 families.

Variable 1	Variable 2	Element									
		B	Ca	Cu	K	Mg	Mn	Na	P	S	Zn
Grain TW'87	Grain TW'88	0.96***	0.72**	0.63**	0.80***	0.76***	0.83***	0.34	0.62**	0.77***	0.39
Grain TW'87	Grain Rud'88	0.88***	0.66**	0.71**	0.42	0.63**	0.50*	0.34	0.44	0.76***	0.44
Grain TW'88	Grain Rud'88	0.90***	0.47	0.55*	0.33	0.67**	0.39	0.32	0.31	0.62**	-0.19
Shoot TW'88	Grain TW'87	0.83***	0.37	-0.05	-0.26	-0.08	0.30	0.54*	-0.02	0.23	-0.20
Shoot TW'88	Grain TW'88	0.87***	0.47	0.14	-0.19	-0.13	0.49	0.55*	0.23	0.34	-0.12
Shoot TW'88	Grain Rud'88	0.67**	0.33	0.07	-0.31	0.15	-0.31	0.18	0.46	0.39	-0.16

LSD (0.05), r=0.50; (0.01), r=0.62; (0.001), r=0.74

*,** and *** significant at P<0.05, 0.01 and 0.001, respectively

Table 8.6 Correlation coefficients for comparisons between the concentration of B and other elements in grain and shoots for the families of (Halberd*(Wl*MMC)) grown at Two Wells in 1987 and 1988 and at Rudall in 1988. Comparisons are between the means of 35 families for the Two Wells 1987 data and 16 families for other comparisons.

B v Element	TW 88 Shoots n=16	TW 87 Grain n=35	TW 88 Grain n=16	Rudall 88 Grain n=16
B v Ca	-0.18	-0.05	0.09	-0.48
Cu	-0.22	0.01	0.25	0.29
K	0.01	0.43**	0.70**	0.07
Mg	-0.26	0.19	-0.02	0.03
Mn	-0.37	-0.25	-0.28	0.41
Na	-0.11	0.23	-0.25	0.11
P	0.10	0.22	0.47	-0.01
S	0.07	-0.05	0.09	-0.21
Zn	0.34	0.02	0.71**	-0.22

LSD n=16 (0.05) r=0.50; (0.01) r=0.62
n=35 (0.05) r=0.33; (0.01) r=0.43
** significant at P<0.01

correlations for grain indicate that selection of genotypes of low B accumulation is unlikely to affect the nutritional status with respect to the other elements analysed.

The variation in B concentration in grain, between sites, was demonstrated in Table 8.3. The concentrations of other elements are also subject to environmental variation, as shown by the comparison of elemental concentrations in grain for the Halberd, Warigal and (W1*MMC) standards included in the F_5 and F_6 experiments (Table 8.7). For the analysis of the standards in the 1989 F_6 experiments, a systematic error in the calibration of the ICP-spectrometer occurred for a number of elements, especially for Mn, K, Ca, Mg and P. The drift in concentration for the analytical standard was in excess of 15% for these elements but less than 5% for several elements, including B. The order of samples was such that sites were randomly arranged but the genotypes were grouped. The error between sites should therefore have been random and so between site comparisons are valid, however the error between the genotypes was systematic. Therefore, the results for the genotypes have been pooled and expressed as the site mean for the F_6 experiments.

8.3.2 Yield

Significant differences among families for grain yield resulted at Two Wells and Rudall in both seasons and also for the F_6 experiment at Minnipa in 1989. There was no significant difference in yield, among families, for the F_6 experiments at Walpeup, Windsor or Waite. The standards provide a measure of environmental variation within each site (Table 8.8). The 1988 Two Wells site and the 1989 Windsor and Waite sites were the most variable and in each case the variability could be related to a soil borne pest or pathogen. Cereal cyst nematode (*Heterodera avenae*) occurred at Two Wells in 1988 and at Windsor in 1989 and a severe outbreak of Take-all (*Gaumannomyces graminis* var. *tritici*) at the Waite resulted in the yield for the Halberd standards ranging from 259 to 1347 g $plot^{-1}$. The lack of significant differences in yield, among families, at Windsor and the Waite in 1989 could therefore be attributed principally to large within family variation arising from the uneven distribution of the pest or pathogen. Within family variation in yield would also have resulted from heterozygosity of F_2 plants for traits of

Table 8.7 Concentration of elements, other than B, in grain of the Halberd, Warigal and (Wl*MMC) standards included in the F_5 and F_6 experiments for the (Halberd*(Wl*MMC)) lines. 1988 data for Halberd and (Wl*MMC) are the means of 20 plots and Warigal the mean of 10 plots and 1989 data are the means of three genotypes and five plots of each genotype.

Site	Genotype	Element								
		P	K	S	Mg	Ca	Cu	Mn	Na	Zn
		(%)				(mg kg^{-1})				
1988										
Two Wells	Halberd	0.19	0.33	0.14	0.10	200	4.14	38.0	83	11.5
	Warigal	0.21	0.35	0.15	0.11	211	3.99	34.7	139	14.1
	(Wl*MMC)	0.21	0.38	0.14	0.11	218	4.37	39.3	124	12.8
	Site mean	**0.20**	**0.35**	**0.14**	**0.11**	**210**	**4.17**	**37.3**	**115**	**12.8**
Rudall	Halberd	0.28	0.32	0.16	0.12	219	4.56	26.8	53	8.3
	Warigal	0.35	0.36	0.18	0.13	242	4.53	25.9	49	12.1
	(Wl*MMC)	0.30	0.35	0.16	0.13	228	4.89	26.3	57	8.5
	Site mean	**0.31**	**0.34**	**0.17**	**0.13**	**230**	**4.66**	**26.3**	**53**	**9.6**
Genotype mean	Halberd	0.24	0.33	0.15	0.11	210	4.35	32.4	68	9.9
	Warigal	0.28	0.36	0.17	0.12	227	4.26	30.3	94	13.1
	(Wl*MMC)	0.26	0.37	0.15	0.12	223	4.63	32.8	91	10.7
LSD (0.05)	**Genotype**	**0.01**	**0.01**	**0.01**	**0.01**	**12**	**0.15**	**1.7**	**9**	**0.7**
	Site	**0.01**	**0.01**	**0.01**	**0.01**	**10**	**0.13**	**1.4**	**8**	**0.6**
	Interaction	**0.02**	**0.02**	**0.01**	**0.01**	**17**	**0.22**	**2.5**	**13**	**1.1**
1989										
Two Wells	Site mean	0.30	0.47	0.14	0.11	193	4.85	43.4	63.0	16.5
Rudall	Site mean	0.27	0.41	0.11	0.11	221	3.41	17.6	79.8	9.7
Minnipa	Site mean	0.24	0.41	0.14	0.11	255	4.13	32.3	48.4	9.5
Windsor	Site mean	0.26	0.44	0.11	0.10	261	4.09	26.8	41.0	11.4
Walpeup	Site mean	0.25	0.46	0.14	0.10	202	3.99	32.0	20.5	11.9
Waite	Site mean	0.24	0.45	0.12	0.08	335	3.93	32.9	22.9	15.4
LSD (0.05)	**Site mean**	**0.02**	**0.03**	**0.01**	**0.01**	**18**	**0.30**	**2.3**	**12.3**	**1.1**

Table 8.8 Yield (g plot^{-1}) and coefficient of variation (c.v.) for the Halberd, Warigal and (Wl*MMC) standards included in the 1988 F_5 and 1989 F_6 experiments for the (Halberd*(Wl*MMC)) lines. In 1988 there were 75 plots each of Halberd and (Wl*MMC) and 20 plots of Warigal, while in 1989 there were 45 plots each of Halberd and (Wl*MMC) and 24 plots of Warigal

Site	Halberd		Warigal		(Wl*MMC)	
	Yield	c.v.	Yield	c.v.	Yield	c.v.
1988						
Two Wells	352	27	296	25	282	25
Rudall	368	12	219	19	244	18
1989						
Two Wells	1337	14	1307	13	1267	10
Rudall	881	12	777	15	970	12
Minnipa	562	12	499	12	524	10
Windsor	439	29	439	22	430	26
Walpeup	416	18	381	17	363	20
Waite	890	31	682	39	862	37

selective importance at individual sites. Such within family variation would have occurred at the high B sites, in particular, because there was a wide range of tolerance to B within the heterozygous families, but within family variation may have also occurred at the low B sites in response to other unidentified characters.

The mean yields of individual families were compared between sites and seasons by correlation coefficient (Table 8.9). Mean yields of families at Two Wells were significantly correlated, between seasons, as was the situation at Rudall. When comparing between sites, yields at Minnipa were significantly correlated with yields at Two Wells in both 1988 and 1989, but most other comparisons were inconsistent between sites and seasons. For example, yields at Walpeup in 1989 were significantly correlated with yields at Rudall in 1988, but not in 1989, and were significantly correlated with yields at Two Wells in 1989 but not in 1988. The yields at Windsor in 1989 were not significantly correlated with yields at any other site and there is the possibility that variation in tolerance to CCN was reflected in the yield.

Correlation between yield and response to B

As the concentration of B in the grain was significantly correlated between sites and seasons and also correlated with the concentation of B in shoots, the full set of analyses for the F4 experiment were used to provide a genetic measure of B uptake for each family for the purpose of comparing between B uptake and grain yield. A significant correlation between mean family B concentration and grain yield resulted at Two Wells for both seasons and at Minnipa for the F_6 experiment, but not at Rudall in either season or at Walpeup, Windsor or Waite in 1989 (Fig. 8.4). Lines of low B accumulation were at a selective advantage at Two Wells and Minnipa.

The concentrations of B in the grain of the standards were similar at Minnipa and Rudall and this suggests a similar degree of toxicity at the two sites and this is supported by the comparisons for concentration of extractable B in the soil (Table 3.1). As tolerance to high concentrations of B was not associated with high grain yields at Rudall other overriding selective pressures must have been in operation and these are discussed further in the following section. B concentrations in the grain of the standards (Table 8.3) were

Table 8.9 Correlation coefficients (r) for comparisons of mean family grain yield between sites and seasons.

Site 1	Site 2						
	Rudall 1988	TW 1989	Rudall 1989	Minnipa 1989	Walpeup 1989	Windsor 1989	Waite 1989
1988							
Two Wells	0.14	0.61**	0.24	0.39*	0.09	0.08	0.08
Rudall		0.30	0.44**	0.19	0.40**	0.05	0.13
1989							
Two Wells			0.48**	0.60**	0.35*	0.03	0.36*
Rudall				0.42*	0.24	0.09	0.48**
Minnipa					0.44**	0.10	0.05
Walpeup						0.14	0.02
Windsor							-0.22

LSD (0.05) r = 0.33; (0.01) r = 0.43

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

Figure 8.4 Relationship between concentration of B in grain at Two Wells in 1987 and grain yield in 1988 and 1989 for the families of (Halberd*(Wl*MMC)).

(a) Grain B, Two Wells 1987 v yield at Two Wells 1988.

(b) Grain B, Two Wells 1987 v yield at Two Wells 1989.

(c) Grain B, Two Wells 1987 v yield at Rudall 1988.

(d) Grain B, Two Wells 1987 v yield at Rudall 1989

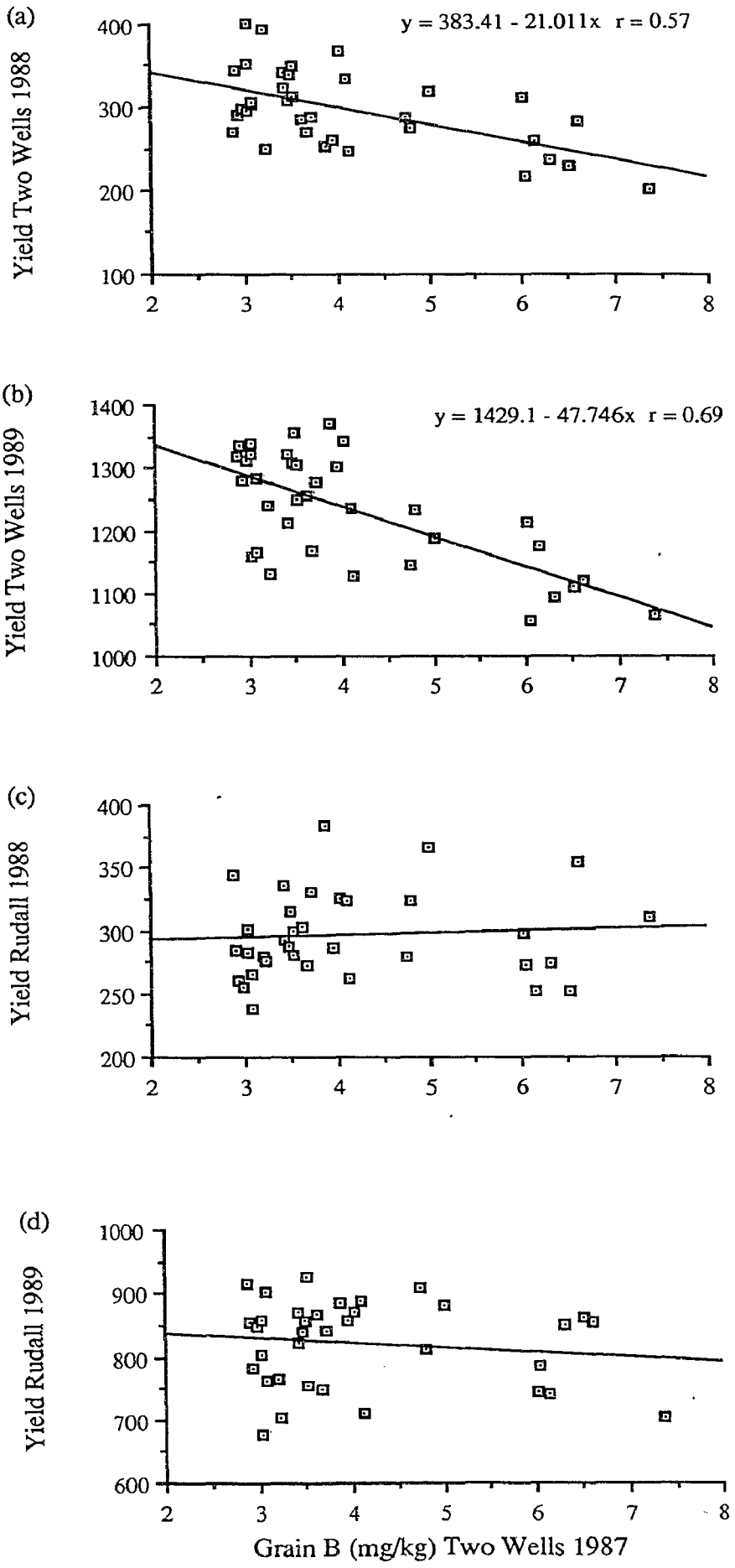

(a)
y = 383.41 - 21.011x r = 0.57
Yield Two Wells 1988
400
300
200
100
2
3
4
5
6
7
8
(b)
y = 1429.1 - 47.746x r = 0.69
Yield Two Wells 1989
1400
1300
1200
1100
1000
2
3
4
5
6
7
8
(c)
Yield Rudall 1988
400
350
300
250
200
2
3
4
5
6
7
8
(d)
Yield Rudall 1989
1000
900
800
700
600
2
3
4
5
6
7
8
Grain B (mg/kg) Two Wells 1987

Figure 8.4 contd.

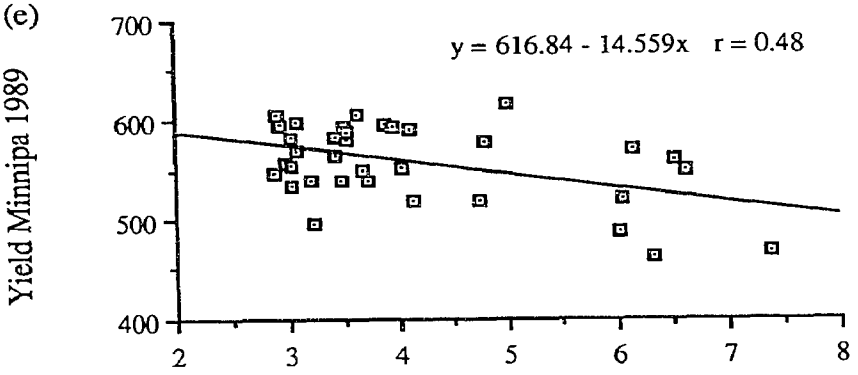
(e) Grain B, Two Wells 1987 v yield at Minnipa 1989

(f) Grain B, Two Wells 1987 v yield at Walpeup 1989

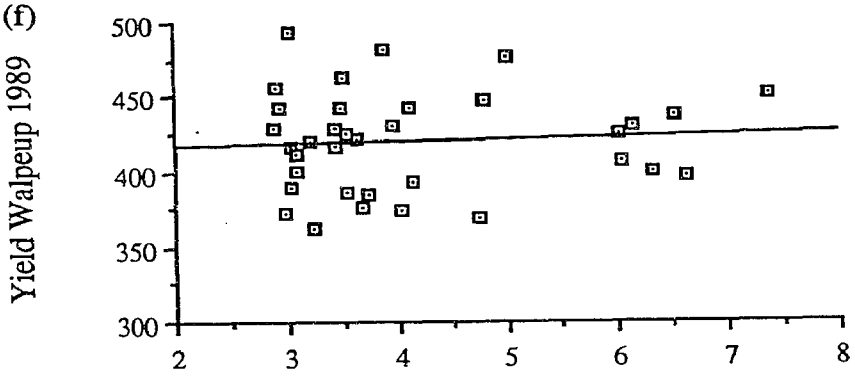
(g) Grain B, Two Wells 1987 v yield at Windsor 1989

(h) Grain B, Two Wells 1987 v yield at Waite 1989

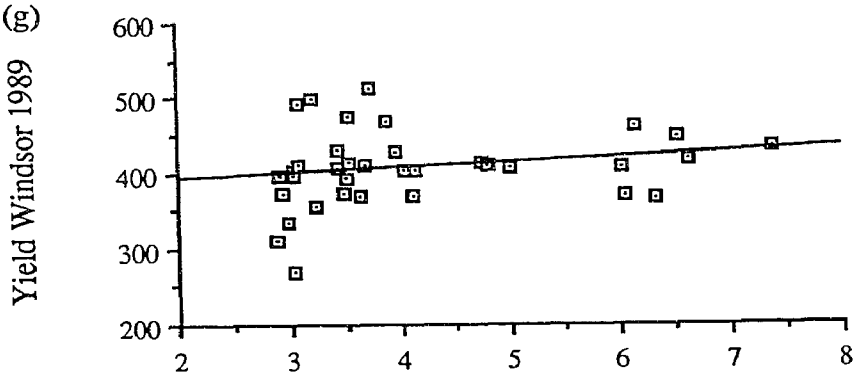
(e)


y = 616.84 - 14.559x r = 0.48
Yield Minnipa 1989
700
600
500
400
2
3
4
5
6
7
8

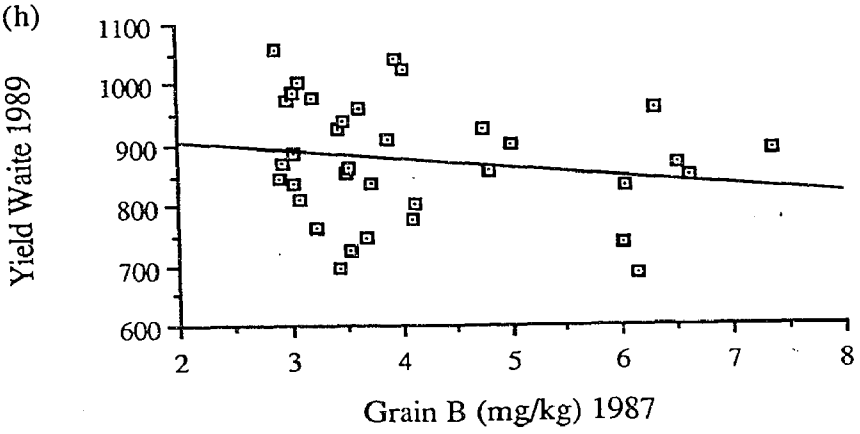
(f)


Yield Walpeup 1989
500
450
400
350
300
2
3
4
5
6
7
8

(g)


Yield Windsor 1989
600
500
400
300
200
2
3
4
5
6
7
8

(h)


Yield Waite 1989
1100
1000
900
800
700
600
2
3
4
5
6
7
8
Grain B (mg/kg) 1987

lowest for Waite, Windsor and Walpeup and so the absence of significant correlations between B tolerance and yield is not unexpected.

Correlation between yield and other elements

The correlation coefficients between grain yields at all sites in 1988 and 1989 and the mean family concentration of elements in shoots of F5 plots grown at Two Wells in 1988 (Table 8.10) and in grain of F4 plots grown at Two Wells in 1987 (Table 8.11), were determined. Significant negative correlations between the B concentration of shoots at Two Wells, 1988 and grain yield at Two Wells, 1988 and 1989 and Minnipa, 1989 are consistent with the previous results for grain B (Fig 8.4). A significant positive correlation between grain yield and concentration of Mn in shoots resulted at Two Wells, 1989 and Minnipa, 1989, while a significant negative correlation resulted between concentrations of both Na and K in shoots at Two Wells, 1988 and grain yield at Rudall, 1989. Further, although the correlations for Mn at Two Wells, 1988 and K and Na at Rudall, 1988 were not statistically significant, they were consistent with the significant results.

Significant negative correlations between the concentration of elements in the grain at Two Wells in 1987 and grain yield in 1988 and 1989 resulted for many elements at one or more sites (Table 8.11). A negative correlation would arise through either toxicity, as is the case for B, or yield related dilution of an element in limited supply. In view of the generally nutritionally deficient soils of South Australia the latter would be the more probable. The significant positive correlation between concentration of Cu in grain at Two Wells, 1987 and grain yield at Windsor in 1989 may be the result of a limiting supply of Cu at Windsor and sufficient genetic variation within the population for the more efficient genotypes to be at a yield advantage. On the other hand, as the concentration of Cu in grain at Windsor was greater than at Rudall and similar to Minnipa, Walpeup and Waite (Table 8.7) it would seem unlikely that Cu was a yield limiting factor at Windsor but not the other sites.

Table 8.10 Correlation coefficients (r) for comparisons between elemental concentration in shoots of F_5 lines of (Halberd*(WI*MMC)) grown at Two Wells in 1988 and grain yield at Two Wells and Rudall in 1988 and at six sites in 1989.

Site	Element									
	B	Ca	Cu	K	Mg	Mn	Na	P	S	Zn
1988										
Two Wells	-0.50*	-0.12	0.46	0.05	0.09	0.46	-0.44	0.28	0.23	-0.46
Rudall	0.06	-0.45	-0.05	-0.28	-0.39	-0.01	-0.23	-0.21	-0.27	-0.03
1989										
Two Wells	-0.69**	0.04	0.40	-0.31	0.12	0.60*	-0.25	0.18	0.14	-0.38
Rudall	0.00	-0.10	0.05	-0.52*	-0.33	-0.01	-0.51*	-0.12	-0.12	-0.18
Minnipa	-0.54*	0.27	0.46	-0.16	0.21	0.61*	-0.10	0.27	0.27	-0.40
Walpeup	-0.12	-0.24	0.31	-0.05	-0.12	0.25	-0.05	0.04	0.00	-0.31
Windsor	0.32	0.07	0.19	0.31	-0.19	0.07	-0.13	0.02	0.20	-0.03
Waite	-0.12	-0.05	-0.23	-0.43	-0.36	-0.11	-0.40	-0.25	-0.33	0.04

LSD (0.05) r=0.50; (0.01) r=0.62

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

Table 8.11 Correlation coefficients (r) for comparisons between elemental concentration in grain of F4 lines of (Halberd*(Wl*MMC)) grown at Two Wells in 1987 and grain yield at Two Wells and Rudall in 1988 and at six sites in 1989.

Site	Element									
	B	Ca	Cu	K	Mg	Mn	Na	P	S	Zn
1988										
Two Wells	-0.58***	-0.20	0.11	-0.12	-0.31	0.09	-0.16	-0.12	0.03	0.01
Rudall	-0.09	-0.16	-0.03	0.11	-0.33*	0.05	0.11	-0.26	-0.20	-0.30
1989										
Two Wells	-0.68***	-0.19	-0.06	-0.12	-0.49**	0.01	-0.16	-0.33	-0.17	-0.24
Rudall	-0.13	-0.17	-0.18	0.22	-0.46**	-0.32	-0.02	-0.44**	-0.36*	-0.41*
Minnipa	-0.48**	-0.09	0.07	-0.13	-0.24	-0.06	-0.16	-0.13	-0.03	0.02
Walpeup	0.05	-0.33*	0.12	0.07	-0.25	-0.01	-0.11	-0.13	-0.19	-0.01
Windsor	0.17	0.10	0.42*	0.08	0.10	0.31	-0.04	0.03	0.06	0.03
Waite	-0.19	-0.16	-0.28	0.28	-0.37*	-0.23	0.25	-0.35*	-0.32	-0.46**

LSD (0.05) r=0.33; (0.01) r=0.43; (0.001) r=0.53

*, ** and *** significant at P<0.05, 0.01 and 0.001, respectively

Effect of time of maturity upon response to boron

The stage of development of all F_5 plots was determined at Two Wells on 23rd September, 1988 (92 days after sowing), at which time individual lines varied between the growth stages Zadoks 39 (flag leaf ligule just visible) and Zadoks 53 (1/4 of inflorescence emerged) and mean family scores ranged from Zadoks 43 to Zadoks 50. These scores were used to determine the effect of time of maturity upon the accumulation of B and grain yield. There were no significant correlations between stage of development and mean family B concentration for either shoots and grain at Two Wells or grain at Rudall in 1988 or for grain at Two Wells in 1987 (Table 8.12). Maturity was significantly correlated with grain yield at Rudall in 1988, but not 1989, and early maturing families were at a relative advantage but this response was not repeated in 1989. The yield advantage of the earlier maturing families in 1988 is apparent in Figure 8.5. To estimate the yield effect due to genetic variation in maturity, the families were grouped into three arbitrary categories, namely Zadoks 43 and 44 (n=12), Zadoks 45 and 46 (n=14) and Zadoks 47-50 (n=9). The mean yields of the three categories were 272, 301 and 319 g $plot^{-1}$, respectively and therefore the yield advantage of the earliest maturing families compared with the latest group was 17%. There was no significant correlation between maturity at Two Wells, 1988 and yield at Two Wells in either season, or at Minnipa, Walpeup, Windsor and Waite in 1989 (Table 8.11). The contrasting response at Rudall could be attributed to differences in rainfall between 1988 and 1989, with 1988 being a severe drought (Appendix A) and earlier sowing of the 1989 experiment.

Table 8.12 Correlation coefficients (r) between stage of development of F5 plots at Two Wells and B accumulation and grain yield at Two Wells and other sites.

Variable 1		Variable 2	Comparisons	Correlation (r)
Maturity	V	Grain B at :		
		Two Wells 1987	35	-0.07
		Two Wells 1988	16	0.31
		Rudall 1988	16	0.29
	V	Shoot B at :		
		Two Wells 1988	16	0.06
	V	Grain yield at :		
		Two Wells 1988	35	0.10
		Two Wells 1989	35	0.17
		Rudall 1988	35	0.47**
		Rudall 1989	35	0.12
		Minnipa 1989	35	0.01
		Walpeup	35	0.19
		Windsor	35	0.01
		Waite	35	0.21

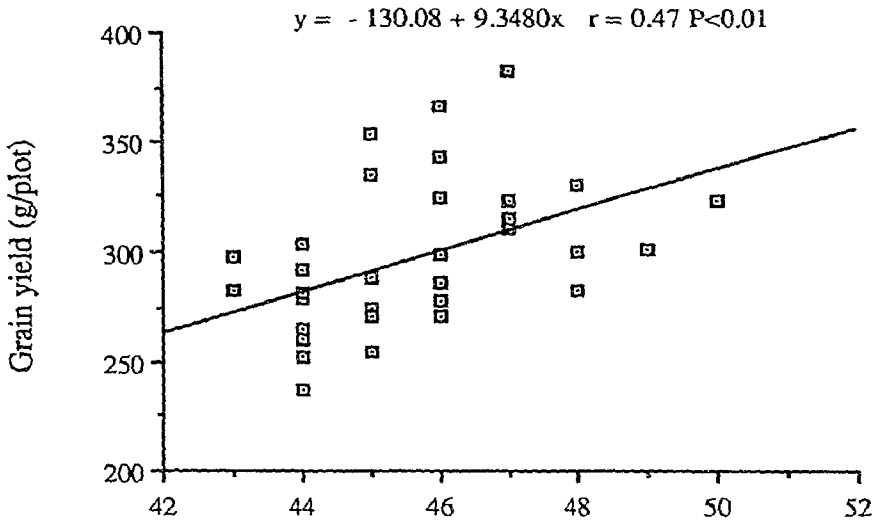
LSD n=16, (0.05) r = 0.50,
n = 35, (0.05) r = 0.33; (0.01) r = 0.43
** significant at P<0.01

Figure 8.5 Relationship between stage of maturity at Two Wells on September 23, 1988 and grain yield for the (Halberd*(Wl*MMC)) families.

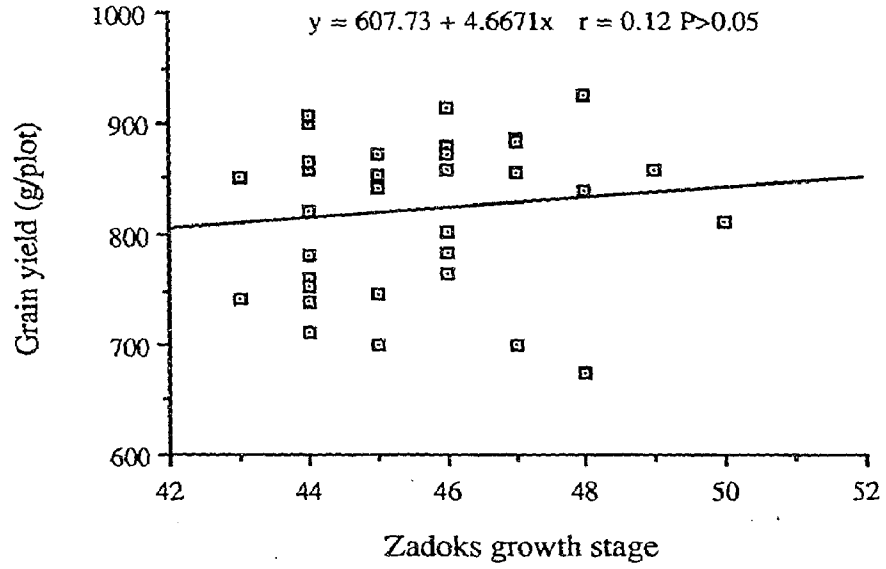
(a) Yield at Rudall 1988.

(b) Yield at Rudall 1989.

(a) Rudall 1988



(b) Rudall 1989



8.4 DISCUSSION

This series of experiments has utilized a population developed from two genotypes of contrasting tolerance to high concentrations of B to investigate the relationship between uptake of B and other elements and the grain yield of lines of contrasting response to B at a number of sites of different B status. Segregation prior to the development of the lines allowed recombination and reassortment of genetically independent characters. Characters which were found to co-vary with B uptake would therefore be those which either (1) are genetically linked or interact pleiotropically with genes controlling uptake of B or (2) are environmental variables which interact with the response to B.

The concentration of B in shoots and grain differed significantly among families and covered the range of the two parents. Significant differences also resulted for the concentration of six other elements in shoots and for most elements in grain. The genetic variation for the other elements allowed comparisons to be made between the uptake of B and the other elements and correlation coefficients were determined for concentrations in both shoots and grain. The families which were sampled for tissue analysis, in 1988, were determined on the basis of concentrations of B in the grain in 1987. Families with the highest and lowest B concentrations were selected, as well as a number of randomly chosen families. This means of selection was adopted for two reasons, firstly to reduce the total number of plant samples to be analysed and secondly to maximise the proportion of extremes for B response and so increase the probability of identifying significant relationships between uptake of B and other variables.

The mean family concentration of B in shoots at boot stage was not significantly correlated with the concentration for any other element in shoots, while correlations between B and other elements in grain were inconsistent (Table 8.6). This indicates that the uptake of B during vegetative growth and accumulation in the grain is genetically and physiologically independent of other elements measured in these trials. This result is consistent with previous reports on the uptake of B. For example, Hill and Jung (1975) reported no genotypic correlations between the concentration of B and 10 other elements

in the shoots of a diallel series for alfalfa, while Loneragan (1975) observed that, with the possible exception of Cu and Mn, micronutrients enter the plant from solution via independent and specific mechansims. Selection of wheat genotypes of low B accumulation should therefore have no effect upon the efficiency of uptake of other nutrients.

The above comments should be qualified and limited to the two genotypes used as parents of the population. In Chapter 6 it was demonstrated that response to B is under the control of at least four independent loci, while Halberd and (W1*MMC) differ at only two loci. It is therefore possible that other loci controlling response to B may be linked to genes controlling uptake of nutrients or response to environmental factors. As the parents were also chosen specifically on the basis of contrasting response to B, the number of significant differences, between families, for concentrations of other elements was fortuitous rather than planned. It is probable that a greater range in efficiency of nutrient uptake exists for all elements, than is found in this population, therefore a more objective means of testing for genetic interactions between uptake of B and other individual elements would be to select parents on the basis of contrasting response to B and uptake of at least one other element. This method should produce a greater range for the test nutrient among the progeny and therefore increase the probability of identifying interactions. This method may require the development of a separate population for each element and even then generalizations could not be made on the basis of the absence of interactions but conclusions would be restricted to the particular combination of parents.

Families of low B accumulation were found to have a yield advantage when grown at Two Wells in 1988 and 1989 and at Minnipa in 1989. The highest concentration of B in grain of the parental standards resulted at Two Wells while concentrations at Minnipa were higher than at other sites, except Rudall, in 1989 (Table 8.3). The results at Two Wells and Minnipa are therefore consistent with toxicity due to excessive amounts of B in the soil and sufficient genetic variation for tolerance to B within the population for the more tolerant families to have a yield advantage when grown under high B conditions.

The contrasting response between Rudall and Minnipa may be related to selective pressures other than B toxicity having an overriding effect at Rudall. For example, in

1988 there was a statistically significant correlation between time of maturity and grain yield at Rudall which indicates seasonal factors favoured early maturing genotypes. Other possible yield determining factors are discussed later.

In order to estimate the yield advantage of the B tolerant families in the Two Wells and Minnipa experiments, the families were divided into three categories based on the mean family B concentration of the F4 experiment conducted at Two Wells in 1987. These categories were <3.5, 3.5-5.0 and >5.0 mgB kg^{-1} grain and the lowest and highest categories were approximately the range for the Halberd and (W1*MMC) standards, respectively. At Two Wells, 1988 the mean yields of the three classes were 319, 297 and 256 g $plot^{-1}$, respectively, therefore the B tolerant families yielded approximately 25% more than the sensitive families. Similarly, at Two Wells, 1989, the mean yields were 1266, 1258 and 1127 g $plot^{-1}$, respectively and the yield advantage of the tolerant families was 12% and at Minnipa, 1989, the mean yields were 561, 569 and 528 g $plot^{-1}$, respectively and the yield advantage of the tolerant families was 6%.

It is significant that a large effect due to B occurred in 1989, a year of extremely high yields. In the early stages of the project the consensus among the group investigating B toxicity was that toxicity would probably be more severe in seasons of low rainfall as this would encourage greater root exploration of the high B subsoils. It was also expected that late sowing would accentuate the toxic effect of B as plants would rely to a greater extent upon stored subsoil moisture rather than current rainfall. This hypothesis was the major reason for the extremely late sowing of the experiments conducted in 1985 and 1986 reported in Chapter 4. A significant interaction between genotypes and sowing dates resulted at Two Wells in 1987 and the yield of the most sensitive genotype, (W1*MMC), was reduced to the greatest extent by the late sowing (Chapter 4). The yield results obtained at Two Wells in 1989 clearly demonstrates that very significant yield differences due to B toxicity may occur among genotypes of contrasting tolerance to B, when sown at the optimum time (during May for most of South Australia) and grown under favourable conditions. The results suggest that the reduction in absolute yield, and hence economic return, may be greater during the more favourable seasons.

The results for B were consistent with the expectation of families with low B uptake having a yield advantage when grown under high B conditions. The families with lowest B concentrations in shoots and grain produced the highest yields at Two Wells in 1988 and 1989 and at Minnipa in 1989. The concentrations of B in the grain of the standards at these two sites were greater than at all other sites other than Rudall. The results of the other elements will now be discussed in a more speculative manner in an attempt to identify the reasons for the anomalous response at Rudall, compared with Minnipa, and to identify nutrients which may be influencing yield at other sites.

The results for the grain analysis will be used as a means of identifying differences in nutritional status between sites and so identify possible yield limiting factors. Grain is rarely used for determining critical concentrations of elements within plants and for certain elements grain has been reported as unsuitable, e.g. Cu (Robson *et al.*, 1984) and Mn (Graham *et al.*,1985), although their concentrations in grain were reported to increase in response to the application of Cu and Mn, respectively. The concentrations of elements in the grain of wheat grown in the field in Australia has been found to be subject to naturally occurring environmental variation, e.g. 12 elements including nine common to the current study (Schultz and French, 1976), seven elements including S, Mn, Cu and Zn (White *et al.*, 1981), Cu (King and Alston, 1975) and Mn (Marcer and Graham, 1986). Analysis of grain may therefore be useful for identifying potential nutritional disorders on a regional scale (Reuter and Hannam, 1987) and this was the rationale behind a survey of the concentration of B (Cartwright and Hirsch, 1986) and other elements in the grain of South Australian barley crops in 1983 (Cartwright, unpublished). A large range in the concentration of all elements occurred and regional differences could be identified.

As there is very little information on critical or optimum concentrations of elements in wheat grain, the results of the 1988 and 1989 experiments are compared with the concentrations published by Schultz and French (1976) for the concentration of 12 elements in 13 plant tissues, including grain, for Halberd wheat grown in 42 experiments at 13 sites in South Australia in a five year period. Although this method will not positively identify nutritional disorders it will provide a measure of the nutritional status

of each site relative to other South Australian sites. The Minnipa Research Station was a site in common between Schultz and French (1976) and the current project.

The concentrations of P and K at all sites were similar to the mean values reported by Schultz and French (1976), the concentrations of Mg, Ca, Cu and Zn at all sites investigated here were below the mean reported values (0.15% and 400, 6 and 18 mg kg^{-1}, respectively), the concentration of Na at all sites was below the minimum reported value (200 mg kg^{-1}) and the concentration of Mn at Rudall in 1989 was below the minimum reported value (19 mg kg^{-1}). The generally lower concentrations of elements for the current study may reflect either differences in analytical procedures, different soil types between the sites for the two studies or changes in fertilizer practices between the two periods. The recent increase in use of high analysis P fertilizer as a replacement for superphosphate, which contains considerable amounts of Zn, may account for the decrease in Zn values between the two studies.

The concentration of Zn in shoots at Two Wells, 1988 was approximately 15 mg kg^{-1} which is marginal for wheat plants in South Australia (N. Wilhelm, pers. comm.). As the Zn concentration of grain at Rudall, 1988 and four sites in 1989 was below the concentration at Two Wells, 1988 (12.0-12.5 mg kg^{-1}) it is probable that Zn deficiency was a common yield limiting factor, and in particular at Rudall and Minnipa where the concentrations of Zn in grain were less than 10 mg kg^{-1}. Symptoms of Zn deficiency on leaves were observed at Rudall in both seasons and at Minnipa in 1989 (A.J. Rathjen, pers. comm.).

The Na concentrations of grain were of the order of one tenth those reported by Schultz and French (1976) (range 200 - 800, mean 300 mg kg^{-1}). As Minnipa was a site in common, this difference is greater than would be expected, even allowing for differences in analytical procedures and approximately only 50% of the Na in grain being extracted by nitric acid digestion (Zarcinas *et al.*, 1987). Despite the difference in absolute values, which would suggest that Na was not present in the soil in high concentrations, there was a statistically significant correlation between the mean family Na concentration of shoots at Two Wells, 1988 and grain yield at Rudall, 1989 and families of low Na accumulation produced the highest yields (Table 8.10). The concentration of Na in grain

in 1989 was highest at Rudall and this is consistent with a significant correlation between genetic differences in Na uptake and grain yield occurring at Rudall and suggests that Na is present in the soil in toxic amounts. Further soil data and information on the relationship between Na in soil and grain is required to substantiate this hypothesis. In 1988, the concentration of Na in grain at Two Wells was high and while not statistically significant the correlation between Na concentration of shoots and grain yield was negative and consistent with a toxic effect of Na.

High concentrations of B in South Australian soils are generally associated with soil sodicity, or a high exchangeable sodium percentage (ESP) (Cartwright *et al*, 1984, 1987) and ESP values in excess of 30% have been measured in the subsoil at Two Wells while the ESP at the Waite is less than 2% (Cartwright *et al.*, 1987). The ESP of the soil at Rudall was not determined during this project, however the map of saline and sodic soils of Australia (Northcote and Skene, 1972) describes the soil in the region of Rudall as AS3, or alkaline and strongly sodic (ESP>15). The results obtained at Rudall, and to a lesser extent at Two Wells, indicate that either Na directly or sodicity as a soil property limit the yield of wheat and that there is sufficient genetic variation in response to Na^+ ions and/or sodicity within the population derived from Halberd and (Wl*MMC) to produce yield responses when grown in sodic soils. The Na concentration of Halberd was considerably lower than for (Wl*MMC) for shoots at Two Wells, 1988 and grain at Two Wells, 1987 and 1988 (Table 8.4). This suggests that Halberd is the source of lower accumulation of Na, although there was no difference between the two genotypes for concentration of Na in grain at Rudall in 1988. Lower accumulation of Na may be another factor which has lead to the persistance of Halberd in southern Australia and may be an additional character required of wheat varieties which are being developed specifically for high B regions. The correlations between concentrations of B and Na in shoots and grain were not statistically significant and this indicates that uptake/exclusion of the two elements is genetically and physiologically independent and therefore independent selection for the two characters will be required. As B and sodicity are often environmentally associated, joint selection may occur for lines selected by progeny methods on the basis of grain yield provided that there is sufficient genetic variation for

both characters. Further information is required on the effect of sodicity on wheat production and genetic variation in tolerance to sodicity.

The efficiency of Mn uptake appeared to be a yield determining factor at Two Wells and Minnipa in 1989 (Table 8.10), however analysis of shoots at Two Wells, 1988 (Table 8.4) and grain from both sites (Table 8.7) would not indicate Mn deficiency based on concentrations of Mn in shoots and grain of Mn adequate plants (Graham *et al.*, 1985). On the other hand, the concentrations of Mn in shoots at Two Wells, 1988 and in grain at Two Wells, 1987 were not significantly correlated with grain yield at Rudall, 1989 (Tables 8.10 and 8.11), the site where the Mn concentration of grain was lowest and below the minimum reported by Schultz and French (1976). It would therefore seem probable that the significant correlations between Mn concentration in shoots at Two Wells and grain yield at Two Wells and Minnipa are a secondary effect rather than the direct result of genetic variation in efficiency of uptake of Mn.

The Rudall site proved to be unpredictable and anomalous results were obtained for correlations between concentrations of B and Mn and grain yield. Despite similar concentrations of B in grain and soil as found at Minnipa, there was no yield effect due to B toxicity in 1988 or 1989. The 1988 result could be attributed to the effect of genetic variation in time of maturity and early maturing families produced greater yields, however there was no effect of maturity in 1989. Analysis of grain of the parental standards derived from all sites in 1989 identified Rudall as being the most nutritionally deficient and in particular for Zn and Mn. The concentration of Cu was also lowest at Rudall and within the range reported by King and Alston (1975) to be associated with sites on Eyre Peninsula where the yield of wheat responsed to the application of Cu while the concentration of S was of the order reported by Randall *et al.* (1981) to be associated with S deficiency for wheat. Genetic variation in response to Na accumulation also appeared to be a yield determining factor at Rudall. The absence of a correlation between response to B and grain yield at Rudall, despite the presence of potentially toxic concentrations of B in the soil, may have resulted from the overriding effect of all other yield limiting factors.

The results of these experiments have demonstrated B to be unique among the elements analysed in that the concentration of B is highly correlated between shoots and

grain and so either shoots or grain may be analysed to determine the relative uptake of B. The concentration of B in shoots and grain was also genetically independent of all other elements, with the possible exception of K in grain, therefore selection of genotypes of low B uptake should not affect the nutritional status with respect to other elements. The genetic variation for accumulation of B in shoots and grain was significantly correlated with grain yield at two high B sites, however genetic and environmental factors other than tolerance to B also affect yields at Rudall. Therefore, although the results are encouraging from the point of view of breeding B tolerant varieties, further study is required to understand other environmental factors and genotype x environment interactions which affect yield in high B environments.

Chapter 9.

GENERAL DISCUSSION

A large range in tolerance to high concentrations of B was recognized among the wheat varieties tested. The responses of plants to high concentrations of B included development of foliar symptoms, reduced vigour, reduction in tillering, delayed development and accumulation of B in shoots. Differences between tolerant and sensitive varieties resulted for all responses. The mechanism of tolerance was reduced uptake and accumulation of B in shoots for the tolerant varieties and this is consistent with the initial investigations of genetic variation in wheat to high concentrations of B (Paull, 1985; Paull *et al.*, 1988). The means of restricting B accumulation in shoots was not investigated, however Nable (1988) reported consistently lower concentrations of B in the roots of tolerant varieties when grown at a range of B treatments in solution culture. It would therefore appear that the tolerant varieties are able to exclude B from the roots.

Genotypes were compared for response to B in pots and under naturally occurring high B conditions in the field. For the pot experiments, the difference between tolerant and sensitive genotypes, as measured by yield response, was greater when assessed during the vegetative growth than at maturity. This could be accounted for by a delay in development due to B treatments, particularly for the sensitive genotypes, and so an extended period of growth. A reduced growth rate did not therefore necessarily result in a reduction in yield when plants were grown to maturity. For a similar reason the apparent critical concentration of applied B differed according to the stage of development at which plants were harvested and the critical treatment increased for plants grown to maturity (Chapter 4).

The difference between tolerant and sensitive genotypes for concentration of B in tissues for plants grown in pots was greater during vegetative growth than for straw at maturity, while the concentration of B in grain did not reflect the genetic differences for concentration of B in shoots. On the other hand, the ranking of genotypes for concentration of B in shoots, YEB's and grain of field grown wheat was consistent over times of sampling and sowing dates. The contrasting result for concentration of B in

grain between the pot and field conditions may be related to the artificial environment of the pot experiment, including unlimited moisture supply during grain filling, an extended period of growth for the more sensitive genotypes and depletion or redistribution of B within the soil.

The genetic differences in the concentration of B in shoots for plants grown at high B treatments in pots were significantly correlated with the concentration of B in shoots and grain for field grown wheat (Chapter 5). Thus, the differences between varieties for concentration of B in tissues when grown in the field was directly related to differences in tolerance to B rather than secondary effects or interactions. The concentrations of B in shoots and grain were consistently significantly correlated for field grown wheat (Chapters 4, 5 and 8) while the concentration in grain was also highly correlated over sites and seasons (Chapters 5 and 8). Expression of tolerance to high concentrations of B is therefore a highly heritable character and the results of chemical analyses at a single site could be extrapolated to other sites and seasons to provide an index of the degree of B uptake, or tolerance, of varieties.

For pot experiments, the concentration of applied B required to distinguish between genotypes for concentration of B in shoots varied with the level of tolerance of the varieties compared. In general, a lower B treatment was required to distinguish between a sensitive and a moderately sensitive variety (e.g. (Wl*MMC) and Warigal) than for comparing between a moderately sensitive and a tolerant variety (e.g. Warigal and Halberd) (Chapters 4 and 6) Significant differences between genotypes for the concentration of B in shoots resulted even at B treatments which did not result in a yield reduction. For example, in Chapter 6 where five genotypes were compared at seven B treatments, the concentration of B in shoots of Halberd was significantly greater than for G61450 and Warigal was significantly greater than Halberd, at the B50 treatment, but the yields of the three genotypes were not reduced relative to the control treatment. It is therefore not necessary to impose severe B treatments for assessing genetic differences in the uptake of B. In contrast to Nable (1988), the difference in B accumulation in shoots between tolerant and sensitive varieties was not consistent at the control treatments. The contrasting results may have arisen from differences in precision between experiments.

For the solution culture experiments of Nable (1988) all genotypes within a replicate were grown in the same pot and by the nature of the cultural system there was no difference in moisture supply to plants within or between replicates. Despite the contrasting response at the control treatments, at the applied B treatments the concentrations of B in shoots of genotypes described in this thesis concur with those reported by Nable (1988).

The concentration of B in tissues associated with yield responses varied between experiments and was generally higher for pot than field experiments. For the pot experiment of Chapter 4, the yields of Halberd and Warigal at the first node stage of development were not significantly reduced at the B20 treatment relative to the control and the concentrations of B in shoots were 68.1 and 84.4 mg kg^{-1}, respectively. Yields of both varieties were significantly reduced at the B60 treatment and the concentrations of B in shoots were 402.1 and 483.1 mg kg^{-1}, respectively. When five genotypes were compared at seven B treatments (Chapter 6) the yields of G61450, Halberd and Warigal at boot stage were not significantly reduced at the B50 treatment relative to the control and concentrations of B in shoots of the three genotypes were 392.3, 696.7 and 1285.0, mg kg^{-1}, respectively. Significant grain yield reductions for the current South Australian varieties grown at Two Wells in 1985 were associated with B concentrations in shoots at the boot stage in the range 40 - 120 mg kg^{-1} (Chapter 5) and a significant negative correlation between B concentation in shoots at boot stage and grain yield for the random lines of (Halberd*(Wl*MMC)) at Two Wells in 1988 resulted over the range 50 - 100 mg kg^{-1} (Chapter 8).

Variation in the critical concentrations of B in shoots of barley, due to environmental factors, has been the subject of a study by Nable *et al.* (submitted for publication). Variation in evaporative demand greatly increased the concentation of B in shoots without having a significant effect upon yield and the majority of the increased uptake of B could be accounted for by the accumulation of B in leaf tips. This is consistent with the previous reports of the distribution of B in leaves (e.g. Eaton, 1935; Oertli and Kohl, 1961). The uneven distribution of B within the plant would enable large changes in the concentration of B on a whole plant basis with little change in the concentration of B in the meristematic region. Further, it has been found that a

considerable amount of B is leached from barley plants when sprayed with water and up to 50% of B was leached from YEB's without affecting the dry matter yield (Nable *et al.*, submitted for publication). The concentration of B in YEB's of field grown wheat is also significantly reduced by rainfall (R.O. Nable, unpublished; D.B. Moody, pers. comm.)

The generally lower critical concentrations of B in shoots of field grown wheat and barley reported in the literature (see Table 2.3) and for the experiments reported in this thesis may arise from a combination of many factors including the generally higher evaporative rates for glasshouse experiments the uneven distribution of B in the plant with most accumulation occurring in physiologically inactive regions and leaching of B by rain, for field grown plants. A further factor which varies between pot and field experiments is the moisture regime. B has an inhibitory effect upon root elongation in solution (Morris, 1931; Lovatt and Bates, 1984) and the growth of roots was inhibited by a high B zone in the soil profile for a pot experiment (Paull, 1985). The naturally occurring zone of high B in the subsoil will reduce root development and so reduce exploitation of stored subsoil moisture. The effect of high concentrations of B in the subsoil upon grain yield could therefore be due primarily to the effects of a reduction in the effective moisture supply rather than a toxic effect within the shoots. In addition, the reduced soil volume available for root growth may also limit the supply of plant nutrients. Plants grown in pots are supplied with adequate moisture and nutrients to maturity and so a reduced root system due to B toxicity may not necessarily result in a reduction in shoot or grain yield.

It is improbable that a critical concentration of B in shoots for diagnosing B toxicity will be determined for field grown wheat. The concentrations of B in shoots associated with toxicity for pot experiments are not appropriate to the field situation, as outlined above. The uneven distribution of B within the soil profile with the highest concentrations occurring in the sub-soil leads to an increase in the concentration of B within the shoots through the growing season (Chapter 4). Therefore, the concentration of B in shoots will only provide a measure of the B already encountered, and this will be modified by the effect of leaching of B from leaves by precipitation, but no indication of the probability of future toxicity. As an alternative, grain may prove to be a more

appropriate tissue for analysis. The concentration of B in grain would reflect the uptake of B during post-anthesis development, the period during which moisture stress is most likely to be a yield limiting factor in southern Australia. In contrast to diagnosis of nutritional deficiencies where remedial action may be taken for the current crop, information regarding the potential for B toxicity would only be of use for selection of future varieties and in this respect grain would be a suitable tissue for analysis.

The historically important Australian wheat varieties together with the current varieties most widely cultivated in South Australia were tested for tolerance to B. The family of varieties derived from Federation and Currawa, including Ghurka, Insignia, Heron, Olympic, Halberd and Spear, were more tolerant than any other Australian varieties examined (Chapter 5), although not as tolerant as some overseas genotypes (Moody *et al.*, 1988). Several varieties within this family, including Ghurka and Halberd, were produced by complex crosses with up to five parents, several of them sensitive to B. The pattern of transmission of tolerance from parent to progeny for such varieties would be consistent with simple genetic control and the presence of high concentrations of B at the selection sites favouring the selection of tolerant genotypes. Concentrations of B of greater than 20 mg kg^{-1} have been measured in the subsoils in the general regions in which many of the tolerant varieties were selected (B. Cartwright, pers. comm.).

The distribution of the B tolerant varieties has been far from uniform across South Australia. The three varieties Insignia, Heron and Halberd were most widely adopted in the same districts (Statistical Register of South Australia, 1960 - 1975) (Chapter 5) and these coincide with the districts where the concentrations of B in the grain of the 1983 barley crop were highest (Cartwright and Hirsch, 1986). This would indicate that B toxicity has been a major factor determining the distribution of wheat varieties in South Australia. Evidence of a yield advantage for B tolerant varieties and genotypes when grown under naturally occurring high B conditions was obtained for the experiments of the current South Australian commercial varieties at Two Wells in 1985 and 1986 (Chapter 5) and for random lines derived from (Halberd*(W1*MMC)) at Two Wells in 1988 and 1989 and at Minnipa in 1989 (Chapter 8). The ranges in grain yield, between

the most sensitive and most tolerant of the South Australian commercial varieties, calculated from the regression equations in Figure 5.7, were approximately 135 - 205 g plot^{-1} in 1985 and 220 - 320 g plot^{-1} in 1986. For the (Halberd*(W1*MMC)) random lines the yield advantage of the tolerant lines over the sensitive lines was 25% and 12% at Two Wells in 1988 and 1989, respectively and 6% at Minnipa in 1989. Very substantial yield effects due to genetic variation in tolerance to B have therefore been measured. Similar responses for B tolerant genotypes have been measured at five further sites in South Australia using near-isogenic lines developed between Halberd and Schomburgk (D.B. Moody and A.J. Rathjen, pers. comm.).

The results of this thesis do not provide any evidence that B tolerant varieties are more susceptible to B deficiency, however Nable (1988) and Paull *et al.* (1988) reported wheat and barley genotypes with low B accumulation at high B treatments to also accumulate less B at the control treatment. Therefore, the possibility of B tolerant lines being more susceptible to B deficiency cannot be excluded. Further evidence that B tolerant varieties are poorly adapted to low B conditions is provided by the origin and distribution of Australian wheat varieties. The only major B tolerant varieties to be released by the wheat breeding programs located at the Waite Institute and in New South Wales, with the exception of Federation, are backcross derived varieties where the recurrent parent was a B tolerant. If there was no yield penalty for B tolerance it might be expected that tolerant varieties would be selected, by chance, by breeding programs located in low B regions, particularly when it is considered that tolerant varieties have been so dominant in Victoria and South Australia and it is customary to use successful varieties as parents. Investigations are being conducted at the Waite Institute, using near isogenic lines, to determine whether B tolerant genotypes do suffer a yield penalty at low B sites. If this proves to be the case it will be necessary to breed varieties of a level of tolerance appropriate to the B status of the soils of the region where they are intended to be cultivated. As the B status of soils is very heterogeneous and can vary considerably over small distances it may arise that varieties of alternative levels of tolerance are required for different soil types within a geographical region and even within a farm.

The pattern of inheritance of tolerance to B within the Federation / Currawa family suggests tolerance is under the control of major genes and this was borne out by the genetic studies of Chapter 6. Single gene differences in tolerance to B could be recognized between successively more tolerant genotypes to the level of Halberd and allelic variation occurred for at least four independent loci among the five genotypes selected for study. Transgressive segregation resulted between the two tolerant genotypes G61450 and Halberd and this would indicate that the tolerance of these two genotypes is under the control of contrasting genes. As tolerance is controlled by major genes, the backcrossing method may be used to incorporate tolerance into sensitive local varieties.

The gene symbol *Bor* was assigned for B tolerance. It is conventional for the upper case gene symbol to represent the dominant genotype but as tolerance is expressed as a partially dominant character it was necessary to decide whether to assign the upper case symbol to the tolerant or sensitive genotype. The upper care *Bor* was assigned to the tolerant genotype and this decision was made on the basis of the agricultural significance of tolerance to high concentrations of B in south eastern Australia, the region where the genetic control of tolerance of wheat to B was determined. Inferences regarding the biochemical basis of tolerance to B should not be made on basis of the gene symbols. It is not known whether tolerance results from the expression of a gene which restricts the uptake of B, or conversely whether the expression of a gene by the sensitive types promotes uptake of B making these genotypes better adapted to low B conditions but more sensitive to high concentrations of B. Evidence supporting both hypotheses was obtained from the experiments of aneuploid lines (Chapter 7). The uptake of B by the Chinese Spring x *Agropyron elongatum* amphiploid was significantly less than for Chinese Spring and this is consistent with tolerance resulting from exclusion of B. On the other hand, for the experiment on the ditelosomics of chromosome 4A of Chinese Spring the uptake of B was reduced when the short arm of chromosome 4A was absent. This would suggest there is a gene located on chromosome $4A_S$ which promotes the uptake of B and therefore supports the second hypothesis.

Major gene control of tolerance would enable the development of isogenic lines with respect to tolerance to B and two sets of isogenic lines are currently being developed

at the Waite Institute. The moderately sensitive variety Schomburgk (a backcross derivative of Aroona, a sister selection to Warigal) has been chosen as the recurrent parent and the tolerance of Halberd and sensitivity of (Wl*MMC) are being incorporated by backcrossing. This will result in lines of common genetic background but of three levels of tolerance to B. These lines could be used for physiological and biochemical studies of the mechanism of reduced accumulation of B in shoots. Isogenic lines could also be compared in the field to determine the yield advantage of B tolerance under high B conditions, whether there is a yield penalty associated with the tolerance gene when grown at normal B levels and to map the extent of B toxicity, either by growing the lines at many sites, or by determining concentrations of B in soil or plants (shoots or grain) at sites at which the tolerant lines have a yield advantage and using these results for either a survey or as the basis of a diagnostic service.

The chromosomal location of genes for tolerance to B was undertaken by intervarietal substitution lines, monosomic analysis and interspecific addition lines (Chapter 7). The results for the substitution lines of Kenya Farmer into Chinese Spring demonstrated that the CS(KF4A) substitution line was more sensitive than Chinese Spring although chromosome 4A did not account for the total difference between Chinese Spring and Kenya Farmer. The segregation ratios for F_2 and F_3 generations of (CS*CS(KF4A)) were consistent with a single gene for B tolerance located on chromosome 4A.

The results for monosomic analysis were more equivocal because experiments were unreplicated and a quantitative character, namely symptom expression, rather than a qualitative character was used to assess the response of individual plants. Nevertheless, the chromosomes of several homoeologous groups, and group 7 in particular, were implicated in the control of tolerance to B. Further support for the significance of the group 7 chromosomes with respect to tolerance to B was obtained from the interspecific addition lines of *Agropyron elongatum* into Chinese Spring. The chromosome 7E addition line was more tolerant than Chinese Spring and the gene(s) for B tolerance was probably located on $7E_L$.

It is possible to compare the results for the experiments for genetic control (Chapter 6) with the chromosomal location of genes (Chapter 7) and hypothesise about the relationships between the genotypes. The most sensitive genotypes, Kenya Farmer (*bor1 bor2 bor3 bor4*)and (Wl*MMC) (*bor1 bor2 Bor3 bor4*), developed a specific symptom referred to as "mid-leaf necrosis" and initially described in Chapter 4. Moderately sensitive varieties, such as Warigal (*bor1 Bor2 Bor3 bor4*), did not develop this symptom at even very high concentrations of B. The CS(KF4A) substitution line was the only Chinese Spring / Kenya Farmer substitution line to develop this symptom, therefore the "mid-leaf necrosis" symptom for Kenya Farmer is under the control of chromosome 4A. As the symptom is a specific response to B, it is possible that other sensitive genotypes, such as (Wl*MMC), have the same allele as Kenya Farmer at the locus for response to B located on chromosome 4A . If this were so, the *Bor3* locus for which (Wl*MMC) and Kenya Farmer have the alternative alleles would be located on a chromosome other than chromosome 4A. On the other hand, the *Bor2* locus for which Warigal and (Wl*MMC) have contrasting alleles would be located on chromosome 4A. The hypothesis of the "mid-leaf necrosis" symptom expressed by (Wl*MMC) being under the control of the *Bor2* locus located on chromosome 4A could be tested by monosomic analysis. This symptom could be considered to be a qualitative character and so it is probable that an unequivocal result could be obtained.

Transgressive segregation was observed for (G61450*Halberd) and this suggests that the two genotypes have tolerance genes at contrasting loci and the genotypes of the two parents were designated G61450 *bor1 Bor2 Bor3 Bor4* and Halberd *Bor1 Bor2 Bor3 bor4*. By this model (G61450*Halberd) would segregate for two loci with respect to tolerance to B and for additive genetic control with both loci of equal effect 5/16 of the progeny would be more sensitive than Halberd and 5/16 more tolerant the G61450. The location of genes of Halberd was not tested by monosomic analysis but Federation, an ancestor of Halberd, was tested by reciprocal monosomic analysis with Chinese Spring. G61450 was also tested by monosomic analysis with Chinese Spring. The two comparisons identified chromosomes of homoeologous group 7 as the most probable locations of genes for tolerance to B, although the critical chromosomes differed between

comparisons. The most probable critical chromosomes were chromosome 7B for reciprocal monosomic analysis between Chinese Spring and Federation and chromosome 7D for monosomic analysis between the Chinese Spring monosomics and G61450. The contrast in critical chromosomes between G61540 and Federation is consistent with the transgressive segregation observed in response to high concentrations of B for the F_2 of (G61450* Halberd).

The uptake of B was genetically independent of all other elements (Chapter 8), therefore breeding for B tolerance (i.e. reduced uptake of B) should not affect the nutritional status of plant with respect to other elements. Despite the genetic independence of uptake of B and other elements, the concentration of B and other elements in plants might be correlated over environments and this would result for edaphically correlated elements. For example, high concentrations of B are generally associated with sodic subsoils. Sodic soils are high in exchangeable Na^+, and Mg^{++} but low in Ca^{++} and so concentrations of these three elements in plants may be correlated over environments. The association between high concentrations of B and sodicity, in the subsoil, would require that for B tolerant varieties to be well adapted to the soil environment they are also tolerant to sodicity. Research is required to assess the genetic variation in tolerance to sodicity, particularly among Australian varieties and the overseas varieties which are being used as donor parents of B tolerance in the Waite Institute wheat breeding program. Tolerance to sodicity could be selected for in the field, in tandem with tolerance to B, by selecting for grain yield at a site with a high concentration of B and a sodic subsoil, provided there is a sufficient degree of tolerance to sodicity among the parents.

This thesis has identified major gene control of tolerance to high concentrations of B with at least four independent loci regulating the response. Within the Australian genotypes studied in detail two major genes were identified and of these, *Bor1*, for which Halberd and Warigal differ, apears to have had a significant role in determining the predominance and distribution of wheat varieties in south eastern Australia. As tolerance to B is under the control of major genes, tolerance could be incorporated into sensitive but otherwise adapted Australian varieties by the backcross method of breeding. Tolerance is expressed at the seedling stage of development and response is significantly

correlated between the glasshouse and in the field. Therefore selection can be conducted during early vegetative growth and as field soils are very heterogeneous for concentration of B and high concentrations of B occur in the subsoil, selection would be more efficient under controlled glasshouse conditions and this could be undertaken for either single F_2 plants or F_3 families.

An area which is the subject of continuing research at the Waite Institute and CSIRO Division of Soils, and which will affect the strategy for recommendation of tolerant varieties, is whether there is a yield penalty associated with tolerance, either due to increased susceptibility to B deficiency or another physiological response, when grown under low B conditions. If this proves to be the case, a range of varieties of differing levels of tolerance would be required for cultivation in specific regions; if there is not a yield penalty associated with tolerance the tolerant varieties could be generally adopted.

APPENDICES

APPENDIX A. Monthly rainfall data (mm) for sites and seasons of field trials. Data provided by Bureau of Meteorology, Waite Agricultural Research Institute and Walpeup Research Station.

Month	Two Wells				
	1985	1986	1987	1988	1989
Jan	4	4	29	10	0
Feb	0	2	21	10	3
Mar	52	4	17	18	5
Apr	32	33	12	7	29
May	29	33	96	83	47
June	34	26	36	74	56
July	30	94	50	49	63
Aug	60	101	25	21	44
Sep	36	43	17	38	37
Oct	53	63	38	9	20
Nov	17	20	4	36	37
Dec	13	12	29	36	10
April-Oct total	274	393	274	281	296
Annual total	360	435	384	391	400

	Rudall 1988	Rudall 1989	Minnipa 1989	Windsor 1989	Walpeup 1989	Waite 1989
Jan	12	0	1	0	23	3
Feb	3	0	0	1	1	2
Mar	6	66	5	12	35	3
Apr	1	10	9	17	12	54
May	30	76	29	53	71	97
June	41	49	53	32	44	105
July	19	93	95	83	33	87
Aug	15	29	30	37	40	89
Sep	26	33	21	21	17	52
Oct	5	5	22	28	12	38
Nov	26	22	16	66	14	34
Dec	43	20	13	40	7	13
April-Oct total	137	295	259	271	229	522
Annual total	231	442	316	410	309	577

APPENDIX B. Effect of high concentrations of B in the grain upon seedling growth and concentration of B in plants.

Grain was derived from the plants of Halberd, Warigal and (Wl*MMC) grown at the B0, B20 and B60 treatments described in Chapter 4. Details of the grain are presented in Table B.1.The grain was sown in nil applied B potting mix. Five grains were sown per pot and the experiment consisted of six replicates arranged as a completely randomized design. Emergence (Table B.2) and plant height (not presented) were recorded regularly and dry weight and concentration and content of B in shoots determined 28 days after sowing (Table B.3). Full results are reported in Nable and Paull (1989).

Table B.1 Concentration (mg kg^{-1}) and content (ng $seed^{-1}$) of B in grain and 1000 grain weight of the grain used for experiments.

	Halberd			Warigal			(Wl*MMC)		
	B0	B20	B60	B0	B20	B60	B0	B20	B60
B conc (mg kg^{-1})	1.1	4.1	21.5	1.2	3.1	17.3	1.3	3.4	16.2
B content (ng $grain^{-1}$)	53	175	959	61	170	920	59	177	770
1000 grain weight	48.0	42.8	44.6	50.9	54.7	53.2	45.5	51.9	47.5

Table B.2 Seedling emergence, expressed as plants per pot, three, four and six days after sowing.

Days after	Halberd			Warigal			(Wl*MMC)		
sowing	B0	B20	B60	B0	B20	B60	B0	B20	B60
Day 3	2.0	2.3	2.2	3.5	3.0	2.8	3.6	3.6	3.2
Day 4	4.8	4.8	4.7	4.8	4.7	4.4	4.8	4.8	4.8
Day 6	4.8	4.8	5.0	5.0	4.8	5.0	5.0	5.0	5.0

Table B.3 Dry matter yield (mg plant^{-1}) and concentration (mg kg^{-1}) and content (ng plant^{-1}) of B in plants four weeks after sowing.

	Halberd			Warigal			(Wl*MMC)		
	B0	B20	B60	B0	B20	B60	B0	B20	B60
Dry matter yield (mg plant^{-1}) LSD (0.05) 5	94	98	94	79	87	85	83	84	84
B concentration (mg kg^{-1}) LSD (0.05) 0.5	2.5	2.5	2.8	3.1	3.2	3.0	3.5	3.6	3.5
B content (ng plant^{-1})	235	245	263	245	278	255	291	302	294

APPENDIX C. Leading wheat varieties in Australia during the twentieth century.

Table C.1 The three leading varieties as a percentage of total area sown to wheat in Australia, South Australia and Victoria for selected years during the period 1929 to 1965. Source of data - Macindoe and Walkden-Brown (1968))

Year	Australia		South Australia		Victoria	
	Variety	Area(%)	Variety	Area(%)	Variety	Area(%)
1929-30	Nabawa	15.3	Gluyas	16.4	Free Gallipoli	22.2
	Federation	12.9	Early Gluyas	10.6	Federation	19.8
	Early Gluyas	6.9	Federation	8.7	Ranee	12.7
1935-36	Nabawa	15.5	Ranee	18.1	Ghurka	34.9
	Ranee	10.8	Nabawa	17.6	Free Gallipoli	24.9
	Ford	8.3	Sword	11.2	Ranee	22.1
1940-41	Bencubbin	24.9	Ranee	16.7	Ghurka	47.6
	Ghurka	11.8	Bencubbin	16.1	Ranee	20.2
	Ranee	10.8	Dundee	11.3	Dundee	8.5
1948-49	Bencubbin	23.0	Bencubbin	19.3	Quadrat	47.2
	Quadrat	13.0	Warigo	15.0	Insignia	11.8
	Bungulla	6.2	Waratah	6.7	Bencubbin	7.3
1953-54	Bencubbin	20.6	Gabo	22.9	Insignia	41.0
	Insignia	12.2	Bencubbin	14.8	Quadrat	26.4
	Gabo	11.0	Insignia	10.3	Pinnacle	21.4
1957-58	Gabo	21.1	Gabo	22.8	Insignia	51.5
	Insignia	16.7	Insignia	18.1	Pinnacle	23.4
	Bencubbin	11.7	Dirk	16.3	Quadrat	9.9
1960-61	Insignia	21.6	Insignia	23.3	Insignia	52.4
	Gabo	17.3	Gabo	20.9	Pinnacle	21.5
	Glenwari	6.3	Dirk	14.6	Olympic	14.2
1965-66	Insignia	25.2	Insignia	37.4	Insignia	49.2
	Gamenya	11.2	Heron	17.5	Olympic	23.2
	Heron	11.2	Gabo	13.6	Pinnacle	18.7

Table C.2 The three leading wheat varieties, expressed as a percantage of the total production for individual states, between 1979/80 and 1989/90. Source of data - Australian Wheat Board.

Year	South Australia	Western Australia	Victoria	Queensland	New South Wales
1979/80	Halberd 39			Cook 35	Condor 26
	Condor 14			Oxley 20	Egret 25
	Kite 11			Kite 15	Songlen 17
1980/81	Halberd 34	Gamenya 50	Egret 29	Cook 42	Egret 43
	Condor 13	Madden 16	Olympic 22	Kite 15	Condor 24
	Kite 12	Halberd 13	Condor 20	Oxley 15	Songlen 8
1981/82	Halberd 35	Gamenya 48	Condor 24	Cook 42	Egret 25
	Condor 12	Halberd 21	Olympic 21	Banks 17	Condor 20
	Kite 12	Madden 13	Egret 19	Kite 13	Songlen 16
1982/83	Halberd 29	Gamenya 45	Olympic 22	Cook 39	Egret 15
	Warigal 15	Halberd 24	Millewa 17	Banks 23	Banks 13
	Festiguay 11	Madden 12	Egret 17	Kite 15	Condor 11
1983/84	Halberd 41	Gamenya 45	Millewa 30	Cook 35	Banks 30
	Warigal 14	Halberd 22	Olympic 21	Banks 25	Condor 12
	Condor 8	Madden 13	Condor 20	Oxley 12	Songlen 12
1984/85	Halberd 31	Gamenya 34	Millewa 40	Cook 31	Banks 32
	Warigal 18	Halberd 29	Olympic 19	Banks 21	Harrier 8
	Millewa 9	Madden 11	Condor 15	Kite 12	Cook 8
1985/86	Halberd 33	Gamenya 23	Millewa 46	Hartog 31	Banks 26
	Warigal 20	Halberd 20	Oxley 18	Banks 22	Quarrion 8
	Millewa 8	Aroona 7	Condor 16	Kite 15	Osprey 8
1986/87	Halberd 34	Halberd 24	Millewa 33	Hartog 30	Banks 19
	Warigal 16	Gutha 17	Condor 24	Kite 17	Hartog 12
	Aroona 10	Eradu 12	Oxley 18	Banks 17	Osprey 10
1987/88	Halberd 25	Halberd 23	Oxley 19	Hartog 35	Hartog 16
	Spear 16	Aroona 18	Condor 19	Banks 15	Banks 12
	Warigal 13	Gutha 15	Cocamba 14	Kite 14	Suneca 11
1988/89	Spear 21	Halberd 21	Meering 23	Hartog 40	Vulcan 20
	Halberd 18	Gutha 16	Cocamba 22	Vasco 12	Hartog 17
	Machete 16	Aroona 14	Condor 14	Kite 10	Suneca 16
1989/90	Spear 23	Halberd 20	Meering 28	Hartog 49	Vulcan 26
	Machete 21	Aroona 16	Cocamba 20	Kite 10	Hartog 18
	Halberd 18	Gamenya 11	Oxley 13	Sunco 9	Suneca 12

APPENDIX D. Concentration of elements, other than B, in shoots and grain for the experiments of the historically important wheat varieties and wheat varieties currently cultivated in South Australia conducted at Two Wells in 1985 and 1986.

Table D.1 Concentration of elements in shoots for the historically important wheat varieties grown at Two Wells in 1985.

Variety	Concentration of elements								
	P	K	S	Mg	Cu	Mn	Zn	Ca	Na
	%				mg kg^{-1}				
Bencubbin	0.27	2.98	0.20	0.14	4.55	55.9	12.8	1330	1280
Condor	0.25	3.36	0.21	0.15	4.36	52.5	13.5	1570	850
Currawa	0.25	3.73	0.24	0.16	5.07	49.7	14.5	1600	980
Dundee	0.24	3.19	0.21	0.13	3.77	43.4	13.1	1250	1320
Federation	0.27	3.58	0.24	0.17	5.26	63.8	17.2	1730	680
Gabo	0.21	2.64	0.18	0.143	4.37	51.9	10.5	1130	1330
Gallipoli	0.25	3.61	0.23	0.15	4.94	55.6	14.3	1380	870
Gamenya	0.22	2.97	0.20	0.16	4.10	53.0	13.1	1380	970
Gluyas	0.28	3.09	0.22	0.15	4.49	56.6	14.9	1400	730
Halberd	0.23	3.31	0.23	0.15	4.80	61.4	14.0	1330	580
Heron	0.27	3.46	0.24	0.16	4.76	63.5	14.5	1600	670
Insignia	0.27	3.41	0.23	0.17	5.25	61.5	16.7	1650	970
Kite	0.26	3.29	0.22	0.15	4.28	55.8	13.5	1420	850
Me C3	0.26	3.40	0.22	0.15	4.36	53.1	15.9	1630	800
Mexico 120	0.25	3.31	0.23	0.15	4.63	53.4	14.9	1520	920
Marshalls No.3	0.28	3.40	0.23	0.14	4.56	43.4	15.2	1380	800
Olympic	0.23	3.22	0.23	0.16	5.13	51.1	15.0	1500	920
Pinnacle	0.27	3.81	0.24	0.16	5.09	49.5	15.4	1520	1120
Purple Straw	0.20	3.48	0.22	0.13	4.54	55.7	14.5	1420	1320
Quadrat	0.23	3.34	0.22	0.14	4.62	46.0	14.8	1530	700
Ranee	0.28	3.35	0.22	0.16	4.37	54.5	16.7	1650	1320
Raven	0.24	3.21	0.21	0.14	4.52	46.5	13.9	1170	970
Siete Cerros	0.23	2.83	0.18	0.14	4.24	48.7	13.7	1280	950
Wards Prolific	0.22	3.04	0.20	0.14	3.92	43.1	12.5	1350	1300
(Wl*MMC)	0.24	3.01	0.21	0.16	4.92	61.2	13.3	1440	780
WW-15	0.25	3.36	0.22	0.15	4.19	50.3	14.6	1430	1000

Table D.2 Concentration of elements in grain for the historically important wheat varieties grown at Two Wells in 1986.

Variety	Concentration of elements								
	P	K	S	Mg	Cu	Mn	Zn	Ca	Na
	%				mg kg^{-1}				
Bencubbin	0.27	0.37	0.13	0.10	3.29	36.9	19.6	309	119
Condor	0.25	0.31	0.13	0.10	2.78	37.0	18.7	363	102
Currawa	0.25	0.38	0.13	0.10	3.26	37.3	20.4	313	132
Dundee	0.25	0.38	0.13	0.10	2.94	33.0	19.7	293	130
Federation	0.25	0.35	0.13	0.11	3.51	38.9	20.2	359	101
Gabo	0.27	0.34	0.13	0.11	3.38	39.2	21.0	312	104
Gallipoli	0.27	0.34	0.13	0.11	3.58	38.1	22.8	333	119
Gamenya	0.27	0.34	0.13	0.11	3.04	42.1	22.2	331	107
Gluyas	0.27	0.35	0.13	0.10	3.37	38.5	20.9	307	124
Halberd	0.24	0.33	0.13	0.11	3.45	36.8	18.2	308	81
Heron	0.25	0.33	0.13	0.10	3.15	38.6	19.5	350	110
Insignia	0.24	0.34	0.13	0.10	3.23	37.0	19.1	342	95
Kite	0.28	0.34	0.14	0.12	3.09	35.4	22.2	309	75
Me C3	0.30	0.36	0.14	0.12	3.43	45.4	25.4	308	118
Mexico 120	0.24	0.30	0.14	0.10	3.12	34.0	20.6	344	114
Marshalls No 3	0.28	0.39	0.14	0.11	2.93	33.7	22.6	307	151
Olympic	0.24	0.36	0.13	0.10	3.16	31.7	18.8	326	135
Pinnacle	0.24	0.38	0.12	0.10	2.89	34.3	19.4	312	104
Purple Straw	0.27	0.37	0.13	0.11	3.50	37.2	21.4	296	107
Quadrat	0.26	0.36	0.13	0.11	3.01	38.1	20.6	362	125
Ranee	0.24	0.34	0.13	0.10	3.02	37.4	19.8	349	143
Raven	0.25	0.35	0.14	0.11	3.81	37.3	21.1	285	111
Siete Cerros	0.25	0.30	0.12	0.11	3.18	39.9	19.2	365	134
Wards Prolific	0.27	0.34	0.14	0.12	3.97	42.3	24.3	370	136
(W1*MMC)	0.26	0.37	0.13	0.11	3.19	40.2	20.3	327	123
WW-15	0.24	0.31	0.13	0.10	2.89	35.4	18.0	345	104

Table D.3 Concentration of elements in shoots for the current South Australian wheat varieties grown at Two Wells in 1985.

Variety	Concentration of elements								
	P	K	S	Mg	Cu	Mn	Zn	Ca	Na
	%				mg kg^{-1}				
Aldirk	0.19	2.56	0.19	0.13	4.06	40.8	10.8	1120	760
Aroona	0.17	2.38	0.17	0.11	3.55	39.5	13.8	960	400
Schomburgk	0.19	2.47	0.18	0.12	4.22	43.2	10.0	860	600
Banks	0.17	2.47	0.18	0.12	4.09	42.0	11.5	1200	1020
Bayonet	0.24	2.77	0.20	0.13	4.66	40.3	13.9	1260	840
Bindawarra	0.25	2.93	0.21	0.15	5.19	50.1	13.6	1460	1100
Condor	0.22	2.63	0.18	0.13	4.61	45.2	12.1	1260	540
Cook	0.22	2.74	0.19	0.13	3.99	42.8	15.5	1420	860
Cranbrook	0.20	2.55	0.16	0.12	4.08	46.4	11.8	1240	500
Dagger	0.19	2.63	0.19	0.12	4.27	44.8	15.2	1260	720
Egret	0.21	2.95	0.20	0.13	4.79	46.6	12.4	1260	700
Festiguay	0.26	2.75	0.22	0.15	4.96	55.3	14.8	1660	880
Gabo	0.15	2.10	0.15	0.12	3.86	44.8	9.4	900	460
Halberd	0.19	2.78	0.20	0.13	4.84	49.6	12.6	1240	480
Katyil	0.23	3.01	0.21	0.15	4.66	45.5	15.8	1460	760
Kite	0.21	2.60	0.19	0.13	3.97	46.6	12.5	1120	720
Lance	0.22	2.60	0.20	0.14	4.99	47.5	12.1	1240	840
Machete	0.20	2.49	0.17	0.13	4.41	49.0	10.5	1060	520
Madden	0.21	2.57	0.19	0.14	4.46	47.4	12.5	1260	950
Meering	0.21	2.70	0.20	0.13	4.25	43.2	12.0	1440	640
Miling	0.21	2.33	0.18	0.13	4.00	46.4	10.9	1280	960
Millewa	0.19	2.58	0.18	0.13	4.15	45.9	12.6	1340	700
Olympic	0.21	3.13	0.21	0.15	4.54	47.1	15.0	1400	820
Oxley	0.22	2.76	0.20	0.14	4.95	48.4	14.2	1360	680
Raven	0.17	2.39	0.17	0.12	4.19	43.1	12.4	1020	540
Sabre	0.23	2.75	0.18	0.14	4.47	48.4	13.6	1140	580
Spear	0.20	2.57	0.18	0.12	4.05	43.8	13.4	1100	600
Sunstar	0.20	2.65	0.18	0.13	4.41	43.6	13.4	1260	660
Takari	0.18	2.21	0.18	0.16	4.18	52.4	13.6	1360	880
Vulcan	0.21	2.54	0.19	0.12	3.94	41.9	12.2	1320	460
Warigal	0.22	3.24	0.21	0.14	4.78	45.5	14.3	1240	1000
Warimba	0.22	2.40	0.19	0.14	4.84	52.4	12.5	1240	860
(WI*MMC)	0.21	2.56	0.18	0.14	4.72	54.4	12.9	1280	900

Table D.4 Concentration of elements in grain for the current South Australian wheat varieties grown at Two Wells in 1986.

Variety	Concentration of elements								
	P	K	S	Mg	Cu	Mn	Zn	Ca	Na
	%				mg kg^{-1}				
Aldirk	0.26	0.37	0.15	0.11	4.53	37.5	20.7	325	118
Aroona	0.26	0.34	0.15	0.11	4.18	41.0	18.9	320	114
Bayonet	0.24	0.35	0.13	0.10	4.48	29.9	19.6	325	127
Bindawarra	0.24	0.39	0.14	0.11	4.71	36.9	18.1	317	139
Condor	0.24	0.32	0.13	0.10	4.02	35.7	17.2	360	108
Cook	0.26	0.31	0.14	0.11	3.93	38.8	17.6	300	149
Cranbrook	0.27	0.34	0.14	0.11	3.87	36.3	19.2	360	105
Dagger	0.25	0.35	0.14	0.10	3.96	34.9	18.0	340	86
Egret	0.26	0.34	0.13	0.10	3.79	34.7	18.9	360	91
Festiguay	0.28	0.32	0.14	0.10	4.48	37.2	22.0	300	117
Halberd	0.28	0.34	0.14	0.11	4.49	37.1	19.0	300	72
Katyil	0.22	0.39	0.13	0.10	4.13	29.9	17.8	300	126
Kite	0.29	0.35	0.15	0.12	4.01	37.1	21.4	300	68
Lance	0.25	0.37	0.14	0.11	4.26	32.0	20.3	320	116
Machete	0.27	0.34	0.13	0.11	3.70	37.5	19.7	260	65
Meering	0.26	0.34	0.14	0.11	4.06	35.3	18.8	367	106
Miling	0.26	0.37	0.13	0.11	3.66	36.6	17.7	300	101
Millewa	0.28	0.31	0.14	0.11	4.01	35.6	20.3	375	113
Olympic	0.25	0.37	0.14	0.11	4.09	33.7	18.3	300	136
Oxley	0.25	0.32	0.14	0.11	3.69	35.8	17.7	333	119
Sabre	0.28	0.34	0.15	0.11	4.09	37.5	21.5	300	75
Schomburgk	0.25	0.33	0.14	0.10	4.00	38.0	18.3	300	99
Spear	0.25	0.36	0.14	0.11	4.09	35.7	18.4	350	90
Sunstar	0.28	0.32	0.14	0.11	3.99	34.3	19.0	360	159
Takari	0.28	0.38	0.15	0.12	4.13	37.3	20.7	350	94
Vulcan	0.27	0.32	0.14	0.11	3.87	35.7	19.3	400	124
Warigal	0.27	0.39	0.15	0.11	4.79	40.4	20.4	340	131
Warimba	0.27	0.38	0.14	0.11	4.01	38.4	22.1	400	107
(Wl*MMC)	0.28	0.40	0.14	0.11	4.20	40.6	19.8	342	134

APPENDIX E. The mean concentrations and standard deviations of the means for all agiculturally significant elements, other than B, determined by ICP-spectrometry for the F4 (1987) and F5 (1988) families of (Halberd*(Wl*MMC) grown at Two Wells and Rudall. Each family consisted of eight lines and experiments were arranged as completely randomized blocks without replication.

Table E.1 Concentration of Ca (mg kg^{-1}) in shoots and grain for the (Halberd* (Wl*MMC)) lines grown at Two Wells and Rudall. Each family consists of eight lines.

Family	Concentration of Ca (mg kg^{-1})							
	Two Wells 1987 Grain		Two Wells 1988 Shoots		Two Wells 1988 Grain		Rudall 1988 Grain	
	Mean	St dev	Mean	St dev	Mean	St dev	Mean	St dev
1	267	23	1243	197	205	31	198	29
2	284	32						
3	261	23						
4	253	28	1189	230	173	14	195	14
5	264	15						
6	267	8	1345	384	182	16	208	26
7	297	29						
8	316	31						
9	291	25	1187	144	192	11	201	27
10	286	19						
11	304	23	1209	202	191	9	214	39
12	299	30						
13	284	24	1126	218	181	22	234	49
14	309	28	1297	176	196	22	206	34
15	309	27						
16	314	24						
17	285	32	1051	121	175	20	205	22
18	310	12	1323	177	220	21	236	44
19	293	23						
20	274	21						
21	331	26	1270	72	212	20	219	34
22	289	25						
23	358	35	1351	328	211	15	235	30
24	305	58						
25	275	28						
26	308	19	1461	302	201	16	219	42
27	283	25	1273	298	195	18	219	28
28	306	22						
29	286	21						
30	326	30	1180	85	216	15	219	28
31	301	24						
32	267	33	1158	339	181	21	198	35
33	297	35	1211	139	207	19	198	35
34	323	37						
35	321	23						

Table E.2 Concentration of Cu (mg kg^{-1}) in shoots and grain for the (Halberd* (Wl*MMC)) lines grown at Two Wells and Rudall. Each family consists of eight lines.

Family	Concentration of Cu (mg kg^{-1})							
	Two Wells 1987 Grain		Two Wells 1988 Shoots		Two Wells 1988 Grain		Rudall 1988 Grain	
	Mean	St dev	Mean	St dev	Mean	St dev	Mean	St dev
1	4.37	0.31	5.71	0.60	4.17	0.37	4.41	0.35
2	5.25	0.54						
3	5.14	0.39						
4	5.28	0.36	6.54	0.68	4.71	0.23	4.94	0.32
5	5.29	0.59						
6	4.96	0.49	5.63	0.30	3.92	0.31	4.81	0.38
7	5.20	0.39						
8	4.87	0.43						
9	5.53	0.68	5.35	0.73	4.58	0.50	5.13	0.32
10	5.56	0.25						
11	4.95	0.51	6.03	0.41	4.28	0.33	4.75	0.25
12	5.29	0.52						
13	4.51	0.54	5.64	0.61	4.02	0.37	4.57	0.64
14	5.08	0.80	6.60	1.08	4.24	0.46	4.74	0.35
15	4.89	0.46						
16	5.13	0.56						
17	4.81	0.55	6.28	0.52	4.42	0.41	4.80	0.37
18	4.83	0.27	5.80	1.14	3.86	0.34	4.47	0.22
19	5.07	0.34						
20	4.83	0.32						
21	5.58	0.37	5.72	0.32	4.54	0.44	4.71	0.60
22	5.04	0.18						
23	5.32	0.45	6.09	0.95	4.46	0.20	5.07	0.49
24	5.00	0.35						
25	4.74	0.39						
26	4.99	0.52	6.50	1.03	4.01	0.34	4.83	0.41
27	4.50	0.49	6.29	1.00	4.29	0.34	4.54	0.28
28	4.54	0.44						
29	4.65	0.60						
30	5.01	0.32	5.36	0.30	4.29	0.35	4.81	0.34
31	5.00	0.44						
32	4.60	0.37	5.83	0.74	4.06	0.27	4.45	0.36
33	4.37	0.29	6.07	0.38	4.08	0.28	4.79	0.31
34	4.65	0.39						
35	5.39	0.49						

Table E.3 Concentration of K (%) in shoots and grain for the (Halberd* (W1*MMC)) lines grown at Two Wells and Rudall. Each family consists of eight lines.

Family	Concentration of K (%)							
	Two Wells 1987 Grain		Two Wells 1988 Shoots		Two Wells 1988 Grain		Rudall 1988 Grain	
	Mean	St dev	Mean	St dev	Mean	St dev	Mean	St dev
1	0.35	0.04	2.11	0.48	0.36	0.02	0.33	0.03
2	0.36	0.03						
3	0.34	0.03						
4	0.32	0.03	2.07	0.47	0.36	0.02	0.34	0.04
5	0.35	0.04						
6	0.34	0.02	2.23	0.31	0.36	0.02	0.34	0.03
7	0.34	0.02						
8	0.32	0.02						
9	0.34	0.02	2.33	0.36	0.36	0.02	0.32	0.04
10	0.31	0.02						
11	0.36	0.04	2.27	0.53	0.36	0.02	0.35	0.05
12	0.32	0.03						
13	0.35	0.03	1.94	0.36	0.35	0.01	0.36	0.04
14	0.30	0.07	2.19	0.28	0.33	0.02	0.32	0.05
15	0.34	0.02						
16	0.34	0.04						
17	0.34	0.04	2.26	0.42	0.33	0.01	0.33	0.04
18	0.33	0.04	2.01	0.40	0.34	0.01	0.32	0.04
19	0.36	0.02						
20	0.34	0.02						
21	0.38	0.05	2.06	0.23	0.37	0.01	0.34	0.04
22	0.35	0.02						
23	0.33	0.06	2.22	0.26	0.34	0.02	0.33	0.04
24	0.34	0.03						
25	0.33	0.03						
26	0.32	0.03	2.29	0.37	0.34	0.01	0.32	0.03
27	0.36	0.04	2.01	0.30	0.35	0.03	0.32	0.05
28	0.35	0.03						
29	0.38	0.02						
30	0.33	0.04	2.20	0.42	0.36	0.02	0.30	0.04
31	0.35	0.03						
32	0.37	0.05	2.16	0.46	0.37	0.02	0.35	0.03
33	0.36	0.04	2.07	0.45	0.38	0.02	0.34	0.04
34	0.32	0.02						
35	0.32	0.03						

Table E.4 Concentration of Mg (%) in shoots and grain for the (Halberd* (W1*MMC)) lines grown at Two Wells and Rudall. Each family consists of eight lines.

Family	Concentration of Mg (%)							
	Two Wells 1987 Grain		Two Wells 1988 Shoots		Two Wells 1988 Grain		Rudall 1988 Grain	
	Mean	St dev	Mean	St dev	Mean	St dev	Mean	St dev
1	0.12	0.009	0.16	0.013	0.11	0.005	0.13	0.005
2	0.12	0.010						
3	0.12	0.010						
4	0.12	0.010	0.17	0.005	0.13	0.006	0.13	0.010
5	0.12	0.006						
6	0.12	0.007	0.16	0.007	0.11	0.003	0.14	0.012
7	0.12	0.010						
8	0.13	0.009						
9	0.13	0.005	0.14	0.012	0.10	0.007	0.13	0.007
10	0.12	0.005						
11	0.12	0.005	0.15	0.013	0.10	0.004	0.13	0.005
12	0.12	0.007						
13	0.11	0.008	0.16	0.015	0.10	0.004	0.13	0.010
14	0.14	0.009	0.18	0.013	0.12	0.007	0.14	0.007
15	0.12	0.006						
16	0.12	0.007						
17	0.11	0.007	0.15	0.015	0.12	0.006	0.12	0.008
18	0.12	0.008	0.15	0.013	0.10	0.006	0.12	0.006
19	0.12	0.005						
20	0.12	0.008						
21	0.13	0.006	0.16	0.007	0.11	0.008	0.13	0.008
22	0.12	0.009						
23	0.13	0.005	0.16	0.024	0.11	0.004	0.13	0.006
24	0.13	0.007						
25	0.12	0.011						
26	0.12	0.009	0.17	0.015	0.10	0.004	0.13	0.008
27	0.11	0.007	0.16	0.021	0.10	0.010	0.12	0.007
28	0.12	0.008						
29	0.11	0.005						
30	0.13	0.006	0.16	0.009	0.11	0.003	0.13	0.006
31	0.13	0.013						
32	0.12	0.010	0.15	0.013	0.10	0.005	0.13	0.005
33	0.12	0.007	0.16	0.011	0.11	0.007	0.13	0.012
34	0.12	0.008						
35	0.13	0.012						

Table E.5 Concentration of Mn (mg kg^{-1}) in shoots and grain for the (Halberd* (Wl*MMC)) lines grown at Two Wells and Rudall. Each family consists of eight lines.

Family	Concentration of Mn (mg kg^{-1})							
	Two Wells 1987 Grain		Two Wells 1988 Shoots		Two Wells 1988 Grain		Rudall 1988 Grain	
	Mean	St dev	Mean	St dev	Mean	St dev	Mean	St dev
1	46.1	2.1	56.4	7.0	39.4	3.9	30.6	5.8
2	48.0	7.8						
3	48.8	4.1						
4	47.1	4.7	73.7	11.8	40.8	4.0	29.0	3.9
5	53.6	8.3						
6	46.3	5.8	65.5	7.6	41.6	3.7	32.0	5.0
7	51.7	3.1						
8	51.5	6.7						
9	53.7	8.0	56.7	7.3	41.4	5.1	34.2	6.7
10	53.4	4.8						
11	46.7	4.5	61.2	8.7	39.9	3.7	28.5	5.1
12	51.0	5.9						
13	43.6	2.4	63.3	5.2	38.4	2.0	24.7	6.8
14	55.3	4.2	78.2	20.3	49.2	5.6	30.7	5.5
15	42.7	3.8						
16	51.1	5.1						
17	47.9	5.3	66.8	7.3	42.5	4.1	28.7	4.9
18	49.3	2.3	69.4	16.4	38.8	6.0	24.3	3.2
19	49.4	3.0						
20	47.7	3.7						
21	49.4	6.6	60.7	12.8	43.6	5.8	27.7	8.6
22	51.3	8.1						
23	51.2	5.1	66.8	17.7	44.3	3.4	28.2	6.8
24	51.4	4.3						
25	48.7	6.7						
26	49.4	5.6	79.7	15.7	42.5	5.1	25.8	3.1
27	52.0	6.4	74.3	16.3	45.0	5.9	27.5	4.5
28	46.3	7.2						
29	44.4	4.8						
30	53.2	5.3	60.7	5.6	43.7	3.3	32.2	3.0
31	47.9	5.4						
32	45.1	4.1	64.3	10.4	37.9	2.7	24.9	7.4
33	41.8	2.8	61.6	9.7	36.9	4.5	26.8	6.5
34	45.1	3.7						
35	57.5	4.7						

Table E.6 Concentration of Na (mg kg^{-1}) in shoots and grain for the (Halberd* (WI*MMC)) lines grown at Two Wells and Rudall. Each family consists of eight lines.

Family	Concentration of Na (mg kg^{-1})							
	Two Wells 1987 Grain		Two Wells 1988 Shoots		Two Wells 1988 Grain		Rudall 1988 Grain	
	Mean	St dev	Mean	St dev	Mean	St dev	Mean	St dev
1	56.7	18.1	1018	494	105.4	20.2	47.7	14.1
2	76.0	32.7						
3	78.5	22.2						
4	43.9	13.0	808	296	105.7	28.5	50.7	16.7
5	43.1	9.9						
6	87.9	85.5	1040	252	124.3	15.8	83.5	21.8
7	68.0	34.7						
8	48.0	16.3						
9	59.4	29.0	1017	258	108.3	16.1	62.1	21.3
10	57.7	20.5						
11	49.9	9.6	791	311	112.5	22.0	80.2	24.7
12	60.8	12.5						
13	57.1	10.3	856	152	110.0	28.1	59.2	10.5
14	63.6	10.8	1091	205	127.2	41.8	66.4	19.4
15	57.1	19.1						
16	50.6	13.9						
17	52.3	19.9	835	189	111.7	36.8	53.2	19.2
18	55.5	17.7	752	137	101.5	24.7	58.4	15.6
19	49.6	12.7						
20	54.7	21.9						
21	71.3	27.9	1197	376	108.9	23.5	50.0	10.2
22	57.8	10.5						
23	58.9	14.5	954	269	85.5	14.2	56.9	15.8
24	62.0	45.7						
25	63.8	25.0						
26	66.4	62.1	1200	368	101.5	29.6	73.8	22.6
27	61.3	22.3	1059	216	120.2	31.4	50.9	12.9
28	79.2	53.6						
29	85.8	45.7						
30	79.6	24.2	1437	807	136.7	19.8	76.1	21.8
31	45.7	9.8						
32	80.6	22.1	796	231	96.2	26.1	54.0	9.1
33	61.3	20.6	849	275	101.1	26.9	84.7	32.0
34	45.5	8.8						
35	55.6	15.5						

Table E.7 Concentration of P (%) in shoots and grain for the (Halberd*(Wl*MMC)) lines grown at Two Wells and Rudall. Each family consists of eight lines.

Family	Concentration of P (%)							
	Two Wells 1987 Grain		Two Wells 1988 Shoots		Two Wells 1988 Grain		Rudall 1988 Grain	
	Mean	St dev	Mean	St dev	Mean	St dev	Mean	St dev
1	0.28	0.025	0.17	0.016	0.21	0.018	0.28	0.015
2	0.29	0.044						
3	0.31	0.040						
4	0.30	0.037	0.17	0.026	0.21	0.019	0.32	0.037
5	0.30	0.024						
6	0.31	0.027	0.16	0.022	0.19	0.017	0.30	0.042
7	0.29	0.023						
8	0.31	0.031						
9	0.33	0.027	0.15	0.023	0.21	0.026	0.301	0.043
10	0.29	0.026						
11	0.29	0.032	0.17	0.014	0.18	0.009	0.295	0.039
12	0.30	0.023						
13	0.26	0.030	0.16	0.010	0.18	0.010	0.30	0.030
14	0.30	0.044	0.19	0.032	0.22	0.020	0.32	0.026
15	0.29	0.028						
16	0.29	0.025						
17	0.28	0.024	0.18	0.017	0.20	0.011	0.30	0.029
18	0.29	0.026	0.17	0.039	0.19	0.024	0.30	0.035
19	0.28	0.022						
20	0.29	0.021						
21	0.34	0.030	0.16	0.019	0.21	0.031	0.31	0.026
22	0.28	0.029						
23	0.33	0.024	0.19	0.043	0.21	0.018	0.32	0.014
24	0.32	0.021						
25	0.32	0.040						
26	0.30	0.030	0.20	0.048	0.21	0.028	0.32	0.024
27	0.28	0.050	0.17	0.031	0.19	0.026	0.26	0.029
28	0.29	0.031						
29	0.28	0.024						
30	0.29	0.030	0.15	0.018	0.21	0.019	0.28	0.020
31	0.24	0.039						
32	0.28	0.034	0.17	0.015	0.20	0.019	0.300	0.025
33	0.29	0.022	0.18	0.024	0.21	0.027	0.31	0.046
34	0.29	0.032						
35	0.31	0.031						

Table E.8 Concentration of S (%) in shoots and grain for the (Halberd*(Wl*MMC)) lines grown at Two Wells and Rudall. Each family consists of eight lines.

Family	Concentration of S (%)							
	Two Wells 1987 Grain		Two Wells 1988 Shoots		Two Wells 1988 Grain		Rudall 1988 Grain	
	Mean	St dev	Mean	St dev	Mean	St dev	Mean	St dev
1	0.14	0.010	0.22	0.019	0.14	0.010	0.15	0.006
2	0.14	0.014						
3	0.16	0.011						
4	0.16	0.011	0.23	0.022	0.15	0.009	0.17	0.011
5	0.15	0.010						
6	0.16	0.006	0.23	0.020	0.14	0.003	0.17	0.016
7	0.16	0.003						
8	0.16	0.010						
9	0.16	0.007	0.21	0.022	0.15	0.010	0.16	0.011
10	0.16	0.008						
11	0.14	0.007	0.23	0.010	0.13	0.006	0.16	0.010
12	0.15	0.007						
13	0.14	0.006	0.21	0.022	0.13	0.004	0.16	0.011
14	0.15	0.013	0.25	0.023	0.15	0.008	0.17	0.008
15	0.15	0.011						
16	0.16	0.016						
17	0.15	0.012	0.23	0.021	0.13	0.005	0.16	0.008
18	0.16	0.008	0.22	0.020	0.14	0.007	0.16	0.011
19	0.15	0.006						
20	0.15	0.011						
21	0.18	0.013	0.22	0.007	0.15	0.008	0.17	0.013
22	0.15	0.008						
23	0.17	0.009	0.23	0.033	0.15	0.004	0.17	0.005
24	0.17	0.008						
25	0.17	0.009						
26	0.15	0.012	0.24	0.021	0.14	0.009	0.17	0.012
27	0.14	0.006	0.23	0.027	0.13	0.008	0.15	0.011
28	0.15	0.007						
29	0.14	0.006						
30	0.16	0.009	0.22	0.013	0.15	0.006	0.16	0.005
31	0.16	0.016						
32	0.15	0.014	0.22	0.023	0.14	0.006	0.16	0.011
33	0.15	0.009	0.23	0.020	0.14	0.007	0.16	0.014
34	0.15	0.007						
35	0.17	0.012						

Table E.9 Concentration of Zn (mg kg^{-1}) in shoots and grain for the (Halberd* (Wl*MMC)) lines grown at Two Wells and Rudall. Each family consists of eight lines.

Family	Concentration of Zn (mg kg^{-1})							
	Two Wells 1987 Grain		Two Wells 1988 Shoots		Two Wells 1988 Grain		Rudall 1988 Grain	
	Mean	St dev	Mean	St dev	Mean	St dev	Mean	St dev
1	12.3	1.0	16.0	3.3	12.1	1.7	7.3	0.9
2	13.4	1.7						
3	14.8	2.7						
4	14.1	0.9	16.3	2.4	12.9	1.1	8.7	1.4
5	14.2	1.0						
6	13.4	2.3	13.8	3.7	11.3	1.3	8.3	1.3
7	12.4	1.2						
8	14.6	2.8						
9	13.8	0.8	14.9	2.2	13.9	1.0	7.8	1.7
10	12.6	1.5						
11	12.6	1.4	16.5	3.4	12.1	1.5	7.9	1.4
12	14.0	0.9						
13	11.2	0.8	14.0	1.3	9.9	0.7	8.4	1.1
14	13.5	2.3	16.6	3.2	12.0	1.3	9.3	1.6
15	12.3	0.6						
16	13.1	0.9						
17	13.5	2.2	15.2	1.4	11.8	1.5	8.4	1.6
18	11.8	1.3	15.4	3.7	11.8	1.9	8.7	1.7
19	12.1	1.2						
20	13.0	1.8						
21	16.4	1.7	14.5	2.4	13.3	0.9	9.2	1.1
22	12.6	2.0						
23	15.7	2.9	14.9	2.5	12.1	1.2	8.4	1.0
24	14.7	3.4						
25	14.8	2.3						
26	13.4	1.3	16.8	4.5	11.7	1.5	8.9	0.7
27	11.6	1.3	14.6	2.7	12.0	1.8	7.4	1.4
28	11.8	1.4						
29	12.1	1.5						
30	13.2	1.8	14.7	3.8	12.7	1.9	7.3	1.1
31	15.8	3.9						
32	11.3	1.8	15.9	3.7	12.4	2.1	8.2	1.6
33	12.3	1.4	16.1	2.6	13.8	1.5	7.6	1.6
34	12.4	1.7						
35	14.6	2.8						

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