Genotypic Variation in Oilseed Rape to Low Boron Nutrition and the Mechanism of Boron Efficiency.

By

James Constantine Roy Stangoulis

University of Adelaide

Waite Campus

Department of Plant Science

South Australia

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Table of Contents

Declaration

I HEREBY DECLARE that the work presented in this thesis has been carried out by myself and does not incorporate any material previously submitted for another degree in any university. To the best of my knowledge and belief, it does not contain any material previously written or published by another person, except where due reference is made in the text. I am willing to make the thesis available for photocopy and loan if it is accepted for the award of the degree.

J. C. R. Stangoulis

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Summary

Boron (B) efficiency in oilseed rape (*Brassica napus* L. and *B. juncea* L.) was investigated in a wide range of genotypes. Using a solution culture screening of 10 day old seedlings, root length best described shoot growth response, and was used to characterise a total of 65 genotypes. Varieties and breeders lines, Huashuang 2, Nangchang rape, Huashuang 1 and Zhongyou 821, and to a lesser extent, Zheyou 2, Dunkeld, Xinza 2, Nangjin 2051, 92-58, 92-13, and Awassa 115, were tolerant of B-deficient growing conditions.

To validate the screening technique a number of genotypes were grown on B deficient soil at Mt Compass, South Australia. Genotypic variation in B efficiency was expressed in both *B. napus* (Zhongyou 821, Dunkeld, Zheyou 2, and 92-13) and *B. juncea* (Pusa Bold and CSIRO 6). Root length response from solution culture screening correlated with field response in *B. napus*, while no correlation was present for *B. juncea*, suggesting that the screening technique is suitable for use in identifying B efficiency in *B. napus* only. B responses in vegetative growth (plants sampled at the 'green bud' stage) also gave an indication of final yield response when plants were subjected to a sufficiently high level of B deficiency, indicating that vegetative growth is important in B efficiency.

Studies were conducted to investigate the mechanism of B efficiency in oilseed rape. Results suggest no association between B efficiency and the capacity to acidify the root rhizosphere, or an increased translocation of B from root to shoot. B efficiency was associated with an increased capacity to accumulate B in the B efficient variety Dunkeld, with B uptake (net B accumulation) into the shoot per unit of root length significantly greater than in the B inefficient Barossa. Varieties also differed in their rate of B uptake which may indicate differences in the uptake kinetics of a B transporter. The rate of B uptake in the variety Barossa appeared non-linear, with kinetic constants I_{max}, K_m and C_{min} of 6.07 μg B g^{-1} DW day^{-1}, 0.26 μM B, and 0.07 μM B respectively.

Boron retranslocation was also studied as a mechanism of B efficiency. B retranslocation was observed in both efficient and inefficient varieties with net movement of ^{10}B out of old leaves corresponding to the B efficiency of individual varieties. The B efficient variety Huashuang 2 demonstrated a greater ability to retranslocate B, and it is hypothesised that its B efficiency is associated with this trait. B retranslocation was hypothesised to occur via the complexation of borate with a polyol. From analysis of leaf tissue extract, no polyol was identified, suggesting B retranslocation occurs by some other, as yet, unidentified means.

Introduction

The research presented in this thesis was undertaken in response to an Australian Centre for International Agriculture Research (ACIAR) grant (9120) to Professor Robin Graham which was to identify the genetic variation in boron (B) efficiency in oilseed rape, and to elucidate a possible mechanism for this efficiency. For the purpose of this research, *B. napus* was predominantly studied as it is the major oilseed rape crop in China and Australia. In both countries, the focus is on breeding for canola[1] quality oilseed rape, while in Australia, high oil quality *B. juncea* (called mustola) is also a breeding initiative. The inclusion of a small number of *B. juncea* genotypes into the following thesis is therefore quite pertinent to Australian agriculture.

ACIAR project 9120 was built on ACIAR project 8603 (Boron and Other Micronutrients for Food Legume Production in Thailand), ACIAR project 8469 (Improvement of the Quality and Yield of Rapeseed in China) and ACIAR project 8839 (Breeding and Quality Analysis of Rapeseed for China). The overall objectives of the project may be summarised as the management of B and zinc fertiliser use of oilseed rape crops in China and Australia. The focus of boron research is mainly centred on China, where B deficiency is a widespread phenomenon (Liu *et al.*, 1981), with up to 80 % of oilseed rape crops grown in the Yangtse River Valley, where soils are commonly B deficient (Liu and Chen, 1986). An understanding of the genetic variation in B efficiency in oilseed rape, provides plant breeders with a potential source of the efficiency trait for breeding high quality cultivars for these problem soils.

To date, there are few reports highlighting the genotypic variation for B efficiency in oilseed rape (Sakal *et al.*, 1991; Yang *et al.*, 1993; Yang *et al.*, 1994), and these reports have only evaluated a small number of genotypes. The initial aim of this project was to

[1]The term canola is used to describe the quality of the seed, where it contains less than 2 % erucic acid in the oil, and less than 30 µmole glucosinolate g^{-1} fresh weight in the seed meal. (M. Vaisey-Genser and N. A. Eskin, Canola Council, Winnipeg, Manitoba, Canada).

determine the extent of genotypic variation in the oilseed rape population for B efficiency. For breeding purposes, a rapid evaluation technique is often desirable to minimise cost and time, for example for B tolerance in wheat (Chantachume *et al.*, 1995). With such a technique, a large number of genotypes can be screened in the early vegetative stage for B efficiency. It was the aim of this study to conduct a similar genotype screening as reported by Chantachume *et al.* (1995). A large number of genotypes from diverse regions of the world were assessed in a solution culture screening, with the relevance of such a technique corroborated by field evaluation.

This project also sought to identify those mechanisms which confer B efficiency. Mechanisms of B efficiency are assumed to operate in a similar way to those proposed by Marschner (1995), with differences in B acquisition by the roots, or in the utilisation of B by the plant (or the involvement of both). Rerkasem and Jamjod (1997) also propose a possible involvement of innate physiological characteristics within the plant; for example cell wall binding capacity, root geometry, rhizosphere effects and B remobilisation.

To date, enhanced rhizosphere acidification has not been examined as a mechanism of B efficiency. Considering B availability and uptake decline with increased soil pH (Goldberg, 1997 and references therein), an increased acidification of the rhizosphere by a B efficient genotype may result in increased B uptake. The following thesis aimed at studying rhizosphere acidification in two genotypes which differ in B efficiency.

Increased B uptake and translocation from root to shoot are associated with mechanisms of B efficiency. Brown and Jones (1971) reported an increased uptake of B in the B efficient tomato cultivar Rutgers over the B inefficient T3238. Bellaloui and Brown (1998) confirmed these observations, citing a reduced rate of B uptake coupled with a reduced translocation of B from root to shoot in the B inefficient T3238. In celery, a mechanism of B efficiency is associated with an increased translocation of B from root to

shoot (Wall and Andrus, 1962; Bellaloui and Brown, 1998). Genotypic variation in B uptake and translocation from root to shoot are examined for a potential mechanism of B efficiency in oilseed rape.

With a foliar application of B to broccoli, B retranslocation reduced the effects of B deficiency broccoli (Shelp *et al.*, 1996), and is postulated as having a role in the B efficiency of rutabaga (Shelp and Shattuck, 1987). B retranslocation may occur via the complexation of borate with a phloem-mobile chelator such as a polyol (Brown and Hu, 1996). To date, information is limited regarding the ability of oilseed rape to retranslocate B. B deficiency symptoms in *B. napus* are most noticeable in the meristematic tissue (terminal buds and leaves) (Chalmers *et al.*, 1992) which may indicate limited B mobility in this species. B retranslocation is quantified in varieties of oilseed rape known to differ in B efficiency to aim at identifying its potential as a mechanism of B efficiency. Soluble carbohydrate concentrations in leaf tissue extract are identified and quantified to indicate the presence or otherwise of a borate-chelator, and hence a mechanism by which B is retranslocated. Any of these mechanisms might operate in oilseed rape.

Chapter 1

Literature review

1.1 Boron Deficiency

1.1.1 Incidence of B deficiency

Since the identification of boron (B) as an essential plant micronutrient by Warington in 1923, over 500 accounts of B fertiliser response within various crops have been reported in the literature (Shorrocks, 1997 and references therein). B deficiency is a significant global problem, leading to reduced crop productivity and farm income. For oilseed rape, its relatively high B requirements (Shorrocks, 1997) make it particularly susceptible to B deficiency. Major growth areas for this crop include China and Australia, with the former country growing up to 80 % of its oilseed rape crop in the Yangtse River Valley, the soils of which are predominantly B deficient (Liu and Chen, 1986). In Australia, B deficiency is not seen as a serious nutritional disorder, with the failure of a *Brassica napus* crop in the Southern Tablelands of New South Wales (NSW) the only published account of B deficiency impacting severely on an Australian oilseed rape crop (Myers *et al.*, 1983). Bell and Huang (1994) provided evidence to suggest that B deficiency may be a problem in canola crops of Western Australia, with 7 farms out of a total of 47 sampled for B nutritional status, found to be within the critical deficiency range. With the expansion of canola into lighter textured acidic soils of NSW and the South East of South Australia, the incidence of B deficiency in canola crops is likely to increase.

1.1.2 Boron deficiency symptoms

In a field situation, B deficiency symptoms in oilseed rape are more noticeable in the meristematic tissue (terminal buds and leaves) (Chalmers *et al.*, 1992). Young leaves develop an interveinal purpling which will often lead to brown patches and necrosis. As

the deficiency increases, the lower leaves become necrotic and brittle, while the plant appears stunted due to the cessation of growth in the apical regions. At flowering, flowers show distorted development and petals fall prematurely, while in the later stages of reproductive growth, pod development and seed set is reduced (Chalmers *et al.*, 1992). Similar deficiency symptoms have also been reported for solution culture-grown oilseed rape (Huang *et al.*, 1996), with the effects of B deficiency on root growth observed 2 days before the onset of B deficiency symptoms in the emerging leaves. The authors report a darkening of the root tissue, with leaves taking on a dark green appearance coupled with an increased thickness. With increased B deficiency, leaves develop adaxial curvature coupled with interveinal purpling which finally results in tissue death.

The effects of B deficiency on seed quality characteristics of oilseed rape is not well known, unlike in many other crops. For example, seed quality of peanut, black gram, green gram, and soybean in Thailand was reduced due to B deficiency (Bell *et al.*, 1990). Over 29 field sites, peanuts grown without the addition of B produced seed with an average of 32 % hollow heart. Hollow heart disorder led to a 35 % depression in peanut seed viability (Rerkasem *et al.*, 1988). Constraints to seed quality brought on by B deficiency also lead to a low germination percentage and a high percentage of abnormal seedlings. In black gram, low seed B reduced the hard seed characteristic, which lead to increased weather damage as fruits matured in the wet and humid conditions of tropical Thailand (Lawn and Ahn, 1985; cited in Bell *et al.*, 1990.). It would be of benefit to the farming community if similar research were conducted for oilseed rape.

1.1.3 Diagnosing B deficiency

Diagnosing B deficiency usually depends on access to analytical equipment (i.e. Inductively Coupled Plasma Optical Emission Spectrophotometry (ICP OES)), although in China, plant analysis is rarely used; application of B fertiliser is often the result of visual diagnosis (Wei *et al.*, 1998).

Due to the relatively low mobility of B in oilseed rape (meristematic tissue is the first to suffer the effects of B deficiency), the youngest open leaf (YOL), is a good indicator of the B status of oilseed rape plants (Huang *et al.*, 1996). Wei *et al.* (1998) also selected the YOL because its B concentrations were responsive to increasing B supply, and correlated with seed yield response in the field. Critical B concentrations for the diagnosis of B deficiency in oilseed rape are 10-14 mg B kg^{-1} DW (Huang *et al.*, 1996), while for the prognosis (the prediction of B deficiency impairing plant growth later in the growth cycle (Bell, 1997) of B deficiency, 20-25 mg B kg^{-1} DW (Wei *et al.*, 1998) and 20-50 mg B kg^{-1} DW (Weir and Cresswell, 1995) have been reported.

1.1.4 Boron requirements

Boron requirements differ at various taxonomic levels. Gupta (1979) observed leaf B concentrations varied among nine plant species grown in the same location under low B supply (Table 1.1). Results are thought to reflect species differences in B requirements for growth, with the high B concentration in brussel sprouts (*Brassica oleracea* L.) of particular interest as it is of the same genus as oilseed rape. Results suggest that the relatively high B concentration may give an indication of a high B requirements one may expect in oilseed rape. A high B requirement is indicated by a high critical deficiency concentration. For example, Bergmann (1983) reported critical deficiency concentrations in a range of species including wheat, red clover, carrot, and sugar beet. In the monocot species, wheat, critical deficiency concentrations ranged from 5-10 mg kg^{-1}, while in the dicot species, values of 25-60 mg kg^{-1} for red clover, 30-80 mg kg^{-1} for carrots, and 40-100 mg kg^{-1} for sugar beet were obtained. These large differences in B requirement between species are now known to be associated with the B requirements of the cell wall, being higher in dicots than monocots (Hu *et al.*, 1996).

The B requirements for reproductive growth are mentioned separately as they differ to vegetative requirements. The B requirement for reproductive growth is generally accepted as being higher than the requirement for vegetative growth (Vaughan, 1977, Dear

and Lipsett, 1987). This can be demonstrated by the high B concentrations in floral tissues relative to vegetative tissues. For example, B concentrations in the leaf, stamen and pistil of B deficient oilseed rape were 10, 19 and 19 mg kg^{-1} dry weight respectively, while in plants adequately supplied with B, B concentrations in these same tissues were 17, 38 and 40 mg kg^{-1} dry weight respectively (Zhang *et al.*, 1994).

Table 1.1. Leaf B concentrations in plant species sampled from the same location (Gupta, 1979)

Plant Species	B Concentration (mg kg^{-1} DW)
Wheat	6.0
Maize	8.7
Timothy	14.8
Tobacco	29.4
Red Clover	32.2
Alfalfa	37.0
Brussel Sprouts	50.2
Carrots	75.4
Sugar beet	102.3

1.1.5 Implications for the thesis research

To breed for B efficiency, Graham (1984) suggests that it is necessary to show (i) a need exists as pressing as other breeders' objectives; (ii) that genetic potential exists to be exploited, and (iii) it is agronomically, economically and ecologically feasible. Using these criteria, the incidence of B deficiency in China alone, and the economic penalty arising from B deficiency in this region, provide for sufficient motivation in breeding B efficient cultivars of oilseed rape.

1.2 The distribution and chemistry of B in soils and plants

Boron is widely distributed in the natural environment (Morgan, 1980) and is found as a constituent of a number of minerals such as axenite, tourmaline, ulexite, colemenite, and kermite (Evans and Sparks, 1983); in which it occurs in chemical combination with oxygen and mainly as hydroxides and silicates. B concentrations in rock range between 10-20 mg kg^{-1}, while soils may have low (<10 mg kg^{-1}) or high (10-100 mg kg^{-1}) B contents (Power and Woods (1997). During the chemical weathering of rocks, B is released forming anions which are predominantly BO_2^-, $B_4O_7^{2-}$, BO_3^{3-}, $H_2BO_3^-$, and $B(OH)_4^-$ (Kabata-Pendias and Pendias, 1992). The species of B which predominates in a given environment depends on the pH of the system; hence the most common forms of B in soil solutions are the undissociated boric acid ($B(OH)_3$), and borate anion ($B(OH)_4^-$).

Boron is the only non-metal in Group III of the periodic table and in terms of structure, normally forms three covalent bonds using sp^2 hybrid orbitals in a plane at angles of 120° (Cotton and Wilkinson, 1976). As the boron atom is small, with only three valence electrons, it readily binds to oxygen to form the weak Lewis acid, boric acid ($B(OH)_3$) (pKa_1 of 9.24; pKa_2 of 12.74 (Dean, 1979)), and has a maximum co-ordination number of four, as observed in the formation of the borate anion (Fig. 1.1). The boric acid molecule is planar with bond angles of around 120° (Loomis and Durst, 1992).

Boric Acid Borate Anion

Fig. 1.1. Boric acid and borate anion configuration.

A characteristic of boron chemistry is the ability of borate to strongly complex with compounds exhibiting *cis*-diol groups (Böeseken, 1949), resulting in an enhanced acidity of the boric acid or borate solutions (Loomis and Durst, 1992). Makkee *et al.* (1985) present the chemistry involved in the interconversion between boric acid and

borate, and the interaction of borate with diols (see Fig. 1.2). The 'borate ion (high pH) can impose its geometry on a diol, or a strongly-complexing diol can impose its geometry on boric acid, producing an anionic borate complex and thus lowering the pH and increasing the electrical conductivity' (Loomis and Durst, 1992).

There are numerous compounds of physiological importance which form stable borate complexes. For example, borate forms stable complexes with ribose and apiose, which exhibit *cis*-diol groups on a furanoid ring (Loomis and Durst, 1992). Fructose, mannitol, and sorbitol all exhibit *cis*-diol groups and exhibit a strong binding capacity with borate (Makkee *et al.*, 1985). Borate complexes with 6-phosphogluconic acid (the first intermediate in the pentose shunt pathway) thereby inhibiting 6-phosphogluconate dehydrogenase (Dugger, 1983) directing carbohydrate metabolism via the glycolytic pathway. Borate complexes the ribityl hydroxyls of the coenzyme NAD^+ (Johnson and Smith, 1976), reducing the formation of NADH.

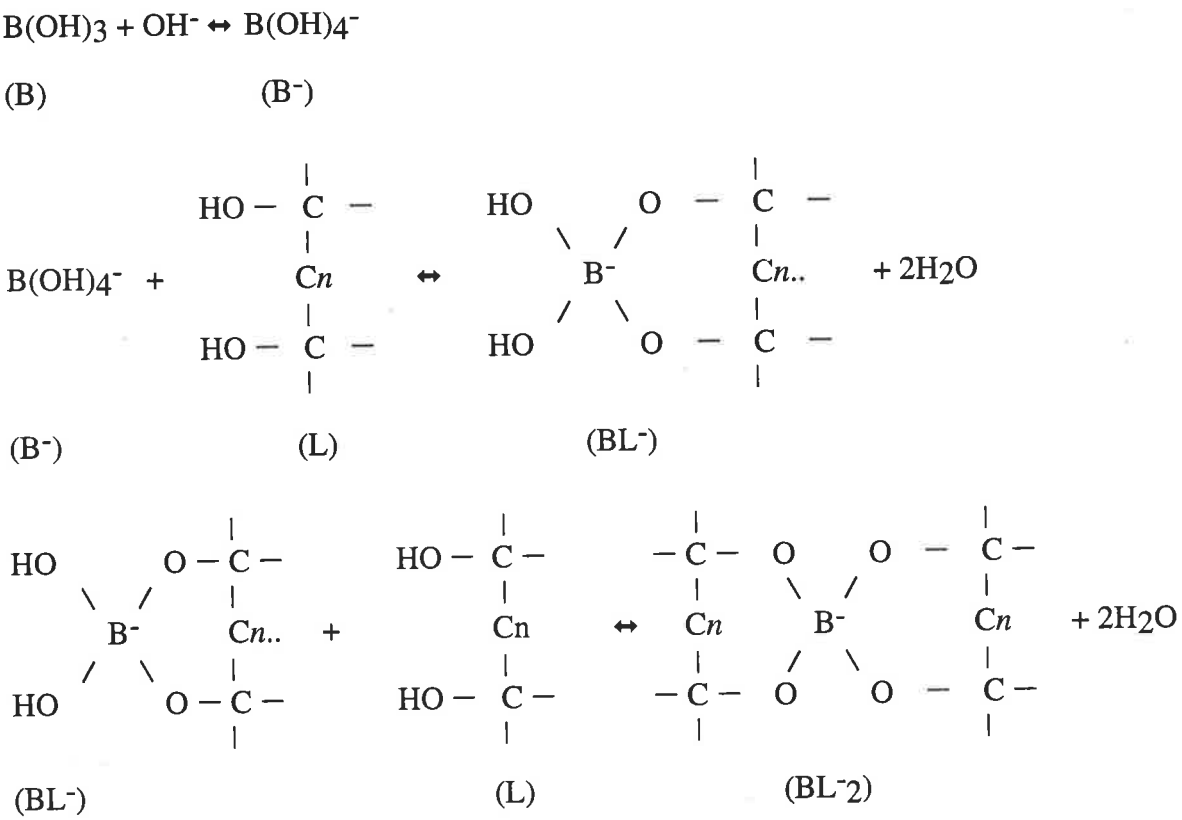


Fig 1.2. Equilibria between boric acid, borate and diols in water (n=0 or 1). Makkee *et al.* (1985). L refers to the ligand which is formed on complexation between borate and diol.

1.3 Factors affecting the availability of B for uptake

1.3.1 Soil adsorbed B

An extensive review of literature regarding the reactions of B with soils is provided by Goldberg (1997). The adsorption and precipitation of B onto the surface of soil particles is of great importance to plant growth as an equilibrium exists between adsorbed and soluble B (Eaton and Wilcox, 1939; Bingham, 1973; Gupta *et al.*, 1985). Boron is strongly sorbed by soils and is predominantly affected by Al and Fe oxides, clay minerals, $CaCO_3$ and the organic matter content of the soil (Goldberg, 1997).

Adsorption of B on oxides of Fe and Al, will in part, govern B solubility (Bingham *et al.*, 1971; Harada and Tamai, 1968; Jin *et al.*, 1988). For example, Jin *et al.* (1988) reported a large amount of B occluded in crystalline Al and Fe oxyhydroxides (up to 74% of the total soil B), and a smaller amount occluded in amorphous Al and Fe oxyhydroxides (up to 34%)

With increased soil pH, B adsorption is enhanced (Bloesch *et al.*, 1987; Goldberg and Glaubig, 1985; Su and Suarez, 1995). Available B (as measured by the hot water extraction method) was positively correlated with pH for acid soils <pH 7, and negatively correlated with pH for alkaline soils >pH 7, at the 95% level of significance (Berger and Truog, 1945). Soluble B content in arid zone soils ranging in pH 6-8, was significantly and inversely correlated with solution pH, with soils having a higher $CaCO_3$ content showing a marked decline in soluble B content (Elrashidi and O'Connor, 1982). $CaCO_3$ not only increases the pH of the soil, thereby reducing soluble B, but also acts as an adsorption surface (Elseewi and Elmalky, 1979; Goldberg and Forster, 1991).

Organic matter can also adsorb B, and therefore affect the soluble B content of soils. For example, organic carbon content was significantly correlated with soluble B content (Berger and Truog, 1945, Elrashidi and O'Connor, 1982; Gupta, 1968), while B adsorption increased in soil with the addition of composted organic matter (Yermiyahu *et al.*, 1995).

1.3.2 Soil environment

Factors affecting the availability of B to plant roots include soil pH, moisture, wetting and drying, temperature and texture (Eaton and Wilcox, 1939; Parks and White, 1952; Biggar and Fireman, 1960; Bingham *et al.*, 1971; Gupta, 1968; Singh, 1964).

The issue of pH affecting B availability has in part been explained previously in section 1.3.1. Put simply, B availability declines with an increase in soil pH (see references above), yet this will also depend on soil texture, which is particularly relevant to the regions of Australia where oilseed rape is currently grown, and where B deficiency is most likely to occur (i.e. NSW; Myers *et al.*, 1983). While acidic in nature, these soils also have a low clay content, indicating a lower fraction of adsorbed B (Wear and Patterson, 1962; Gupta, 1968; Wild and Mazaheri, 1979; Elrashidi and O'Connor, 1982). A low clay content coupled with a low pH, increases the potential for leaching B down the soil profile (Wilson *et al.*, 1951; Aubert and Pinta, 1977) thereby reducing B availability.

The availability of B to plants is also affected by soil moisture. As soils become dry, diffusivity of B decreases as a result of reduced soil solution mobility and an increase in the diffusion path length (Scott *et al.*, 1975). Mezuman and Keren (1981) reported an increased adsorption of B for three Israeli soils was positively correlated with soil to solution ratio. Berger (1949) reported an increased complexation of B to organic matter due to the reduced activity of microbial action because of drought conditions.

Though published data are scarce regarding the effects of soil temperature on B availability in the soil, there does appear to be a negative effect on availability as a result of increased soil temperature. Bingham *et al.* (1971) reported a slight increase in B adsorption as the temperature of an amorphous Mexican soil was increased from 10-40°C.

Fleming (1980) reported an increased B fixation as soil temperature increased, though this may also have been due to an interactive effect with soil moisture.

1.3.3 Non-soil environment

In biological and soil solutions, B occurs mainly as boric acid, $B(OH)_3$, which is a very weak acid that accepts OH^- rather than donates H^+:

$$B(OH)_3 + 2H_2O \rightarrow B(OH)_4^- + H_3O^+ \quad pK_a = 9.25 \text{ (25°C; Greenwood, 1973)}$$

With such a high pK_a, at physiological pH values, the primary B species in solution entering the plant root, is the undissociated boric acid (Raven, 1980), with absorption into root cells affected by a number of non-soil factors, including the humidity, temperature, light intensity and duration, in which plants are grown (Bowen, 1972).

In a recent study, Rawson (1996) demonstrated the effect of high humidity on B supply to the inflorescence of wheat. By enclosing wheat plants in plastic bags, a humid environment was maintained and transpiration reduced. This led to limited xylem supply of B to the inflorescence which resulted in sterility. This also occurred while B concentrations in the growth medium remained adequate.

In his comprehensive study, Bowen (1972), reported a relationship between B concentration of sugarcane plants and air temperature over the range of 8 °C to 37 °C. This relationship also held for water utilisation by the plants, although a 1:1 stoichiometric relationship between B accumulation and water utilisation was not observed, corroborating observations made by Oertli (1963), which questions the premise of a passive absorption of B.

Forno *et al.* (1979) found that at low solution temperatures (i.e. 18 °C compared to 28 or 33 °C for the controls) severe B deficiency symptoms developed in cassava. The development of B deficiency in cassava at suboptimal root temperatures was due both to a lowering of the rate of B uptake per unit root weight and a reduction in the relative size of

the root system. As the authors noted, short term B uptake experiments with sugarcane leaf tissue (Bowen, 1968) and excised barley roots (Bowen and Nissen, 1977) had already shown that the rate of B absorption depended on temperature, but their results revealed that B absorption was apparently more sensitive to root temperature than was plant growth. This was supported by the concentration of B in the tops and roots being affected at both sub-optimal and supra-optimal temperatures.

Mozafar *et al.* (1993) studied the effect of root-zone temperature on the growth and mineral nutrient status of maize (*Zea mays* L.), but included the interactive effects of photoperiod. Bowen (1972) reported a stimulatory effect of increasing photoperiod on B and water uptake, while Mozafar *et al.* (1993) also report a strong interaction between photoperiod (6, 12 and 18 h) and root-zone temperature (9, 15 and 21 °C) on the growth and concentration of B in the plant tops and roots. At the root zone temperature of 21 °C, increasing the photoperiod from 6 to 18 h reduced the concentration of B close to twofold, while at the photoperiod of 18 h, increasing root-zone temperature from 9 to 21 °C did not significantly change the B concentration. This lead the authors to suggest that B concentrations in the tops and roots was more sensitive to photoperiod than to root-zone temperature. The reason for this effect is unknown, though they postulate an effect on the phytochrome and/or phytohormone system.

1.3.4 Implications for the thesis research

The behaviour of B in the soil can vary according to a range of factors, including the innate characteristics of the soil type, the environment, or as a result of the interaction between soil and environment. Genotypes of oilseed rape grown in soils low in B are confronted by these soil factors when trying to access a limited B resource. Their ability to adapt or manipulate this soil environment to reduce the adverse effects of B deficiency will give an indication of their survival mechanisms and their tolerance to low B supply.

Factors affecting B uptake and hence plant growth in a non-soil environment are particularly relevant in the following studies. For developing a suitable screening

technique to evaluate B efficiency in oilseed rape, these factors need to be considered. Screening genotypes in a controlled environment such as a growth cabinet, will minimise the environment interaction and allow for the expression of B efficiency without the variation in the environment which often affects a field situation.

1.4 Boron uptake and translocation

1.4.1 B absorption

There are numerous reports citing active uptake of B (Thellier and Ayadi, 1967; Thellier and LeGuiel, 1967; Bowen, 1968; Bowen, 1972; Bowen and Nissen, 1976, 1977; Bowen, 1981; Dannel *et al.*, 1998), and passive uptake of B (Oertli and Kohl, 1961; Tanaka, 1967a,b; Oertli, 1969; Bingham *et al.*, 1970; Oertli and Grgurevic, 1975; Oertli and Kohl, 1976; Thellier *et al.*, 1979; Nable *et al.*, 1990; Seresinhe and Oertli, 1991; Brown and Hu, 1994). In a recent review on this subject, Hu and Brown (1997) concluded uptake to be most likely a passive, non-metabolic absorption of boric acid, though the authors concede that support for a passive uptake is still not absolute and there is need for more scientific evidence.

Passive B absorption can be argued from both a theoretical and an empirical basis. From theoretical calculations, Raven (1980) derived a permeability coefficient for boric acid across cell membranes of around 10^{-6} cm s^{-1}. He concluded that active transport away from thermodynamic equilibrium would be energetically expensive and unlikely to occur.

There is considerable experimental evidence that B is taken up by passive absorption. Bingham *et al.* (1970) investigated the absorption of B in excised barley roots and noted it to be accumulative, rapid, and unaffected by pH variations of the substrate in the acid range. At a pH above 7, B absorption was reduced in a manner consistent with the decrease of the fraction of undissociated boric acid at more alkaline pH values. At pH 6, changes in substrate temperature, salt composition and level, or the addition of inhibitors

such as KCN and DNP failed to exert any influence on B absorption. The authors postulate that the entire plant is a free space for B.

Oertli and Grgurevic (1975) reported a similar effect when investigating the relative importance of $B(OH)_3$ and $B(OH)_4^-$ for B absorption. They observed a decrease in relative B uptake (B uptake at pH 6 = 100%) with increased solution pH. B in root tissue and external solution approached a diffusion equilibrium which was governed by the undissociated acid.

Brown and Hu (1994) reported a passive absorption of B in cultured tobacco cells, and in the roots of sunflower and squash plants. Passive absorption of B was observed between 0-10 mM B, and was unaffected with the application of inhibitors, DNP and KCN, or temperatures ranging between 2 and 47 °C.

In long term uptake studies over a number of days, B uptake by barley genotypes which differed in their tolerance to B toxicity was linearly related to B supply over a range of B concentrations from normal to excessive (1-1000 μM B), while differing root temperature (5, 10, 15, 20 and 25 °C) did not effect B uptake (Nable *et al.*, 1990). The authors postulate a passive absorption of B, although genotypes differed in their rates of B accumulation. While the mechanisms underlying these effects are unknown, Hu and Brown (1997) have put forward a number of potential reasons. They include: (i) B uptake is under partial metabolic control, and may include an active exclusion mechanism; (ii) root exudation of B complexers restricts B uptake; (iii) differences in root B-adsorption capacity; (iv) differences in physical barriers within the root cell wall; (v) inherent differences in membrane permeability. This last hypothesis is also favoured by Huang and Graham (1990), who studied B uptake into wheat callus. At a cellular level, genotypes varied in their uptake of B, with uptake linear over a range of external B supply indicating a passive absorption of B.

Despite strong arguments for passive uptake of B, there is considerable evidence to suggest an active absorption of B. Bowen (1968, 1972) studied the uptake of B by sugar

cane leaf tissue, meristematic tissue and excised root, and reported a fraction of B uptake was regulated metabolically, correlating with the concentration of the singly charged species $B(OH)_4^-$.

Wildes and Neales (1971) proposed a dual uptake system in carrot (*Daucus carota*) and beet (*Beta vulgaris*) disks, with active transport of $B(OH)_4^-$ and the passive diffusion of $B(OH)_3$. The addition of inhibitors, DNP and anoxia, inhibited B uptake.

Bowen and Nissen (1977) questioned the results presented by Oertli and Grgurevic (1975), and Bingham *et al.* (1970), citing an insufficient desorption period (around 30 s to 1 min) in their methodology which may have resulted in an overestimation of adsorbed B. Bowen and Nissen (1977) using a 30 min desorption period, observed the uptake of B into excised barley roots as an active process, although there was no evidence for an accumulation of B against a concentration gradient. B transport was inhibited with the addition of 0.05 mM DNP, 0.05 mM NaN_3 and 5 mM dicoumarol, and low temperatures. A double reciprocal plot of the B uptake data indicated the presence of six phases, or transitions, which are consistent with multi-phasic uptake of B. The phase shifts were postulated as due to all-or-none allosteric transitions within the single transport mechanism (Bowen, 1981).

Very recent studies have alluded to an active uptake of B in sunflower (*Helianthus annuus* L. cv. Frankasol) (Dannel *et al.*, 1998). From B compartmentation studies, the authors reported an active B uptake at very low external B concentrations. Activity was down-regulated when supply was increased from 0.1 to 10 μM B. At B supply above 100 μM B, passive diffusion of boric acid from the external solution across the plasma membrane appeared responsible for B uptake.

The failure to consider intracellular complexation of B, which gives the appearance of active accumulation of B against a concentration gradient, can question the validity of many reports citing active uptake of B (Thellier and Ayadi, 1967; Thellier and LeGuiel, 1967; Wildes and Neales, 1971; Bowen, 1972). The finding of internal

complexation lead Thellier *et al.* (1979) to change their earlier hypotheses of active B uptake (Thellier and Ayadi, 1967; Thellier and LeGuiel, 1967) to passive diffusion of B.

With support for both active and passive B uptake in the literature, there is insufficient evidence to conclude that passive uptake is the exclusive mechanism of B uptake. Further research is required before a more definitive answer can be deduced.

1.4.2 Long distance B translocation

Long distance translocation of B from root to shoot occurs apoplastically in the xylem, with the accumulation of B into various shoot tissues influenced by the rate of transpiration (Eaton, 1944; Kohl and Oertli, 1961; Oertli and Kohl, 1961; Bowen, 1972).

B concentration gradients within the various tissues of the shoot generally correspond to organ age, and by implication, to transpiration. B concentrations in old leaves are generally higher than in young leaf tissue (Shelp, 1993), while gradients are also present within individual leaves, with petioles and midribs<middle lamina<margins and tips under an excess supply of B (Oertli and Roth, 1969; Oertli, 1993).

Tissue concentration gradients also give an indication of the mobility of B in the phloem. In the species *Isomems arborea* and *Ulmus parvifolia*, B concentrations are always higher in older leaf tissue, whereas in *Apium graveolens* and *Osmanthus fragrans* the opposite is observed (Brown and Shelp, 1997). The former two species show minimal retranslocation whereas the later two species possess the ability to retranslocate B. To try and explain the lack of B mobility, Oertli and Richardson (1970) postulate that B readily enters and is translocated within the bark. It remains water-soluble in plants and therefore its immobility cannot be due to the chemical fixation of B, the inability of B to enter the phloem or the absence of phloem transport. They postulate that B passively enters the phloem where the B concentration is high, with subsequent phloem to xylem leakage at regions of the phloem where its concentration is low. The highly permeable nature of boric acid across plant membranes (10^{-6} cm s^{-1}; Raven, 1980) facilitates such a transfer.

This does not explain the many incidences where B has been reported as phloem mobile, for example in *Vitis vinifera* (Scott and Schrader, 1947); *Gossypium hirsutum* (McIlrath, 1965); *Arachis hypogaea* L. and *Trifolium subterraneaum* L. (Campbell *et al.*, 1975); *Brassica napus* L. ssp *rapifera* (Shelp and Shattuck, 1987); *Brassica oleracea* (Shelp, 1988; Liu *et al.*, 1993); *Prunus*, *Pyrus* and *Malus* genera (Brown and Hu, 1996); *Apium graveolens* and *Osmanthus fragrans* (Brown and Shelp, 1997) to name but a few. Brown and Hu (1996) propose that in the genera *Prunus*, *Pyrus* and *Malus*, sorbitol facilitates B retranslocation. They postulate that in all species where the polyol sorbitol is a major sugar, B is freely mobile. This property of phloem mobility associated with a polyol such as sorbitol (they also hypothesise phloem B mobility where mannitol and dulcitol are present) is associated with the chemical characteristics of the polyol, namely that its structure exhibit *cis*-diol groups, enabling a stable complex between borate and the polyol to be formed (Loomis and Durst, 1992). Recent analyses of celery phloem sap and vascular exudate, as well as sap collected from phloem fed nectaries of polyol producing species, has shown that the majority of B is complexed to the ligands mannitol, sorbitol and fructose (Hu *et al.*, 1997).

1.4.3 Implications for the thesis research

The mechanism underlying the uptake of B is complex and in need of further scientific investigation. Studying the uptake of B in oilseed rape will contribute toward the knowledge surrounding this complex topic. Net B uptake can differ within a species (Nable *et al.*, 1990), and for oilseed rape similarly, the potential exists for genotypic differences in B uptake. The identification of active or passive uptake of B in oilseed rape may also provide further information leading to a better understanding of the function of B. The expenditure of energy in an active uptake system may exist to maintain an important intracellular function of B. Research in this thesis will explore this possibility.

While studies into the mobility of B in oilseed rape are virtually non-existent, the mobility of B in the related species *B. oleracea* and *B. napus* ssp. *rapifera*, indicate a

potential for B retranslocation in oilseed rape. The reported presence of mannitol in *B. oleracea* (Trip *et al.*, 1963) may give an indication of the mechanism involved in enabling retranslocation to occur.

1.5 Function of B

The primary mechanism of action for B within the plant is still not well understood. Parr and Loughman (1983) postulate 10 roles for B based on their review of literature: (1) sugar transport; (2) cell wall synthesis; (3) lignification; (4) cell wall structure; (5) carbohydrate metabolism; (6) RNA metabolism; (7) respiration; (8) IAA metabolism; (9) phenol metabolism; (10) membranes. Marschner (1995b) postulates that this list may indicate that B is involved in numerous metabolic pathways, or the effects of B are due to a cascade effect, as is observed for the phytohormones. The following section will give an appraisal of the literature in relation to the most likely action of B in higher plants. An extensive review on the function of B is provided by Goldbach (1997).

1.5.1 The role of B in the cell wall

Very early reports of B effects on cell wall properties, indicate a role for B in the structure of the cell wall. Palser and McIlrath (1956) observed thinner xylem vessel cell walls in B deficient tomato, turnip and cotton. In his comprehensive study on the effect of B on cell wall structure in celery, Spurr (1957) reported a pronounced effect of B on cell wall thickness. Under B deficiency, collenchyma cell walls were markedly thinner, while the cell walls of the phloem parenchyma and the ground parenchyma become thicker. In B deficient collenchyma, fewer lamellae exist, and the angular corner thickenings of the cells are absent. B was hypothesised to affect the rate and process of carbohydrate condensation into wall material.

Lee and Aronoff (1966) reported an effect of B in the cell wall of sunflower leaves. Using electron microscopy, the authors examined leaf mesophyll cells of B deficient sunflower plants, reporting a change in cellular structure within 3 days after the removal of B from the nutrient solution. At this stage no visual deficiency symptoms were

recognisable. Damaged cells were localised in groups of a few to several cells near the basal margin of the leaf, with increased B deficiency leading to a thickening of leaf mesophyll cells, indicating a reduced structural integrity of the cell wall.

Matoh *et al.* (1992) studied the effect of B supply on cultured cells of tobacco (*Nicotiana tabacum* L. cv. Bright Yellow 2) and observed thicker, less tightly packed cell walls under B deficiency, while Golgi bodies were accompanied by larger numbers of secretory vesicles. Yamauchi *et al.* (1986) postulate a structural role for B in the cell wall, with B decreasing the amount of Ca associated with pectin constituents in B deficient leaf tissue. Ca and B were shown to be associated with different cell wall components, and it is hypothesised that B may act to maintain the Ca-pectin association.

The amount of B in the cell wall is an indication of its importance in this cellular fraction. Matoh *et al.* (1992) reported up to 98 % of cellular B of tobacco was located in the cell wall. Hu and Brown (1994) examined the localisation and chemical fractionation of B in squash plants and cultured tobacco cells. As squash plants were subjected to increased B deficiency, a greater proportion of cellular B was localised to the cell wall (96 to 97 % of cellular B), and in particular, was bound within the pectin fraction. The differential B requirement of a species was also found to be associated with the concentration of cell wall pectin in plant tissues (Hu *et al.*, 1996), and helps to explain the differences in B requirements between monocots (3-10 μg B g^{-1} DW; Jones *et al.*, 1991) and dicots (20-30 μg B g^{-1} DW; Jones *et al.*, 1991).

Recent research efforts have now isolated and characterised the borate-polysaccharide complex from cell walls (Matoh *et al.*, 1993; Matsunaga and Nagata, 1995; Ishii and Matsunaga, 1996; Kobayashi *et al.*, 1996; Matoh *et al.*, 1996; O'Neill *et al.*, 1996). Matoh *et al.* (1993) purified a B-polysaccharide complex from a Driselase digest of cell walls of radish roots. The complex had a molecular weight of 7.5 kDa and contained B (0.232 %), w/w), uronic acid (52.3 %, w/w) and neutral sugars (32.4 %, w/w). ^{11}B-NMR spectroscopic analysis suggested that B was present as a tetravalent 1:2 borate-diol

complex. O'Neill *et al.* (1996) reported that borate esters covalently cross-link a pectic polysaccharide, rhamnogalacturonan II (RG-II), in the cell walls of suspension-cultured sycamore cells, and etiolated pea stems. Kobayashi *et al.* (1996) demonstrated that boric acid forms cross links between two identical RG-II chains, through borate-diol ester bonding in the radish B-polysaccharide complex. Matoh *et al.* (1996) examined the presence of the RG-II complex in 24 species from higher plants. In all the species examined, the majority of cell-wall B was associated with RG-II in a 1:2 complex.

1.5.2 The role of B in the membrane

While the concentration of B in cellular membranes is lower than in the cell wall (Pollard *et al.*, 1977; Parr and Loughman, 1983; Tanada, 1983), evidence suggests a requirement for B in maintaining membrane function (Robertson and Loughman, 1974; Pollard *et al.*, 1977; Parr and Loughman, 1983; Blaser-Grill *et al.*, 1989; Schon *et al.*, 1990; Goldbach *et al.*, 1990; Roldán *et al.*, 1992; Barr *et al.*, 1993).

B deficiency appears to impair ion transport. Robertson and Loughman (1974) demonstrated an inhibition of phosphate transport in *Vicia faba*, as well as the potassium analogue (^{86}Rb) under B deficiency; this effect could be reversed within 90 minutes of addition of B. Similar effects were also witnessed by Pollard *et al.* (1977) who observed a reduced capacity for phosphate absorption in B deficient *Zea mays* and *Vicia faba*. Phosphate absorption was restored within 1 h of reintroducing B.

The effect of B is observed in the polarisation of the root membrane, FeCN dependent H^+ release and ATPase activity. While previous authors had demonstrated an effect of B on the hyperpolarisation of the membrane (i.e. Blaser-Grill *et al.*, 1989), a lack of washing in the research protocols led to some ambiguity in their results. Schon *et al.* (1990) propose that without an adequate washing procedure, the membrane potential may also include cell wall potential, or a portion of the diffusion potential of the cells. Using electrophysiological techniques coupled with appropriate washing procedures, Schon *et al.* (1990) demonstrated a direct effect of B on the membrane potential of sunflower root tip

cells. Exposure to B induced a significant plasmalemma hyperpolarisation within 20 minutes and a greater accumulation of K^+ after 48 h, which they propose may have stimulated the H^+-ATPase (proton pump) and led to the observed hyperpolarisation of root cell membranes. Alternatively, they suggest that B may stimulate the proton pump, with the subsequent hyperpolarisation resulting in an increased driving force for K^+ influx.

Goldbach *et al.* (1990) also reported a reduced FeCN induced net H^+ release in suspension cultured carrot and tomato cells of up to 50 %. This effect was reversed within 60-90 minutes on the addition of B. As proton release was completely suppressed by vanadate, this indicated an ATPase driven process.

Roldán *et al.* (1992) studied the effect of B excess and deficiency on the H^+ efflux from excised roots of sunflower (*Helianthus annuus* L. cv. Enamo) seedlings and on plasma membrane H^+-ATPase in isolated microsomes. In both toxic and deficient levels of B, or when exposed to light, the capacity of roots to externally acidify the growth medium declined.

Boron has also been demonstrated to affect auxin activity, linking it to the regulation of growth. Robertson and Loughman (1974) demonstrated an increase in exogenous levels of IAA in B deficient *Vicia faba*, which was proposed as a means of explaining many of the physiological responses observed under B deficiency (i.e. inhibition of root elongation, induction of lateral roots, and modified carbohydrate translocation). Pollard *et al.* (1977) examined the effect of IAA on ion absorption in B deficient *Zea mays* and *Vicia faba* and demonstrated an inhibition of ion uptake at IAA concentrations as low as 10^{-5}-10^{-4} M. Goldbach *et al.* (1990) reported that B deficiency reduced FeCN induced net H^+ release in suspension cultured carrot and tomato cells by up to 50%, but differences between B treatments did not appear when auxin was omitted from the growth medium; these differences were again observed 30 minutes after the addition of auxin to auxin deficient cell cultures. This suggested that an adequate supply of B was required for auxin action to take place and its presence is required to regulate growth .

This also supports the work of Jarvis *et al.* (1984), who postulate a role for B in regulating the activity of IAA-oxidase. An increased activity of this enzyme would reduce auxin concentrations in the root and maintain root growth.

Goldbach *et al.* (1990) recently proposed an interaction of B with auxins by '(1) B stabilising the plasma membrane in such a way that auxins can be bound to receptor sites; (2) B deficiency reducing protein synthesis probably by inhibiting DNA synthesis; (3) the signal transduction from the receptor-bound auxin to protein synthesis being thought to be mediated by secondary messengers such as Ca^{2+}/calmodulin and or inositol phosphates'. At this stage, this is only speculation.

Recently, B has also been implicated in NADH oxidase activity, which is a significant role as it seems to impact directly on growth (Barr *et al.*, 1993). Barr *et al.* (1993) studied the effect of B deficiency on plasma membrane electron transport reactions and associated proton secretion in carrot cells. In keeping with previous reports (see above) of the effect of B on proton secretion, the authors reported a stimulatory effect of B on H^+ secretion in the presence of FeCN. The hormone sensitive plasma membrane NADH oxidase (also called ascorbate free radical (AFR) oxidoreductase) was inhibited by boron deficiency, and on the addition of B or 2,4-dichlorophenoxy acetic acid to the deficient cells, activity could be restored. Gramicidin, a channel-forming protonophore, further stimulated NADH oxidase indicating a coupling of the oxidase and proton movement. These results indicate that B is directly associated with cell growth through its effect on the plasma membrane NADH oxidase and H^+ secretion.

Lukaszewski and Blevins (1996) recently reported a negative effect of B deficiency on ascorbate (ASC) metabolism and root growth through a proposed inhibition of NADH oxidase and the subsequent altering of the ASC redox state. This arose from a decline in root tip ASC concentration, with no subsequent effect on the oxidised forms of ASC (ascorbate free radical (AFR) and dehydroascorbate (DHA)).

The effects of B deficiency on enzymatic activities may also point to a role for B in maintaining the structural integrity of the membrane. Parr and Loughman (1983) postulate that 'B could interact with the membrane via glycoprotein or glycolipid components to maintain the most efficient conformation'. If B acts in this way, it could aggregate the components of the membrane into domains, or change the lipid packing and hence fluidity of the membrane. The complex formation between borate and sugars may also affect transport capacity (Parr and Loughman, 1983). The increased enzymatic activity often witnessed under B deficiency may in part support such a theory (see Shkolnik, 1984, and references therein), with B acting to complex these enzymes onto the plasma-membrane. This is also supported by recent reports where membrane-bound protein in Petunia (*Petunia hybrida*) pollen tubes declined under B deficiency (Jackson, 1991). Cakmak *et al.* (1995) also reported a stabilising effect of B on the plasma-membrane of *Helianthus annuus* leaves. Under B deficiency, K^+ leakage was 35-fold higher than in B sufficient leaves, while for sucrose, 45-fold higher, and for phenolics and amino acids, 7-fold higher. Their results may indicate a role for B in maintaining the structural integrity of the plasma-membrane.

While the effect of B deficiency on plasmalemma bound reactions is considered a secondary event (Findeklee and Goldbach, 1996; Goldbach, 1997), a structural requirement for B in the plasma-membrane may, when further evidence is presented, be deemed a primary function for B.

1.5.3 The role of B in sugar metabolism and transport

There are numerous accounts of B controlling sugar metabolism and for a detailed account of these reports, Goldbach (1997) is recommended. One of the most significant ways in which B may act to regulate sugar metabolism is via its complexing with 6-phosphogluconic acid (the first intermediate in the pentose shunt pathway) and inhibition of 6-phosphogluconate dehydrogenase (Lee and Aronoff, 1967; Dugger, 1983). The presence of B ensures carbohydrate metabolism via the glycolytic pathway, while under B

deficiency, minimal regulation occurs leading to phenolic acid metabolism via the pentose phosphate pathway, and hence adverse effects due to phenol oxidation to reactive quinones and the enhancement of toxic oxygen species which impact on membrane integrity (Cakmak and Römheld, 1997).

The role for B in sugar transport is less clear. Gauch and Dugger (1953) postulate a role for B in carbohydrate transport, suggesting the negatively charged sugar-B complex can more easily traverse cell membranes, or that B may be a constituent of the membrane site across which the sugar moves. In their study, sugar uptake and transport was enhanced in the presence of B. Their theory is supported in part by studies which show reduced C translocation in B deficient plants (Sisler *et al.*, 1956; van de Venter and Currier, 1977; Boyce and Blevins, 1993; Hoddinott, 1993), although a direct role of B in C transport has recently been questioned, as borate only weakly complexes sucrose and there appears to be little evidence to suggest a B requirement in the phloem loading of sucrose. Reduced translocation of carbohydrate in B deficient tissue may merely result from induced callose formation in the sieve tubes, or reduced sink activity (Marschner, 1995b). This is also supported by McIlrath and Palser (1956), who demonstrated an impaired carbohydrate translocation only after the effects of B deficiency were sufficient to cause necrosis in the sieve tubes. Odhnoff (1957) corroborates these results, by showing that carbohydrate synthesis was unimpaired in the leaves prior to the onset of severe B deficiency, an event commensurate with a continued sugar translocation to the root. Before translocation was fully blocked, root growth was inhibited and carbohydrates accumulate. The shoot is not affected until some time later, when the effects of deficiency are more severe, and only then do carbohydrates accumulate in the shoot. Van de Venter and Currier (1977) reported a reduced sugar translocation in B deficient bean (*Phaseolus vulgaris*) plants, commensurate with callose formation in the sieve tubes. Callose deposition was attributed to cellular damage. What was unusual from both this report and that of McIlrath and Palser (1956), was that B deficiency did not lead to disorganisation of the cambium or phloem in cotton (*Gossypium hirsutum*), even though C transport was significantly reduced in this species. This lead van de Venter and Currier (1977) to

suggest that other factors may contribute to a reduction in assimilate movement in B deficient plants. This may merely reflect a reduced sink activity, or may indicate a more direct role of B in the transport mechanism.

1.5.4 Implications for the thesis research

In recent years the identification of a structural role for B in the cell wall has contributed greatly to our knowledge of a possible function of B in higher plants. Without the regulation of B uptake to maintain a critical intracellular function, it is hard to determine a primary function of B outside of this structural role in the cell wall and membrane.

1.6 B in reproductive growth

Schmucker (1933, 1934) was perhaps the first to demonstrate an effect of B on pollen germination and pollen tube growth. In the species *Nymphaea,* pollen germination was enhanced with the addition of stigmatic extract, which contained a high concentration of B. In the absence of B, pollen tubes swell and burst (Schmucker, 1934). Visser (1955; cited in Vasil, 1963) demonstrated a direct relationship between pollen tube length and boric acid concentration. Similar effects were also reported by Vasil (1963) and Cheng and Rerkasem (1993), though unlike Visser (1955), a direct effect of B on germinating pollen grains was observed.

In cereals, B deficiency lead to atrophy of anthers, while the embryo sac and the surrounding tissues remained unaffected (Löhnis, 1937, 1940; Whittington, 1957). Birnbaum *et al.* (1974) studied the interaction of B and phytohormones (indoleacetic acid (IAA) and gibberellic acid) on unfertilised cotton ovules grown *in vitro.* A constant supply of B was necessary to maintain fiber elongation and prevent callusing of epidermal and subepidermal cells. Dugger and Palmer (1980) demonstrated that in fibers adequately supplied with B, both ß-1,4- and ß-1,3- water soluble polymers formed, while in B deficient fibers, ß-1,3- water soluble polymers predominate. Dugger and Palmer (1985) observed an increased incorporation of glucose into ß-1,3-glucan in cotton ovules. Given

the problems associated with increased callusing in the supply of photoassimilate through vascular tissue (van de Venter and Currier, 1977), a number of effects witnessed as a result of B deficiency may be due to an increased deposition of callose into vascular tissue of floral structures.

Due to the high B requirements of the cell wall (Matoh, 1997), a likely role for B in reproductive tissues is as a structural component of the cell wall. This does not exclude other potential roles for B. Moewus (1950) reported a regulatory role for B in reproduction, with the application of B reducing the inhibitory effects of rutin and quercitrin on self pollination in *Forsythia.* Self pollination occurs only in the presence of B. Blevins and Lukaszewski (1998) postulate a role for B in pollen tube growth as a chemotactic agent. This is proposed due to a recent report where pollen tubes of petunia grew toward a higher B concentration from stigma to ovary (Robbertse *et al.*, 1990). Jackson (1991) presented evidence to support a role for B in the movement of protein into petunia (*Petunia hybrida*) pollen tube membranes, indicating a possible role for B within the membrane itself, either in maintaining the structural integrity of the membrane, or in a regulatory function. Borate-complexation with sugar residues is proposed as a mechanism for the supply of protein structural units into the cell wall and membrane.

Sidhu and Malik (1986) studied the metabolic role of B in germinating pollen and concluded that since pollen does not contain lignin, B having a primary role in lignin biosynthesis was unlikely. Not disputing this, one may envisage an indirect effect of B on lignin deposition into the cell wall of the pollen tube. For example, Liu and Ger (1997) studied the enzymatic activity during pollen germination in maize (*Zea mays* L.) and observed an increased activity of phenylalanine ammonia-lyase (PAL) and peroxidases, accompanied by a decline in phenolic compounds. PAL catalyses the deamination of phenylalanine to cinnamic acid (Elkind *et al.*, 1990; Bate *et al.*, 1994), and peroxidases polymerise phenolic precursors such as ferulic and cinnamic acids into lignin (O'Malley *et al.*, 1993; Liu *et al.*, 1994; McDougall *et al.*, 1994), a component of the pollen tube cell wall. The presence of an adequate supply of B reduces phenol oxidation, maintaining the

synthesis of phenolic alcohols, the precursors of lignin (Cakmak and Römheld, 1997). In the absence of B, reactive products of phenol oxidation (i.e. quinones) are enhanced, ultimately leading to impaired cellular function and reduced lignin biosynthesis.

1.6.1 Implications for the thesis research

While the effects of B deficiency on reproductive development are quite well known, the role of B is less clear. Genotypes which differ in B efficiency can provide the basis for further studies into processes which confer reproductive success. This will contribute to the knowledge surrounding the role of B in reproductive development.

1.7 Boron efficiency

1.7.1 Definition and occurrence

Nutrient efficiency is defined as the ability of a cultivar to grow and yield well in soils too deficient in the nutrient for a standard cultivar (Graham, 1984). B efficiency by this definition does not imply a mechanism. Likewise, B efficiency can be the crop's ability for nutrient uptake and metabolic nutrient utilisation (Haneklaus and Schnug, 1993).

Using the above criterion, genotypic variation in response to B is a widespread phenomenon, canvassing a large range of species. The degree of genotypic variation is highlighted in a recent review by Rerkasem and Jamjod (1997) (Table 1.2). Genotypic variation has also been reported in garden beet (Walker *et al.*, 1945), cotton and sunflower (Agarwala *et al.*, 1984), tomato (Brown and Jones, 1971) and in sesame and mustard (Sakal *et al.*, 1991).

To date there are few published reports of B efficiency in oilseed rape. From field evaluation using the efficiency definition of Graham (1984), Sakal *et al.* (1991) reported B efficiency in the *B. juncea* variety Pusa Bold, while Yang *et al.* (1993) reported B efficiency in the *B. napus* breeders' line 92-13. Yang *et al.* (1994) studied the B efficiency in a range of varieties and breeders' lines of oilseed rape in both the field (at

Tonglu County, Zhejiang Province, China) and in a pot study. In the field, both Zhongyou 821 and 92-13 expressed a high level of B efficiency, while in a pot study, the hybrid canola variety, Huashuang 2, expressed a high level of B efficiency.

Table 1.2. Crop and other domesticated plants reported to have genotypic differences in their response to low boron (Source: Rerkasem and Jamjod, 1997).

Plant	Reference
Apple	Tagliavini *et al.* (1992)
Barley	Rerkasem and Jamjod (1989)
Beet	Kelly and Gabelman (1960)
Black Gram	Rerkasem (1990)
Oilseed Rape	Yang *et al.* (1993)
Broccoli	Shelp *et al.* (1992)
Cotton	Smithson and Heathcote (1974)
Celery	Pope and Munger (1953)
Cacao	Tollenaar (1966)
Citrus	Smith (1966)
Green Gram	Rerkasem (1990)
Grape	Scott (1944)
Maize	Mozafar (1993)
Peanut	Keerati-Kasikorn *et al.* (1993)
Pinus caribaea	Martinez *et al.* (1989)
Pinus ponderosa	Wright *et al.* (1969)
Pinus radiata	Lambert and Turner (1977)
Plum	Benson *et al.* (1966)
Pomegranate	Singh *et al.* (1990)
Strawberry	Willis (1945)
Sunflower	Blamey *et al.* (1979)
Soybean	Rerkasem *et al.* (1993)
Tomato	Wall and Andrus (1962)
Wheat	Singh *et al.* (1976)

1.7.2 Mechanism/s of B efficiency

An increased B uptake is thought to be one mechanism underlying B efficiency. Differential B uptake appears to be under the control of the root (Eaton and Blair, 1935; Brown and Jones, 1971; Bellaloui and Brown, 1998). Eaton and Blair (1935) reported a differential accumulation of B in sunflower leaves was dependent on the root stock. On artichoke rootstock, B accumulation was higher than when grown on its own sunflower rootstock. Likewise, Brown and Jones (1971) reported that the B efficient tomato cultivar Rutgers was around 15 times more efficient than the B inefficient T3238 in absorbing the B in the growth medium. Reciprocal grafts showed that the controlling process for B transport was located in the root. This is also the case for barley genotypes known to differ in their tolerance to B toxicity (Nable *et al.*, 1990). Nable *et al.* (1990) reported a passive uptake of B over a range of external B supply with B tolerant (tolerant to B toxicity) genotypes exhibiting a lower rate of B uptake (net B accumulation).

Yang *et al.* (1993) also reported an increased capacity to take up B in the B efficient oilseed rape cultivar 92-13, while Sakal *et al.* (1991) screened six varieties of mustard and sesame under low B supply and recorded yield responses up to 38% in sesame, while in the mustard lines, yield responses in the order of 27% were recorded. Efficiency in yield response was associated with an increased B uptake.

B efficiency may be associated with an increased translocation from root to shoot. For example, Pope and Munger (1953) reported significant genotypic variation in B efficiency in celery. The B efficient Summer Pascal produced significantly more dry matter than the B inefficient S48-54-1. Bellaloui and Brown (1998) studied this effect further and concluded that the inefficient S48-54-1 exhibited a restriction in B translocation from root to shoot. A similar result was observed between the tomato genotypes Rutgers (B-efficient) and T3238 (B-inefficient), though with these two cultivars, there were also differences in the rate of B uptake.

Rerkasem and Jamjod (1997) in reviewing variation in genotypic response to low B supply cited a number of potential mechanisms which may contribute toward the mechanism of B efficiency. Two of these mechanisms were root geometry and rhizosphere effects. To date there has been no detailed research into the effect of these two factors on B uptake. With regard to rhizosphere acidification enhancing B uptake, considering that soluble B content in soils is significantly correlated with solution pH (Elrashidi and O'Connor, 1982), with B uptake increased at lower soil solution pH (Wear and Patterson, 1962), it is unusual that the effects of rhizosphere acidification on B uptake have not been considered before.

To date, there is little evidence to suggest a mechanism of B efficiency due to variation in cell wall B requirement. Given that the majority of cellular B is located in the cell wall, one may envisage small changes in cell wall B requirements would impact on a genotypes efficiency in utilising B. Recently, Bellaloui and Brown (1998) provided evidence to exclude cell wall composition as a means to explain differences in B efficiency in celery, wheat and tomato.

While B remobilisation is generally limited in most species (Brown and Shelp, 1997 and references therein), there is evidence which supports a possible role between B efficiency and B remobilisation. Blamey *et al.* (1979) reported a higher B concentration in the young leaf tissue of the efficient sunflower genotype than in the same tissues of its inefficient counterpart. Rerkasem *et al.* (1990) reported higher B concentrations in the young leaves of black and green gram genotypes less affected by B deficiency. Shelp *et al.* (1992) reported a higher floral B concentration in the B efficient broccoli cultivar when grown in a B deficient environment, while Shelp and Shattuck (1987) reported an increased capacity to remobilise B in two rutabaga cultivars was correlated with their tolerance to B deficiency. Clearly, more research is required to indicate a role for B remobilisation in the B efficiency mechanism across a range of species.

In the majority of the above cases, the efficiency mechanism has been located in the vegetative tissue (i.e. root and shoot). In wheat this does not appear to be the case (Rerkasem *et al.*, 1993). The authors studied the effect of B deficiency on reproductive development and grain set in two genotypes, SW41 (B-inefficient) and Fang 60 (B-efficient). Early vegetative response to B was measured in the elongation of the youngest emerged blade. According to vegetative response, SW41 was B efficient and Fang 60 inefficient. At grain set, the opposite response was observed. The lack of relationship between vegetative and reproductive response indicates that the mechanism of B efficiency in Fang 60 is within the reproductive structures. In a similar study, though using the B efficient Sonora 64 instead of Fang 60, Cheng and Rerkasem (1993) found no differences in pollen viability between the two genotypes, while they postulate a difference in the supply of B to the germinating pollen in the stigma and style.

1.7.3 Implications for the thesis research

With the need for introducing B efficient oilseed rape cultivars onto B deficient soils, the identification of B efficient germplasm will allow plant breeders the opportunity to access a broader gene pool of B efficiency, which is as yet still relatively small. The identification of a B efficiency mechanism may also provide a marker for future selection of B efficient genotypes, as well as a site for genetic manipulation. It will also provide information regarding the physiological processes involved in conferring B efficiency.

Chapter 2

Genotypic Variation in Oilseed Rape (*Brassica* spp.) Grown in Low-Boron Solution Culture.

2.1 Introduction

Boron (B) deficiency is a world-wide problem (Liu *et al.*, 1981; Sillanpää, 1982; Welch *et al.*, 1991) which reduces the yield of many agriculturally important crops (Bussler, 1962; Tollenaar, 1968; Gupta and Cutcliffe, 1975; Myers *et al.*, 1983; Porter, 1993). The introduction of B efficient cultivars onto these problem soils is one option in helping to maintain a high yield potential. Nutrient efficiency is defined as the ability of a cultivar to grow and yield well in soils too deficient in a nutrient for a standard cultivar (Graham, 1984). B efficient genotypes of sesame and mustard (Sakal *et al.*, 1991) and oilseed rape (Yang *et al.*, 1993; Yang *et al.*, 1994) have been identified using such a definition. Haneklaus and Schnug (1993) also defined nutrient efficiency as a crop's ability for nutrient uptake or metabolic nutrient utilisation. B efficient genotypes of celery (Pope and Munger, 1953) and tomato (Wall and Andrus, 1962; Brown and Jones, 1971) have been identified using this definition. In oilseed rape, the number of genotypes screened has been very small (Sakal *et al.*, 1991; Yang *et al.*, 1993; Yang *et al.*, 1994), giving no real understanding of the extent of B efficiency in the oilseed rape gene pool. A more comprehensive evaluation of germplasm is required because of its sensitivity to B deficiency (Shorrocks, 1997).

The 'ideal' measure of B efficiency is a field based evaluation. This requires considerable time and cost, and there are environmental constraints to successfully completing such an exercise. Corroboration of field results with an early growth screening technique would allow plant breeders to minimise these constraints. Such an approach has been proposed by Chantachume *et al.* (1995); root length is used to discriminate between genotypes of wheat with different levels of tolerance to B toxicity. The success of the

technique appears related to the sensitivity of root growth to B toxicity. Likewise, B deficiency also reduces root growth, and its effect is measurable within hours of removing B from the growth medium (Warington, 1923; Odhnoff, 1957; Whittington, 1958; Neales, 1960; Cohen and Lepper, 1977). The methodologies employed by Chantachume *et al.* (1995), or some variation of it, may also serve to identify B efficient oilseed rape genotypes.

The following study aims to examine a number of plant growth parameters known to be affected by B deficiency (relative root length, root elongation rate and total root dry weight), to indicate the vegetative response of a genotype to low B supply. The definition of B efficiency proposed by Graham (1984) will be used to characterise genotypic responses. Plants are grown in solution culture; hence preliminary experiments will determine optimum levels of external B supply for growing oilseed rape. A subsequent chapter (Chapter 3) examines the effectiveness of the screening technique proposed in this chapter.

2.2 Materials and Methods

Experiment 2(a) Defining the critical concentration of external boron for deficiency in oilseed rape grown in solution culture

2.2.1 Design

The experiment was designed with the canola cultivar Barossa grown for up to 30 days in 6 B treatments and duplicated.

2.2.2 Seed

Seeds of the canola cultivar Barossa, supplied by the canola breeding program in Horsham, Victoria, were imbibed for 36 h at 20°C in distilled water in petri dishes lined with moistened filter paper. Germinated seed were selected for uniformity and transferred to support collars in lids of aerated 2.2 L acid washed Monbulk© PVC containers filled with 2 L of aerated complete nutrient solution.

2.2.3 Nutrient Solution and Growth Conditions

The plants were grown using a chelate-buffered nutrient solution system. The composition of solution in which plants were grown was: $Ca(NO_3)_2$, 1 mM; KNO_3, 1.5 mM; $MgSO_4$, 250 µM; KCl, 50 µM; $NH_4H_2PO_4$, 50 µM; MoO_3, 0.1 µM; FeHEDTA, 20 µM; NiHEDTA, 0.1 µM; ZnHEDTA, 10 µM; MnHEDTA, 0.4 µM; CuHEDTA, 1 µM; MES, 5 mM; K_3HEDTA, 25 µM (adjusted to pH 6.0 with KOH). B treatments were applied at rates of 0, 0.01, 0.05, 0.2, 0.8, 12.5 and 100 µM B (as boric acid). Rates of B were chosen after a preliminary investigation into external B concentrations, using rates of 0, 0.8, 1.6, 3.1, 6.3, 12.5 and 25 µM B, in which a response was observed in 10 day old canola plants only at 0 and 0.8 µM B (Appendix I). Hence for the current experiment, it was necessary to lower the rates of B, as well as incorporate a higher B treatment. Exclusion of light and prevention of nutrient contamination in culture solutions was achieved by adopting the methodology described by Norvell and Welch (1993); in addition the nutrient solution was changed every 5 days to minimise nutrient depletion and pH fluctuations. Plants were grown at 25/20°C day/night temperature, with a 14/10 h light/dark photoperiod supplied by high-pressure mixed-metal halide lamps delivering a photon flux density of 350 µmol m^{-2} s^{-1}.

2.2.4 Measurements

Two harvests were taken, at day 20 (D20) and day 30 (D30). At each harvest, roots was rinsed for 30 s in high purity water (18 MΩ cm^{-1} conductivity, prepared by reverse osmosis and ion exchange), blotted dry with tissue paper, and plant tissue separated into young leaves, old leaves, stem and root, and oven dried at 80°C for at least 24 h. Samples were weighed and digested at 140 °C in 70 % (w/w) HNO_3, then analysed for all essential elements (except N) by inductively coupled plasma optical emission spectrometry (Zarcinas *et al.*, 1987), using an ARL 3580B ICP analyser. Critical external B concentrations were derived from Mitscherlich and rescaled Mitscherlich equations (Barrow and Mendoza, 1990):

Mitscherlich: $Y = a - b\,(\mathrm{Exp}\,(-cx))$

Rescaled Mitscherlich: $Y = a - \dfrac{b}{(1 + m\,c\,x^{n})^{1/m}}$

where Y indicates the yield, in this case plant tissue dry weight, and a, b, c are coefficients, while m and n are indices.

Experiment 2(b) Determining appropriate selection criteria for evaluating boron efficiency in oilseed rape.

2.2.5 Design

Nine oilseed rape (*Brassica napus* L.) genotypes (Table 2.1) were grown in solution culture at low (0.1 µM B, designated B0.1) and adequate (13 µM B, designated B13) levels of B. Sixteen plants of each genotype were grown together with all other varieties in aerated 12 L acid washed PVC containers filled with 10 L of complete nutrient solution (Plate 2.1). For root growth measurements, each plant was considered a single replication (ie. 16 replications in total), while for ICP analyses, plants were separated, bulked and analysed, giving in total, 3 replications of each treatment, with 2 replications consisting of 5 plants each, and the third replication, 6 plants.

Table 2.1. Cultivar name, seed quality characteristic and origin of nine oilseed rape cultivars

Variety	Seed Quality	Breeding Institution
761	Double High	Zhejiang Province, China
Zhongyou 821	Double High	Chinese Agricultural Academy, China
Ningyou 7	Double High	Jiangsu Agricultural Academy, China
92-58	Double High	Zhejiang Agricultural Academy, China
Nangjin 2051	Double High	Unknown (from Nangjin, China)
Xiangyou 11	Canola	Hubei Agricultural Academy, China
Zheyou 2	Canola	Zhejiang Agricultural Academy, China
Huashuang 2	Canola	Hubei Agricultural Academy, China
Barossa	Canola	New South Wales Agriculture, Australia

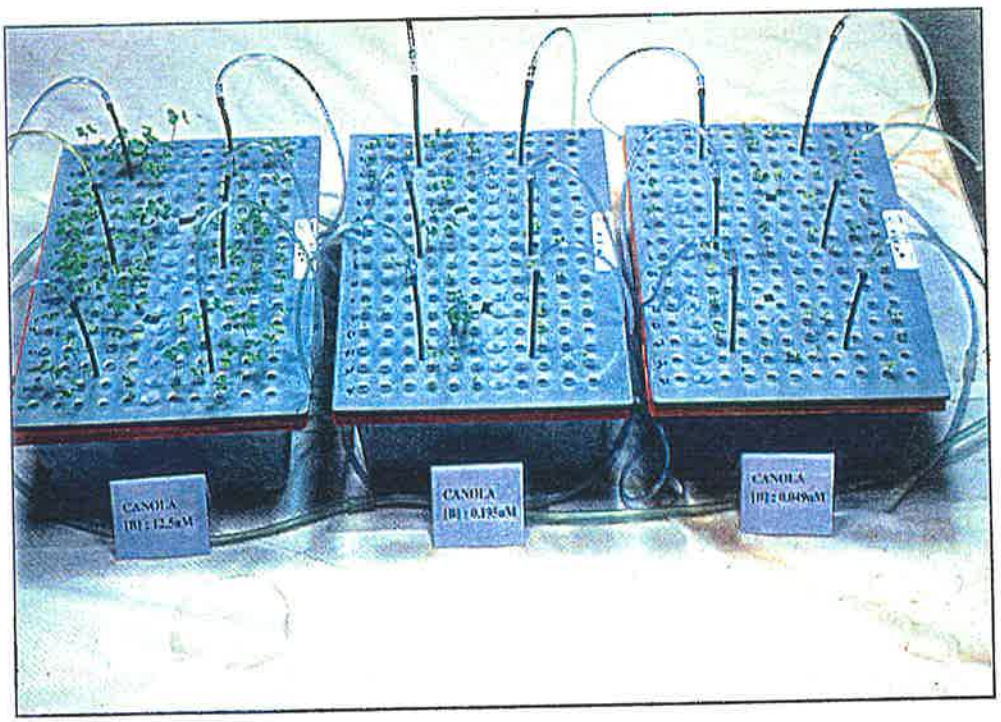


Plate 2.1. Solution culture system used for screening oilseed rape seedlings for tolerance to B deficiency.

2.2.6 Seed

Seed of all but one variety was supplied by Professor Y. Yang (Zhejiang Agricultural University, China), while Barossa was supplied by Dr. P. Salisbury (University of Melbourne, Australia). Seed B contents of the genotypes were determined by selecting 100 seeds from each genotype and analysing by ICP according to procedures previously reported in section 2.2.4. Seed B content ($\mu g\ seed^{-1}$) for the nine genotypes are presented in Table 2.2. Prior to planting seed into support collars housed in the lids of the growth containers, seed were imbibed and pre-germinated on moistened filter paper lined petri dishes for 36 h at 20°C.

2.2.7 Nutrient Solution and Growth Conditions

The composition of the nutrient solutions and the growth conditions at which the plants were grown were identical to those of experiment 2(a).

2.2.8 Measurements

Root length (measured from the junction of the shoot/root, and down the full length of the tap root to the apex) of each variety was measured on day 6 (D6) and day 10 (D10), with plants harvested on D10. Following harvest, roots were rinsed for 30 s in high purity water (18 $M\Omega.\ cm^{-1}$ conductivity, prepared by reverse osmosis and ion exchange) and plants were then separated into shoot and root and oven dried at 80°C for at least 24 h. Root and shoot samples were weighed and analysed by ICP as previously reported in section 2.2.4.

2.2.9 Statistical Analyses

The data were subject to analysis of variance using the Genstat 5 statistical package (Lane *et al.*, 1988). Significant mean separation is indicated by the use of the least significant difference (LSD) at the 5% level where the F value is significant.

Table 2.2. Oilseed rape genotypes and their initial seed B content (μg seed^{-1}) for experiment 2.3. Genotypes in bold print are *Brassica juncea* while the rest are *B. napus*.

Genotype	Seed B content	Genotype	Seed B content
CSIRO 6	0.04	Eureka	0.06
Pusa Bold	0.08	BLN 706	0.05
84004	0.07	826	0.04
Ningyou 8	0.06	Xiangyou 11	0.05
Wuyou 8	0.04	Wangyou 324	0.06
Huaozao 2	0.05	Yickadee	0.05
Xisuibai	0.05	Nindoo	0.06
Caijingti	0.06	BLN 702	0.06
CRGE 6/9/22/85	0.04	Oscar	0.05
Dizhao-2	0.06	Siren	0.06
Bechyne 47	0.05	**CSIRO 1**	0.04
141-5	0.05	Wangyou 5	0.05
Dandi	0.06	761	0.07
Wesbarker	0.05	**TM 18**	0.04
China D	0.05	China A	0.06
28-1	0.06	Ningyou 7	0.06
7755-2	0.06	Hua Kuang 1	0.05
China C	0.05	Shiralee	0.06
Xinza 1	0.06	Qingyou 2	0.05
Rainbow	0.06	China B	0.05
81007	0.05	Taparoo	0.05
Bechyne 3	0.05	Awassa 115	0.06
Budounuofusiji	0.06	92-13	0.05
609	0.05	92-58	0.06
Narendra	0.04	Nangjin 2051	0.04
Bechyne 41	0.05	Xinza 2	0.06
BLN 716	0.05	Dunkeld	0.06
Xiangyou 12	0.06	Zheyou 2	0.05
Barossa	0.05	Huashuang 1	0.04
BLN 704	0.04	Zhongyou 821	0.05
601	0.06	Nangchang rape	0.04
BLN 602	0.04	Huashuang 2	0.04
Hyola 42	0.06		

Experiment 2(c) Genotypic variation among 65 oilseed rape genotypes in root elongation index at low solution B.

2.2.10 Design and Measurements

Seven consecutive experiments (a total of 65 genotypes screened) were conducted and results pooled to determine the B efficiency of the genotypes screened. Experimental procedures for growing plants were the same as those of Experiment 2(b), with the exception that plant roots were measured on D10 only. This preceded harvest on the same day. To normalise results of the seven experiments in this study with each other, a standard variety (Barossa) was included in each experiment and the data corrected as follows: Root length = $c_i*(X_{aj}/x_i)$; where c_i is the mean root length of a given variety in experiment i; X_{aj} is the grand mean of the variety Barossa over experiments a to j; x_i is the mean of Barossa in experiment i at each level of B.

2.2.11 Seed

Seed for the following experiments were obtained from three sources, Professor Y. Yang (Zhejiang Agricultural University), Professor Y. Zhang (Xinjiang August 1st Agricultural College) and Dr. P. Salisbury (University of Melbourne). Information regarding their seed B contents prior to experimentation are given in Table 2.2.

2.3 Results

Experiment 2(a) Defining the critical concentration of external boron for deficiency in oilseed rape grown in solution culture

2.3.1 General observations

At solution concentrations below 12.5 μM B, both shoot and root growth were reduced (Plate 2.2 and 2.3). In B deficient plants, symptoms included an inward curling of the leaf; 'puckering' of areas in the leaf lamina; interveinal leaf chlorosis coupled with some reddened regions, particularly on the underside of the leaf; stunted shoot growth (in particular the apex) and reduced root growth, including poor lateral root development.

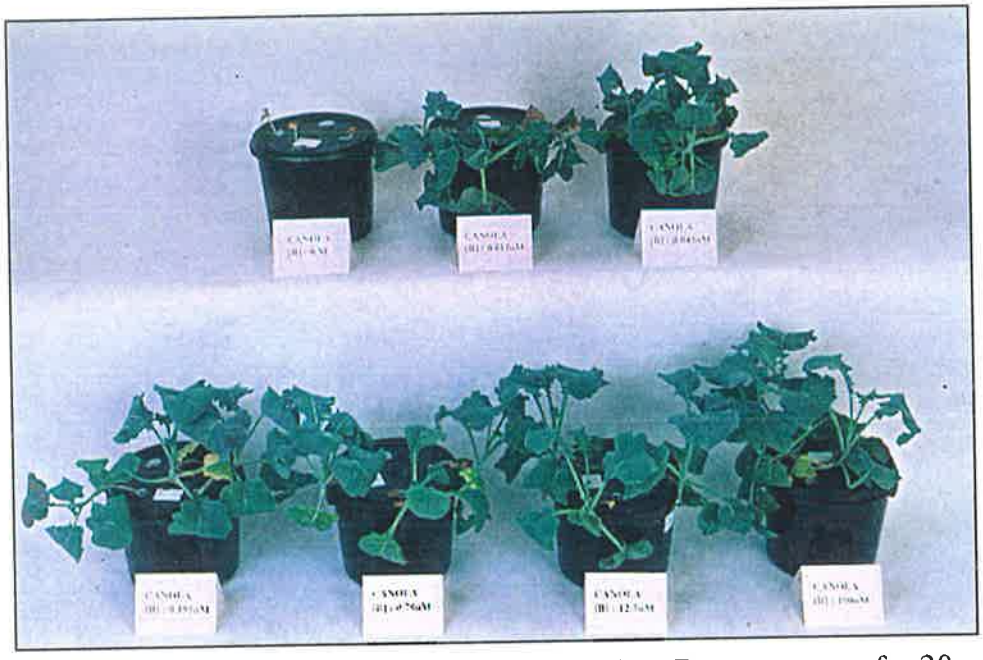


Plate 2.2. The effect of B supply on shoot growth in the variety Barossa grown for 30 days in solution culture. Rates of B are 0, 0.01, 0.05, 0.2, 0.8, 12.5, 100 μM B.

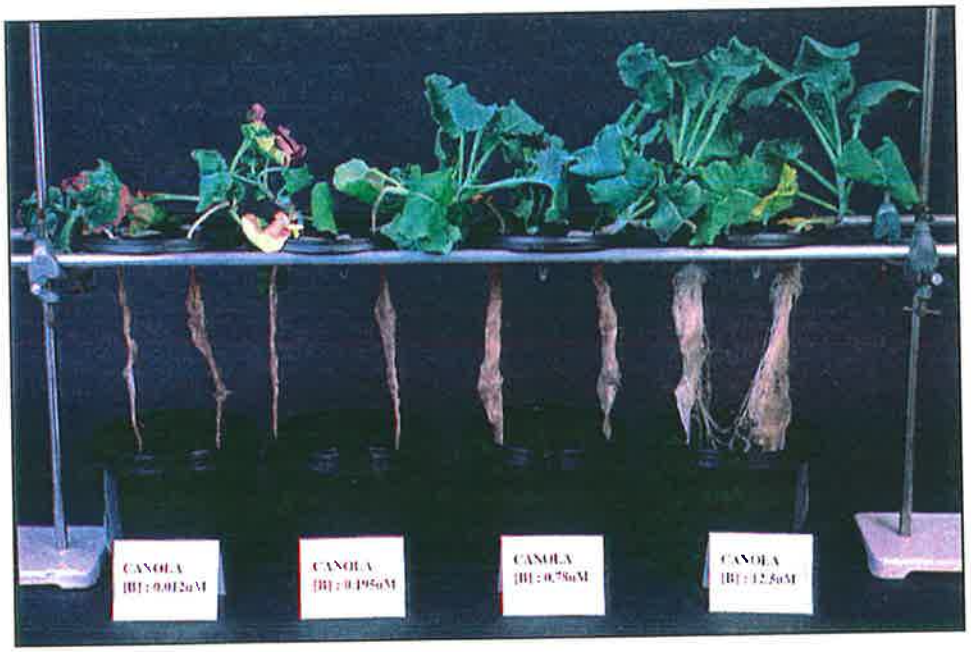


Plate 2.3. The effect of B supply on root and shoot growth in the variety Barossa grown for 30 days in solution culture. Rates of B are 0.01, 0.2, 0.8, 12.5 μM B.

2.3.2 Plant growth

According to published tissue concentrations for B deficiency in canola (6-13 mg B kg^{-1}; Reuter *et al.*, 1997), plants grown at B rates below 12.5 µM B were B deficient (Fig. 2.1). A 62-fold difference in plant dry weight was observed between the lowest and highest B treatments (Table 2.3). Root growth appeared more sensitive to B deficiency than shoot growth with root:shoot dry weight ratios varied 2 fold between the lowest and highest treatment at D20, and 6 fold at D30 (Fig. 2.1). With increased plant age the differential between treatments increased with the root:shoot dry weight ratio maximised at 12.5 µM B. At 100 µM B, partitioning of dry matter between root and shoot did not differ significantly from the 12.5 µM B treatment.

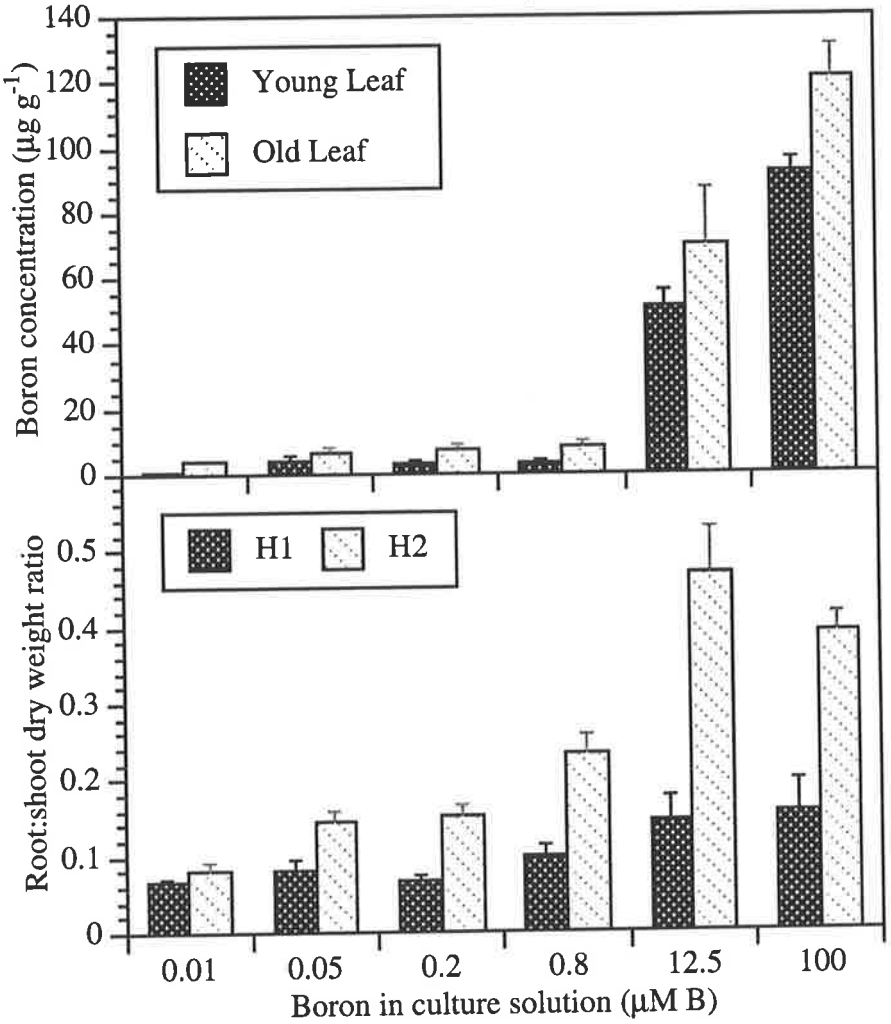


Fig. 2.1. The effect of external B supply on B concentration in leaf tissue of 20 day old canola plants, and the root:shoot dry weight ratio at day 20 (H1) and day 30 (H2). Values represent the mean of 2 replications ± se.

2.3.3 External and internal B requirement of oilseed rape

To minimise any effect of sub-optimal B supply during the growth of Barossa, a large volume (10 L) of solution was used, and nutrient solutions were changed at D5. Solution B concentration at D5 in 13 μM B treatment was 11 μM B.

Using a rescaled Mitscherlich equation, critical B concentrations (90% maximum yield) were derived from the experimental data. The external B requirement for the canola variety Barossa at D20 was 8 μM B, and 9 μM B at D30 (Fig. 2.2; D30 data only are presented due to the similarity in result). The internal B requirement was 6 μg B g^{-1} dry weight at both D20 and D30 (Fig. 2.3).

Experiment 2(b). Determining appropriate selection criteria for evaluating boron efficiency in oilseed rape grown in solution culture.

2.3.4 Visual Symptoms of B Deficiency

After D5, cotyledons of plants grown at 0.1 μM B started to take on a shiny appearance with very small necrotic lesions developing. By D10, the apical regions of the plants more seriously affected by B deficiency were purplish in colour. The symptoms were not evident in plants grown at 13 μM B. At 0.1 μM B supply, a visible reduction in root and shoot growth was present. Plants grown at the lower B concentration were visibly shorter with reduced root growth, compared with those grown at 13 μM B, but there was some variation in this respect between genotypes.

Table 2.3. Effects of B supply on whole plant dry weight and B concentration in the canola variety Barossa grown for 30 days in solution culture. Values represent means of 2 replications (2 plants per replication) ± se.

B in culture solution (μM B)	Whole plant dry weight (mg plant^{-1})	Whole plant B concentration (mg kg^{-1})
0	29±8	2±0.3
0.01	1093±140	4±0.3
0.05	1031±186	5±0.2
0.2	865±20	6±0.4
0.8	1234±56	8±0.6
12.5	1646±71	40±1.3
100	1804±75	79±5.9

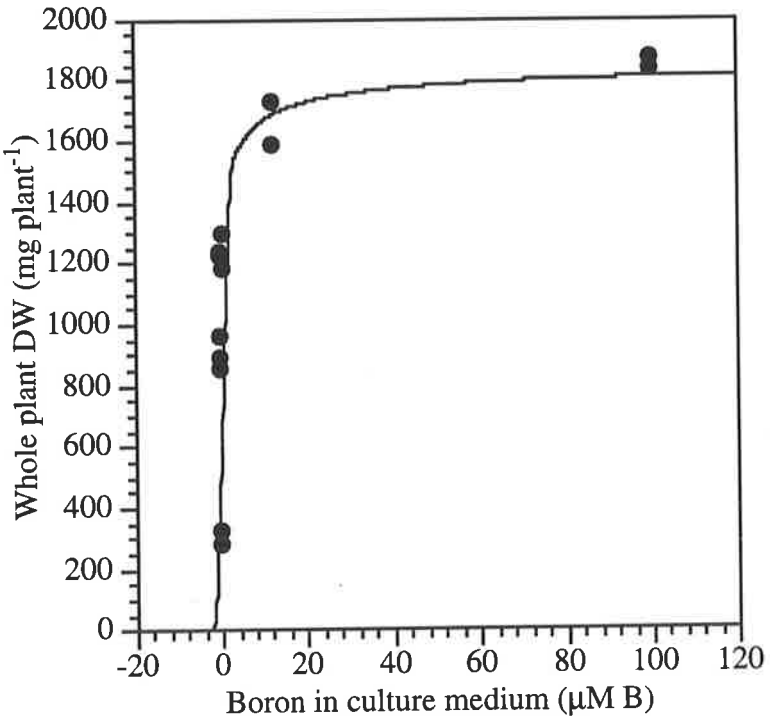


Fig. 2.2. External B requirement for the canola variety Barossa grown in solution culture for 30 days. Values represent means of 2 replications (2 plants per replication) ± se. Equation: Whole plant dry weight=1845-(1348/(1.48(Boron in culture medium)$^{0.36}$)$^{3.83}$; r^2=0.78.

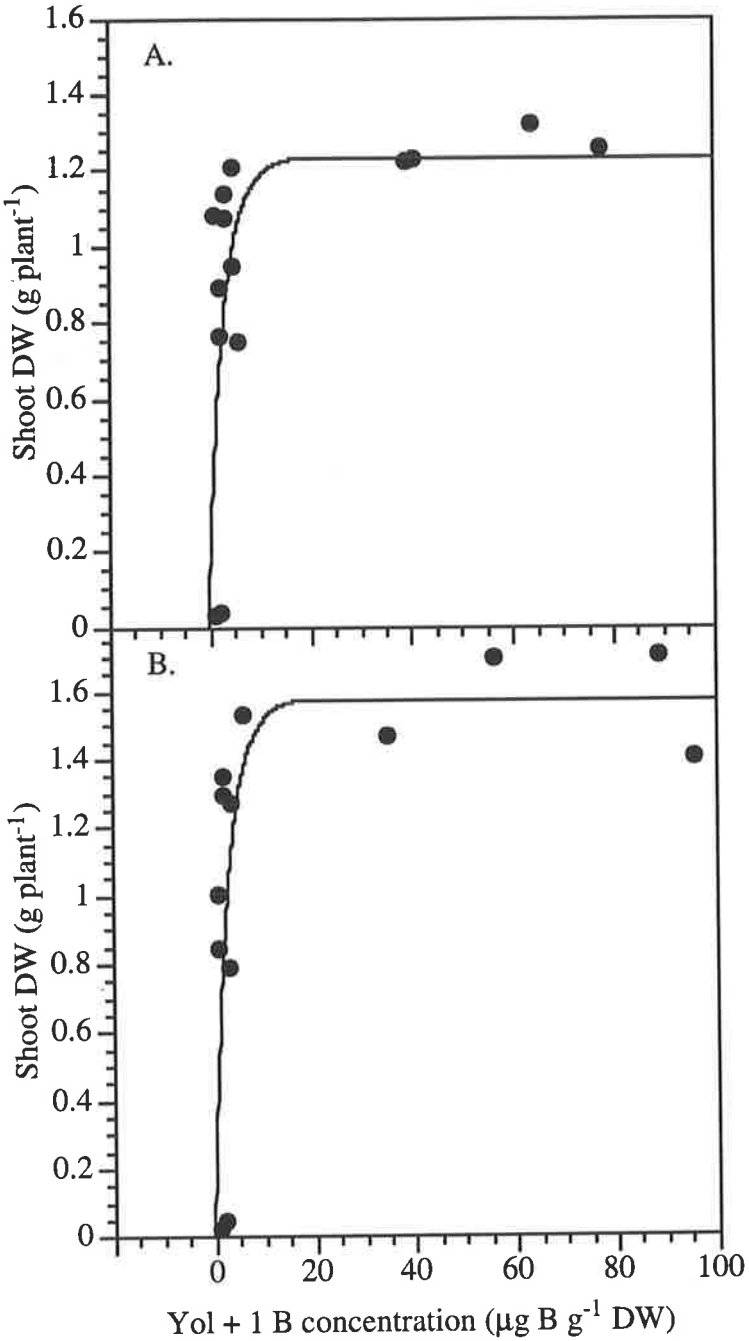


Fig. 2.3. Internal B requirements for the canola variety Barossa grown in solution culture for 30 (A) and 20 days (B). Equation for curve A: Shoot dry weight = 1.24 (0.21 Exp (-0.30 (YOL +1 B concentration))); $R^2 = 0.41$. Equation for curve B: Shoot dry weight = 1.58 (0.16 Exp (-0.31 (YOL +1 B concentration))); $R^2 = 0.51$

2.3.5 Root length and elongation rate

Root length and elongation rate were dependent on external B supply. Although there was genotypic variation present for root length at both D6 and D10, there was no correlation between the two parameters at D10 (Fig. 2.4).

While root length was reduced by low B overall, several varieties were able to maintain a relatively high root length and elongation rate (Table 2.4); Zhongyou 821 and Huashuang 2 maintained relatively good root growth at 0.1 μM B, in contrast to Barossa and Xiangyou 11 which were seriously affected by B deficiency and appeared B inefficient.

2.3.6 Plant Growth

Significant ($p<0.001$) genotypic variation in plant growth was present at both adequate and low B supply, with a 2 fold difference between genotypes in both treatments (Fig. 2.5 and 2.6). 92-58 and Huashuang 2 showed the highest and lowest whole plant dry weights under B adequacy, while under B deficiency, 92-58 and Xiangyou 11 showed the highest and lowest plant dry weights respectively. While 92-58 appeared to have a higher growth potential, its relative yield (0.1/13 μM B plant growth) was greatly reduced and it appeared B inefficient. On a relative yield basis, both Huashuang 2 and Zhongyou 821 are B efficient, with an identical efficiency ranking between genotypes in both whole plant dry weight (Fig 2.5) and shoot dry weight (Fig. 2.6).

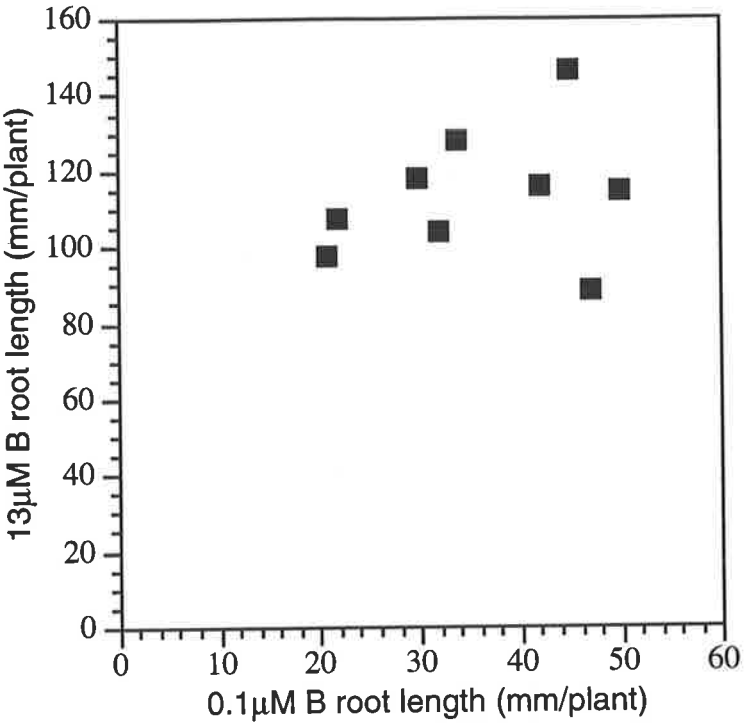


Fig 2.4. Relationship between root lengths of 10 day old oilseed rape genotypes grown at low (0.1 µM) and adequate (13 µM) levels of B.

Table 2.4. Root length (mm plant^{-1}) and elongation rate (mm 24 h^{-1}) of rapeseed cultivars grown in solution culture for six and ten days at low (B1; 0.1 µM B) and adequate (B13; 13 µM B) B supply. Values represent means of 16 plants ± se. Elongation rate was determined as the average rate in the interval from D6 to D10.

	Root length (mm plant^{-1})								
	D6			D10			Elongation rate (mm 24 h^{-1})		
Variety	B0.1	B13	B1/B13	B0.1	B13	B1/B13	B0.1	B13	B1/B13
761	23±4	51±7	0.45	30±4	118±10	0.25	1.8	16.8	0.11
Zhongyou 821	34±4	63±5	0.54	50±5	114±7	0.44	4.0	12.8	0.31
Ningyou 7	26±3	63±5	0.41	34±4	128±7	0.27	2.0	16.3	0.12
92-58	32±3	70±4	0.46	45±3	146±5	0.31	3.3	19.0	0.17
Xiangyou 11	17±3	51±4	0.33	21±3	97±7	0.22	1.0	11.5	0.09
Zheyou 2	37±3	63±5	0.59	42±3	116±4	0.36	1.3	13.3	0.10
Huashuang 2	36±3	48±6	0.62	47±3	88±9	0.53	2.8	10.0	0.28
Nangjin 2051	24±2	55±3	0.44	32±2	104±6	0.31	2.0	12.3	0.16
Barossa	18±2	56±5	0.32	22±3	107±9	0.21	1.0	12.8	0.08

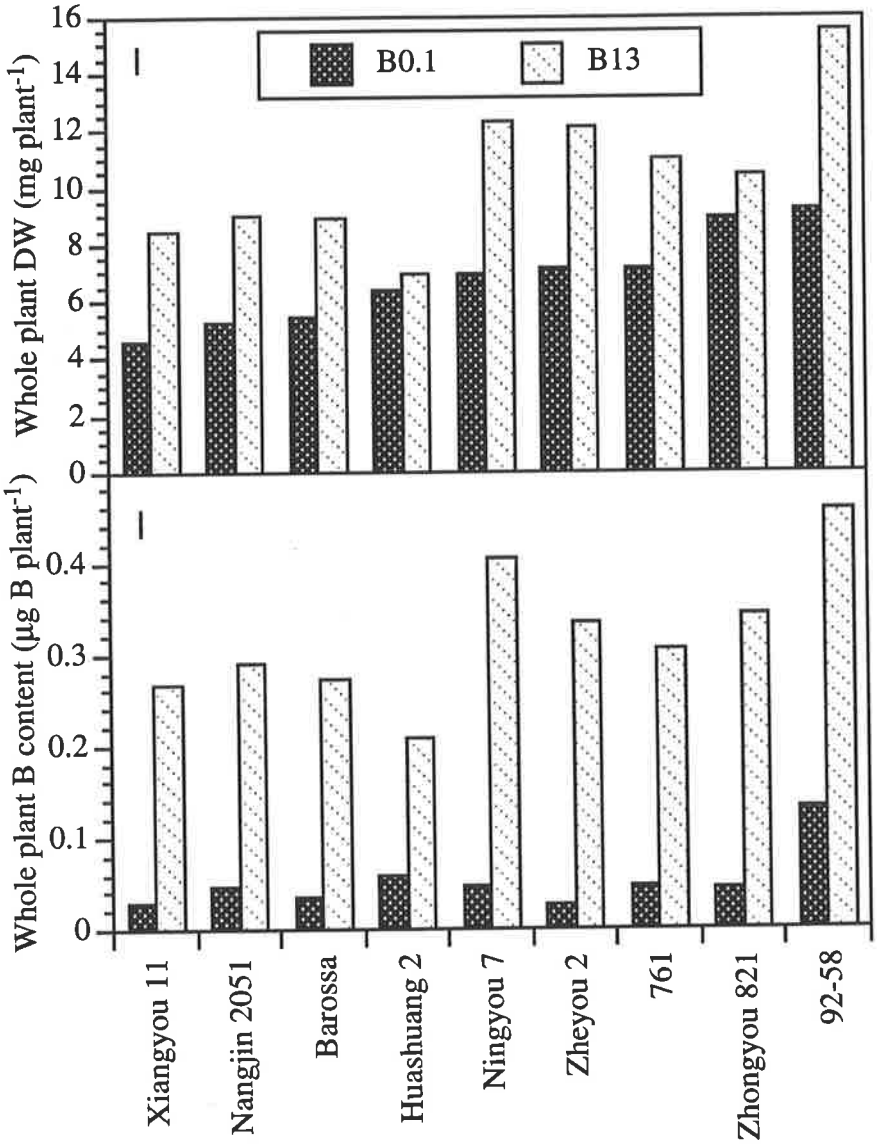


Fig. 2.5. The effect of low (0.1 µM B) and adequate (13 µM B) B supply on whole plant dry weight and B content in nine genotypes of oilseed rape grown for 12 days in solution culture. Bars represent means of 3 replications. Vertical lines represent the LSD ($P<0.05$), for the Var x Boron interaction.

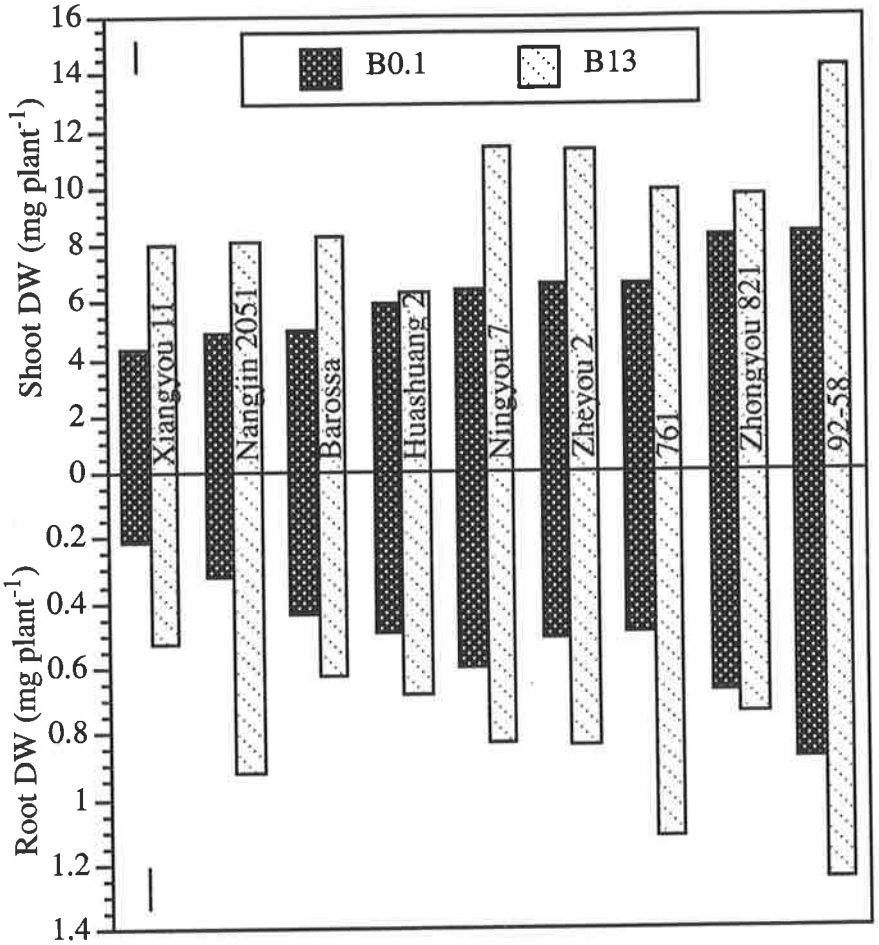


Fig. 2.6. The effect of low (B0.1; 0.1 μM B) and adequate (B13; 13 μM B) B supply on shoot and root dry weight in nine genotypes of oilseed rape grown for 12 days in solution culture. Bars represent means of 3 replications. Vertical lines represent the LSD ($P<0.05$), for the Var x Boron interaction.

2.3.7 Net Boron Uptake

Net B uptake declined under B deficiency, with significant ($p<0.001$) varietal differences in the net uptake of B in both 0.1 and 13 μM B treatments (Fig. 2.5). Varieties varied 2 fold in B uptake when grown at adequate B supply, which increased to a 5 fold difference under B deficiency. Both 92-58 and Ningyou 7 exhibited a higher capacity to take up B at 13 μM B, with Huashuang 2, the lowest. In the 0.1 μM B treatment, 92-58 exhibited a greater capacity to take up B compared with other genotypes, while the genotypes most seriously affected by B deficiency were Zheyou 2, Xiangyou 11 and Barossa.

2.3.8 Selection Criterion for B Efficient Genotypes

Root length, elongation rate, and dry weight can all be used to assess B efficiency in oilseed rape (Fig 2.7). Relative root length was most strongly correlated with relative shoot growth, while root elongation rate and root dry weight were less well correlated with relative shoot growth. These results indicate that root length ratio can adequately measure genotypic responses to B deficiency, and therefore could be useful as a screening method for tolerance to low B supply, if indeed dry weight reflects field performance.

Experiment 2(c) Genotypic variation among 65 oilseed rape genotypes in root elongation index at low solution B.

2.3.9 Genotypic variation in 65 genotypes of oilseed rape

The good relationship between root length efficiency and shoot dry weight efficiency provided the stimulus to evaluate further a total of 65 oilseed rape genotypes. Root length varied greatly among the 65 lines both at low and adequate B supply (Fig 2.8). This genotypic variation could not be attributed to the initial seed B content. A three fold range in root length under B sufficiency was increased to 5 fold under B deficiency, with a number of genotypes able to produce roots similar in length to those grown at an adequate B supply. On a relative growth basis (B0.1/B13), and as an indication of the degree of B efficiency in the lines screened, 34 genotypes in this study were ranked sensitive to B deficiency, 20 moderately sensitive, 7 moderately efficient, 3 efficient and 1 very efficient (Table 2.5). B efficiency was expressed in Huashuang 2, Nangchang rape, Huashuang 1 and Zhongyou 821, and to a lesser extent, Zheyou 2, Dunkeld, Xinza 2, Nangjin 2051, 92-58, 92-13, and Awassa 115.

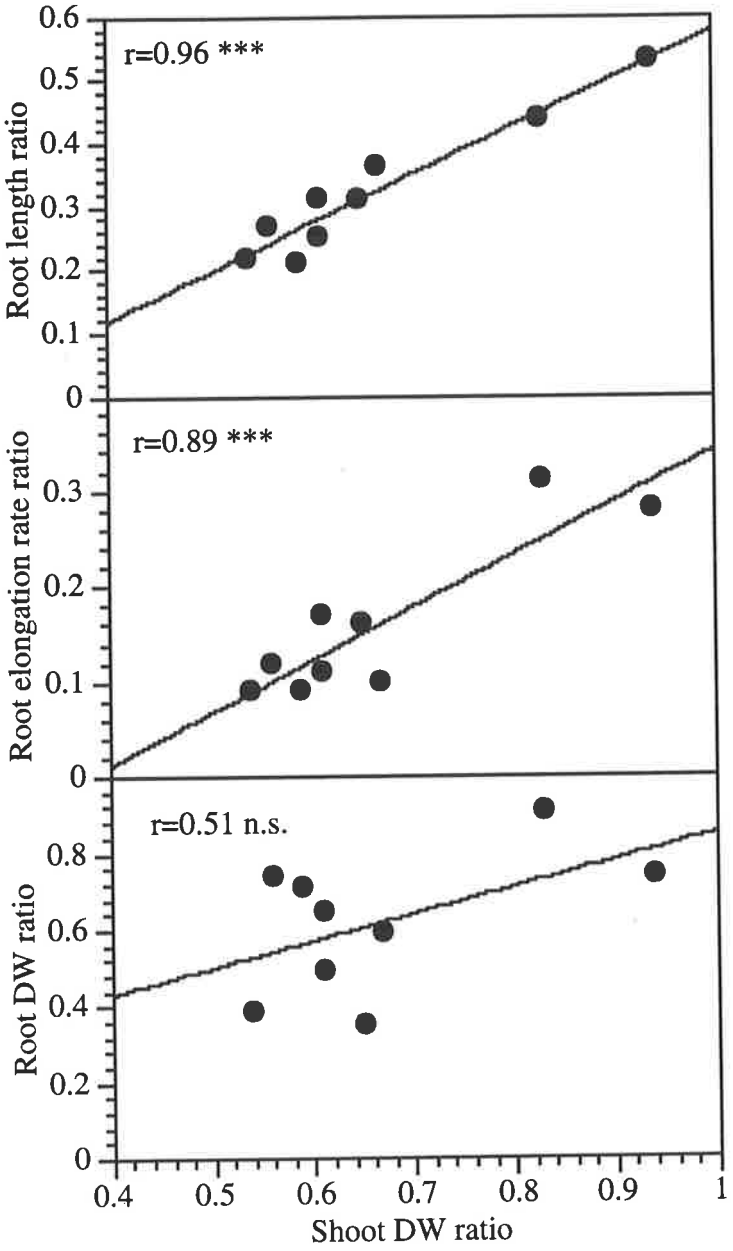


Fig. 2.7. The relationship between relative shoot dry weight (B0.1/B13) and relative root length, relative root elongation rate and relative root DW in nine varieties of oilseed rape grown for 10 days in solution culture. Correlation coefficient, r, *** $P<0.001$; n.s. Not significant. The linear relationships are best described by: Root length ratio = 0.77 (Shoot DW ratio) - 0.19; Root elongation rate ratio = 0.55 (Shoot DW ratio) - 0.21; Root DW ratio = 0.70 (Shoot DW ratio) - 0.15.

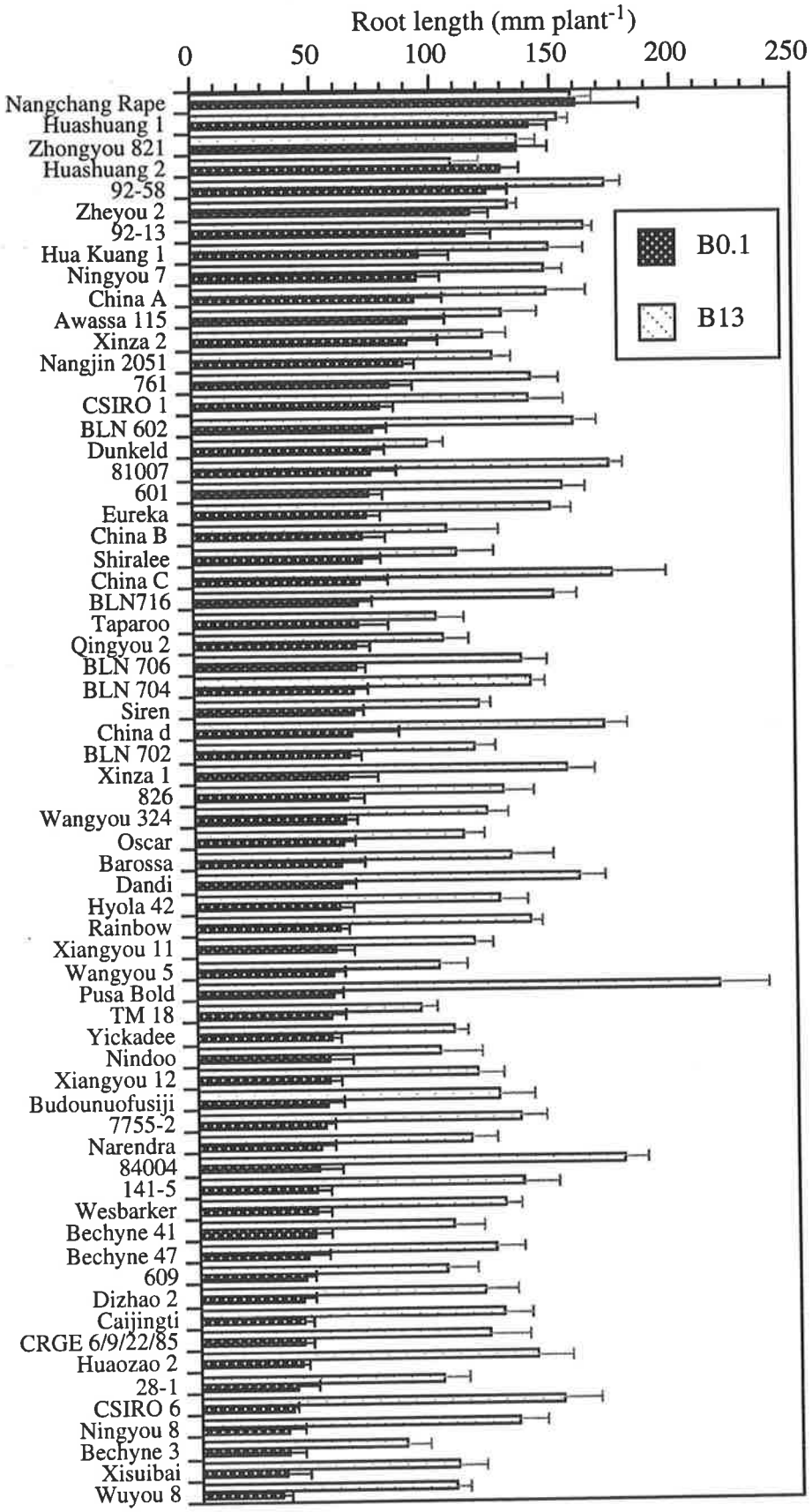


Fig 2.8. Effect of B supply on adjusted root length for 65 oilseed rape genotypes grown for 10 days in solution culture. Bars represent mean of root length of 16 plants ± se.

Table 2.5. Relative root length (RRL, 0.1 /13 μM B) of 65 oilseed rape genotypes. RRL is arbitrarily divided into six B efficiency ranks (E) as follows: 0-19% (1, very sensitive), 20-49% (2, sensitive), 50-69% (3, moderately sensitive), 70-89% (4, moderately efficient), 90-109% (efficient), and >109% (6, very efficient).

Variety	RRL (%)	E	Variety	RRL (%)	E
CSIRO 6	25	2	Eureka	49	2
Pusa Bold	26	2	BLN 706	50	3
84004	28	2	826	50	3
Ningyou 8	28	2	Xiangyou 11	51	3
Wuyou 8	31	2	Wangyou 324	52	3
Huaozao 2	31	2	Yickadee	53	3
Xisuibai	33	2	Nindoo	54	3
Caijingti	34	2	BLN 702	55	3
CRGE 6/9/22/85	36	2	Oscar	56	3
Dizhao-2	37	2	Siren	56	3
Bechyne 47	37	2	CSIRO 1	56	3
141-5	37	2	Wangyou 5	57	3
Dandi	38	2	761	59	3
Wesbarker	39	2	TM 18	60	3
China D	39	2	China A	63	3
28-1	40	2	Ningyou 7	64	3
7755-2	40	2	Hua Kuang 1	64	3
China C	40	2	Shiralee	64	3
Xinza 1	41	2	Qingyou 2	65	3
Rainbow	43	2	China B	67	3
81007	43	2	Taparoo	69	3
Bechyne 3	43	2	Awassa 115	70	4
Budounuofusiji	43	2	92-13	70	4
609	44	2	92-58	71	4
Narendra	45	2	Nangjin 2051	71	4
Bechyne 41	45	2	Xinza 2	74	4
BLN 716	46	2	Dunkeld	76	4
Xiangyou 12	47	2	Zheyou 2	88	4
Barossa	47	2	Huashuang 1	92	5
BLN 704	48	2	Zhongyou 821	100	5
601	48	2	Nangchang rape	101	5
BLN 602	48	2	Huashuang 2	118	6
Hyola 42	48	2			

2.4 Discussion

Ulrich (1952) gave three formal definitions of the critical nutrient concentration (CNC): (i) the internal nutrient concentration that is just deficient for maximum growth; (ii) the nutrient concentration that is just adequate for maximum growth, or (iii) the concentration separating the zone of deficiency from the zone of adequacy. To accommodate all three definitions, the CNC should be seen as a range and not as an individual single value (Smith and Loneragan, 1997). From results presented in this chapter, the critical B concentration (CBC) for Barossa was around 6 μg B g^{-1} dry weight at D20 and D30, while the external B requirement was around 8 μM B, and varied little over the 10 day period. The CBC was lower than recent reports of the CBC for oilseed rape of around 10-14 mg B kg^{-1} dry weight (Huang *et al.*, 1996), which may be due to differences in experimental procedure as Huang *et al.* (1996) used the 'nutrient addition program' (Asher and Edwards, 1983) to maintain a constant supply of B to the plant. Differences in the ratio of B to other nutrients in solution may also contribute toward the observed differences in the CBC between the two experiments.

In determining appropriate external B concentrations for the screening of 65 genotypes of oilseed rape, 13 μM B was chosen as an adequate B level for the control, around 5 μM B more than the critical external B concentration determined for Barossa. This level of B was within the 'adequate zone' (Smith and Loneragan, 1997) of the critical nutrient curve (Fig. 2.2), where this plateau was maintained up to 100 μM B without any indication of a decline in yield. A further consideration in choosing this level of B was the apparent maximising of the root:shoot dry weight ratio around the 12.5 μM B treatment in the critical level study (Fig. 2.1). Below this level root growth was depressed, with root growth more sensitive than shoot growth; a characteristic of B deficient plants (Blamey *et al.*, 1997) which may be associated with a reduced partitioning of carbon to the root as an effect of limited B mobility (Marschner *et al.*, 1996).

It should be highlighted that genotypes may vary in the utilisation of B in the shoot, which may result in genotypic variation in external and internal B requirements. Critical deficiency concentrations for the large number of genotypes in this study is a large task to complete. The level of B chosen in the control was made relatively high to try and compensate for these differences, thereby reducing the confounding effect which would eventuate from an inadequate supply of B to adequate control plants of some genotypes.

The B deficient concentration of 0.1 μM B was chosen with the objective of maintaining a very high level of B stress without causing cessation of plant growth. The root elongation rates between D6 and D10 showed that root growth was maintained, albeit at a very low rate, and this level of B allowed differential expression of response between genotypes.

Chantachume *et al.* (1995) proposed a selection criterion for diagnosing B tolerant wheat genotypes with the use of solution culture in filter paper. Using this quick and relatively easy method, root length was used as a selection criterion as it best described shoot B concentration and growth changes. Results from this study also support the use of root length as a selection parameter, more than root elongation rate and root dry weight as it closely reflected changes in shoot growth. The lack of correlation between B treatments, one deficient and one adequate, suggests the presence of genetic variation in response to deficiency as early as 10 days into plant growth. Based on the responses in root length (Table 2.5), genotypes which exhibit some degree of B efficiency according to this tentative index, are: Huashuang 2, Nangchang rape, Huashuang 1 and Zhongyou 821, Zheyou 2, Dunkeld, Xinza 2, Nangjin 2051, 92-58, 92-13, and Awassa 115.

Genotypes screened in this study have originated from diverse regions of the world which vary substantially in the availability of B in soil. Very few of these genotypes have been evaluated for B efficiency in the field. B efficiency has been reported in field grown 92-13 (Yang *et al.*, 1993, 1994), Zhongyou 821 (Yang *et al.*, 1994), and Pusa Bold (Sakal *et al.*, 1991). In pot studies, Huashuang 2 has also been identified as B efficient (Yang *et*

al., 1994). Apart from Pusa Bold, the results presented in this chapter are in reasonably good agreement with published reports on B efficiency in a number of these genotypes. In terms of relative root length, 92-13 was moderately B efficient, Huashuang 2, very efficient, Zhongyou 821, efficient, while Pusa Bold was classified as sensitive. This now raises the question of whether vegetative response during early stages of plant growth can adequately indicate a genotype's B efficiency in the field, a question also raised in respect of wheat by Rerkasem *et al.* (1993). Further research utilising field based screening of oilseed rape genotypes on low B soil needs to be conducted before this screening technique can be used to further evaluate germplasm for B efficiency.

Chapter 3

Boron Efficiency in Field and Pot Experiments of Oilseed Rape (*Brassica* spp.) Grown to Maturity.

3.1 Introduction

Graham (1984) suggests that to breed for nutritional characters, it is necessary to show (i) a need exists as pressing as other breeders' objectives; (ii) that genetic potential exists to be exploited, and (iii) it is agronomically, economically and ecologically feasible. The need for boron (B) efficient cultivars is warranted given the vast areas of low B soil in which oilseed rape (including the high oil quality canola) is grown. Soils such as the Lower Yangtse region of China can be acutely deficient in B (Liu *et al.*, 1981), yet this area contributes a large portion of the world's oilseed rape crop. In breeding cultivars suitable for these problem soils, the identification of genetic potential remains an imperative for a successful breeding program.

In a previous study (Chapter 2), variation among genotypes of oilseed rape in response to low solution B was identified in their vegetative growth stage, by measuring relative root length of 10 day old plants. Relative root length under B deficiency correlated with relative shoot growth. The use of relative root length as a selection criterion was based on the definition of nutrient efficiency; that is the ability of a cultivar to grow and yield well in soils too deficient in B for a standard cultivar (Graham, 1984). B efficiency by this definition does not imply a mechanism. This same definition will be used in the following study, which aims at screening genotypes of oilseed rape for B efficiency in both field and pot studies, and to evaluate the relationship between vegetative response (including relative root length (Chapter 2) and seed yield.

3.2 Materials and Methods

Experiment 3(a) B efficiency in field grown oilseed rape

3.2.1 Design

Sixteen varieties of oilseed rape (*Brassica napus* L. and *B. juncea* L.; Table 3.1) were sown at Mt. Compass, South Australia, a site low in B (0.1-0.3 mg B kg^{-1} ($CaCl_2$ extractable B)). The site consisted of the Myponga Sand (pH 5.4 in 0.01M $CaCl_2$), a podzolic soil, Uc 2.32 (Northcote, 1971), developed on sand of Permian fluvioglacial origin that has been reworked by aeolian activity into dune and drift formations (Maud, 1972). The trial was modelled on a randomised complete block, split plot design with blocks (4), whole plots (varieties), and sub-plots (B treatments). *Brassica napus* varieties were kept in a separate trial, but adjacent to the *B. juncea* varieties. This was due to the vigorous growth habit of *B. juncea* plants which had the potential to compete adversely with the *B. napus* plants if grown together.

Table 3.1. Identification of the 17 genotypes grown in the field at Mt. Compass, 1994.

B. napus	*B. napus*	*B. juncea*
Zhongyou 821	Barossa	397-23-2-3-2
Dunkeld	Hyola 42	CPI 81792
Zheyou 2	Oscar	Pusa Bold
92-13	BLN 702	CSIRO 6
Yickadee	Siren	TM 18
Narendra	761	

3.2.2 Nutrient additions

Two weeks prior to sowing, a micro-nutrient mix consisting of Cu, Zn, Mo was sprayed onto plots at a rate of 2:2:0.1 kg ha^{-1}. Because of the low soil pH, Fe deficiency was not considered a risk. Genotypes were sown at a rate of 4 kg seed ha^{-1} and B

treatments were sprayed onto plots at a rate of 2 kg B ha^{-1} (source: Boric acid) (B+) immediately following sowing. Low B treatment was applied by omission (B0). On day 94, an extra 0.4 kg B ha^{-1} was applied as a foliar spray to the B+ plots. Since the Mt. Compass site was also low in elements other than B, macro-elements were applied either as a split application or at sowing. N, P, K, S were supplied at a rate of 115 kg N ha^{-1} (applied in a split application of 25 kg predrilled into the soil before sowing, 20 kg applied with the seed at sowing, 30 kg six weeks post sowing, and 40 kg post stem elongation), 28 kg P ha^{-1} (15 kg at sowing and the remainder in the vegetative stage), 18 kg K ha^{-1} (at sowing) and 23 kg S ha^{-1} (10 kg at sowing and 13 kg prior to stem elongation).

3.2.3 Measurements

In the vegetative stage (just prior to 'green bud' stage (Sylvester-Bradley, 1985)), 10 plants per plot were harvested, dried at 80 °C for 36 h, and dry weights recorded. To indicate the B status of the plants, the second youngest open leaf (YOL + 1) was sampled from 10 plants, dried at 80°C for 36 h, weighed and analysed for all essential elements (except N) according to previously reported procedures in section 2.2.4. At plant maturity, a one metre square area was harvested at ground level from each plot and yield components (branch number m^{-2}; pod number m^{-2}; seed yield ha^{-1}, dry weight $seed^{-1}$) were determined.

3.2.4 Statistical Analyses

The data were subject to ANOVA using the Genstat 5 statistical package. Significant mean separation is indicated by the use of the least significant difference (LSD) at the 5% level where the F value is significant. Since the screening of *B. napus* and *juncea* genotypes was conducted in separate experiments, accordingly, ANOVA was conducted on each species separately.

Experiment 3(b) Boron efficiency in three *Brassica napus* cultivars grown in pot culture

3.2.5 Design

The experiment was modelled on a factorial design with three cultivars of *Brassica napus* L. (Dunkeld and Barossa both canola quality cultivars bred for the Australian canola industry, while Zhongyou 821 is a low oil quality cultivar grown in southeast China) sown in unwashed sand, extracted from the Mt Compass field site (experiment 3(a)). Two B treatments (0 and 0.25 mg B kg^{-1} soil) were applied, and the experiment was replicated four times.

3.2.6 Seed

Information on seed source and B content are given in Chapter 2, section 2.2.6 and Table 2.2 respectively.

3.2.7 Growth Conditions and Nutrient Additions

Dry, sieved (2 mm) Mt. Compass sand (9 kg) (pH 5.4 in 0.01M $CaCl_2$; $CaCl_2$ extractable B, 0.1 mg B kg^{-1} soil) was placed in clear plastic bags with the following nutrients (mg kg^{-1} dry soil) added to each bag and mixed thoroughly throughout. $CaCO_3$, 1000 (mixed through before addition of other nutrients); $Ca(NO_3)_2 \cdot H_2O$, 918; $MgSO_4 \cdot 7H_2O$, 140; K_2SO_4, 114; KH_2PO_4, 72; $ZnSO_4 \cdot 7H_2O$, 6.6; NaCl, 3.2; $MnSO_4 \cdot 4H_2O$, 3.7; $CuSO_4 \cdot 5H_2O$, 2.3; $CoSO_4 \cdot 5H_2O$, 0.23; $H_2MoO_4 \cdot H_2O$, 0.23. After thorough mixing by hand, individual bags were placed inside black plastic garden pots (10 kg maximum capacity) and treatments lacking B (B0) were imposed by omission, while controls had 0.25 mg B kg^{-1} (source: boric acid) (B+) added. This adequate level of B was determined from previous critical level studies on oilseed rape conducted at the Waite Institute by Dr M J Webb (data unpublished). Pots were watered to 10 % (w/w), with high purity water prepared by reverse osmosis.

Ten seeds were sown into each pot at a depth of 1 cm, and at D10 plants were thinned to leave 7 uniform plants.

3.2.8 Measurements

Three plants per pot were harvested at D14 and a further 2 plants at D28. Plants from each pot were bulked together, with the shoot washed for 5 s in high purity water and separated into the following plant components: Youngest open leaf + 1 (YOL+1) blade and petiole; YOL+3 blade and petiole; old leaf (all remaining leaves) blade and petiole; stem. Plant material was dried at 80 °C for 48 h and dry weights recorded. Samples were analysed for all essential elements (except N) according to procedures in section 2.2.4 in Chapter 2. At plant maturity, yield components on the remaining 2 plants per pot were determined by counting branch and pod numbers, and recording seed yield plant^{-1}.

3.2.9 Statistical Analyses

The data were subject to ANOVA using the Genstat 5 statistical package. Significant mean separation is indicated by the use of the least significant difference (LSD) at the 5% level where the F value is significant.

3.3 Results

Experiment 3(a) B efficiency in field grown oilseed rape

3.3.1 General observations

Immediately following sowing, very cold night and day temperatures (including 2 severe frosts) caused slow plant growth. At D30, *B. juncea* genotypes appeared more vigorous in growth than those of *B. napus* and were at the 3-4 leaf stage, while *B. napus* genotypes were at the 2 leaf stage. At the time of taking the vegetative harvest (D77), no visible B deficiency symptoms were evident other than a small reduction in shoot growth in B0 plots of the genotype 761.

3.3.2 Plant Growth in the Vegetative Stage

In B0 plants, B concentrations in the YOL+1 ranged from 13-18 mg kg^{-1} in *B. napus*, and 22-24 mg kg^{-1} in *B. juncea* (Fig. 3.1). These levels of B in the YOL+1 (a standard tissue used to diagnose the nutritional status of oilseed rape (Reuter *et al.*, 1997)) indicate marginal B deficiency in *B. napus*, with the most seriously affected being Narendra. *B. juncea* plants were all within the B adequate range.

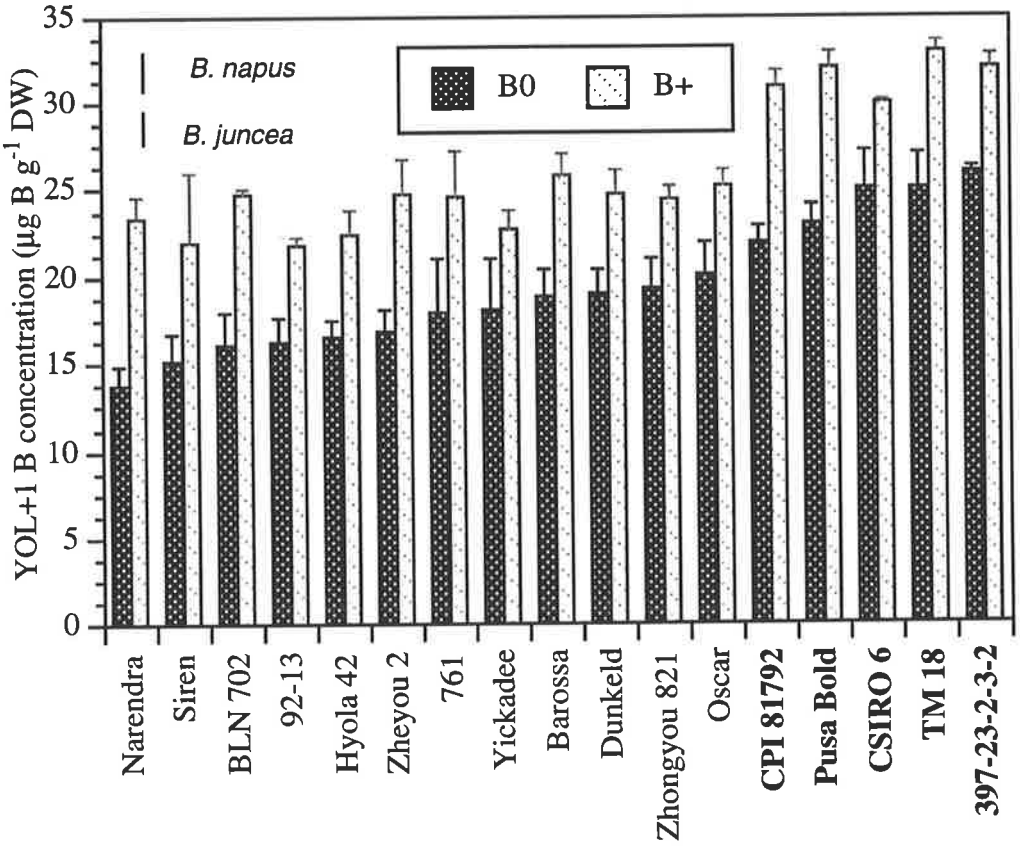


Fig. 3.1. Ranking of oilseed rape genotypes according to the YOL+1 B concentration in plants grown without the addition of B (B0). Control plants were grown with the addition of B (B+). Plants were sampled in the late vegetative stage. Genotypes in bold type are *Brassica juncea* while the remainder are *B. napus*. Bars represent means of 3 replications ± se. Vertical lines depict LSD ($P<0.05$). *B. napus*, Boron. *B. juncea*, Variety * Boron.

At D77, only 761 showed a real decline under B deficiency (Fig. 3.2). In B+ plants, Hyola 42 and 761 exhibited the greatest growth, 2 to 3 fold more than Oscar and Siren, while the remaining genotypes all exhibited similar dry weight. In *B. juncea* genotypes, while treatment effects were not significant, there was a decline (around 15 %) in shoot growth in B0 grown TM 18 and CSIRO 6. The major variation at this stage of growth were differences in shoot growth between the two *Brassica* species, with *B. juncea* exhibiting much more shoot growth than *B. napus* genotypes. Variation in shoot growth sampled in the vegetative stage, gave some indication of genotypic responses in seed yield for *B. napus* genotypes (Fig. 3.3A), though not for *B. juncea* (Fig 3.3B). This lack of correlation probably reflects the unresponsive nature of the field site at Mt. Compass.

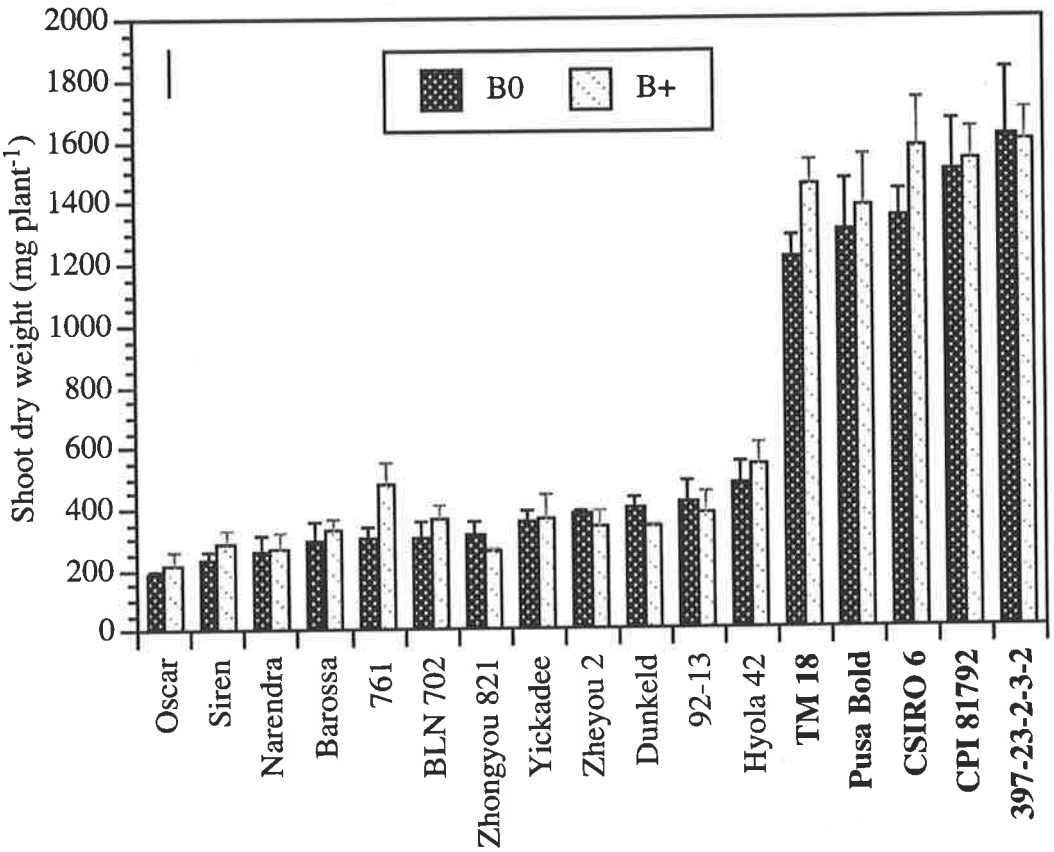


Fig. 3.2. Ranking of oilseed rape genotypes according to shoot dry weight in plants grown without the addition of B (B0). Control plants were grown with the addition of B (B+). Genotypes in bold type are *Brassica juncea* while the remainder are *B. napus*. Bars represent means of 3 replications ± se. Vertical line depicts LSD (P<0.05). *B. napus*: Variety.

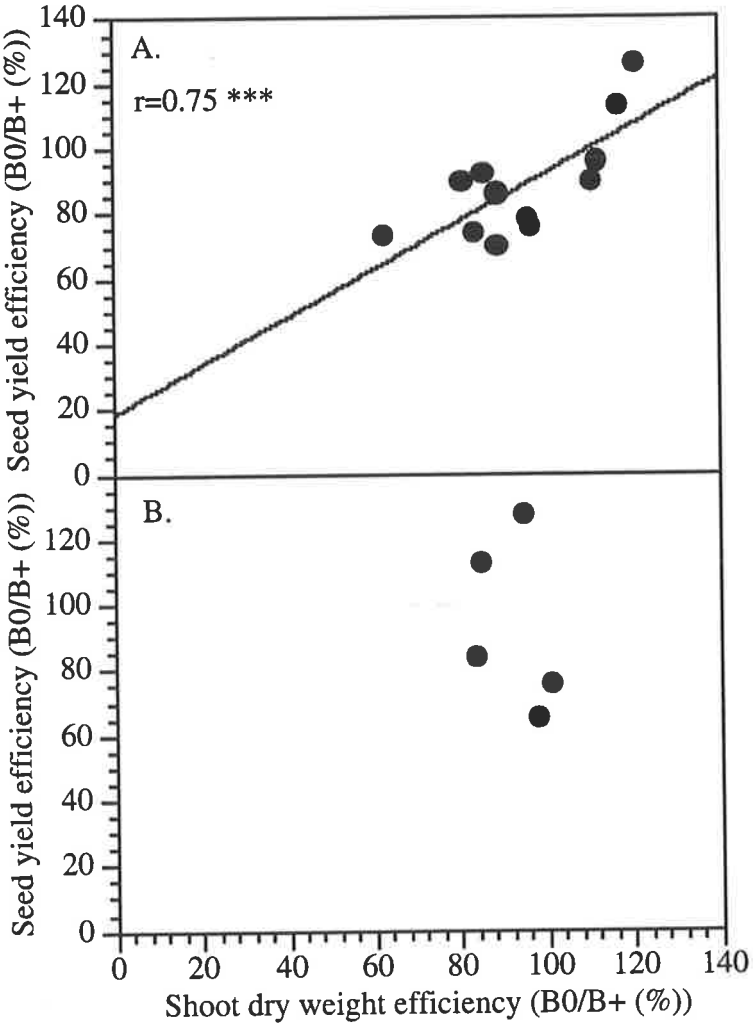


Fig. 3.3. The relationship between shoot dry weight efficiency, as observed in the vegetative stage of plant development, and seed yield efficiency for *Brassica napus* (3A) and *B. juncea* (3B) varieties grown on low B Mt. Compass sand. Equation for graph A: Seed yield efficiency = 0.7 (shoot dry weight efficiency) + 18.3. Correlation coefficient, r, *** ($P<0.001$).

3.3.3 Yield Components

Brassica napus genotypes matured up to 14 days later than *B. juncea* genotypes. Species differences in seed yield and its components were present (Table 3.2), with branch and pod number in the majority of *B. juncea* genotypes higher than in *B. napus*, though seed yields were lower, most likely due to the smaller seed size in this species (Table 3.3).

The effect of B deficiency on seed yield varied with the genotype; B deficiency affected yield at different stages of plant growth and reproduction. For example, in the *B. napus* genotype BLN 702, branch number in B0 plots was maintained (B0/B+, 98 %), yet pod set (B0/B+, 74 %) and final seed yield declined (B0/B+, 74 %). By contrast, in 761, both branch number (B0/B+, 88 %), pod number (B0/B+, 95 %) and seed size (B0/B+, 111 %) were maintained in B0 plots, but seed set was depressed (B0/B+, 66 %). This was also the case for other *B. napus* genotypes, Barossa and Yickadee. For *B. juncea*, CPI 81792 and TM 18 branch number, pod number and seed yield were depressed by omitting B. In contrast, *B. napus* genotypes Dunkeld and Zhongyou 821, and *B. juncea* genotypes Pusa Bold and TM 18, showed no decline in yield or its components.

While there was an indication of a reduced seed dry weight in B0 grown plants of *B. napus*, no significant treatment effect was observed (Table 3.3). In contrast, a significant variety effect was present, with the variety's 761 and Zhongyou 821 having the highest and lowest seed dry weights respectively. Similar effects were observed in seed B contents, with the variety 761 exhibiting a very high seed B content compared to the other varieties. In *B. juncea,* main effects for variety and treatment were not significant for seed dry weight and B content.

Table 3.2. Effect of applying B on yield of oilseed rape genotypes grown on low B Mt. Compass sand. Values represent means of 3 replications. Genotypes in bold type are *Brassica juncea* while the remainder are *B. napus*. E: B efficiency (B0/B+ (%)).

Variety	Branch No. m^{-2}			Pod No. m^{-2}			Seed Yield (kg ha^{-1})		
	B+	B0	E	B+	B0	E	B+	B0	E
BLN 702	80	79	98	1548	1249	81	553	411	74
761	61	54	88	1242	1184	95	474	312	66
Dunkeld	67	80	119	1057	1237	117	548	617	113
Zheyou 2	72	75	104	1469	1472	100	583	570	98
Siren	67	59	89	1240	1020	82	387	308	80
Barossa	94	95	102	1502	1304	87	640	449	70
Oscar	99	97	98	1328	1350	102	523	458	88
Narendra	79	95	120	1000	1444	144	724	615	85
92-13	93	85	85	1715	1712	100	502	452	90
Yickadee	97	86	88	1879	1428	76	650	500	77
Hyola 42	97	110	113	2049	1972	96	769	625	81
Zhongyou 821	95	97	102	1482	1755	118	580	597	103
CPI 81792	120	96	80	2723	2074	76	278	182	65
397-23-2-3-2	184	128	70	3644	1726	47	376	282	75
Pusa Bold	109	137	126	1549	2742	177	207	263	127
CSIRO 1	115	105	91	2658	1715	65	296	264	89
CSIRO 6	123	184	150	2126	2702	127	216	241	112
TM 18	138	103	75	2473	1889	76	173	146	84
LSD (P<0.05) *B. napus*	Var, 11			Var x Boron, 217			Var x Boron, 14		
B. juncea	Var, 39								

Table 3.3. Effect of applying B on seed weight and B content of oilseed rape genotypes grown on low B Mt. Compass sand. Values represent means of 3 replications. Genotypes in bold type are *Brassica juncea.*

	Dry weight (mg seed^{-1})		B content (ng seed^{-1})	
	B+	B0	B+	B0
BLN 702	2.8	2.3	52	53
761	3.5	3.9	83	84
Dunkeld	3.0	3.1	64	61
Zheyou 2	2.8	3.0	61	58
Siren	2.8	2.5	62	65
Barossa	2.7	2.7	55	56
Oscar	2.7	2.3	55	61
Narendra	2.6	2.5	49	44
92-13	2.8	2.6	48	55
Yickadee	2.8	2.7	53	55
Hyola 42	3.1	3.1	59	55
Zhongyou 821	2.1	2.3	49	54
CPI 81792	1.0	1.2	44	36
397-23-2-3-2	1.2	1.6	42	53
Pusa Bold	0.9	1.0	37	35
CSIRO 1	1.0	1.4	55	37
CSIRO 6	0.8	0.9	36	36
TM 18	0.8	0.9	35	42
LSD, P<0.05. *B. napus*	var, 0.3		var, 5	

In *B. napus*, the ranking of genotypes according to relative root length in solution culture (Chapter 2) correlated (r= 0.84, P<0.001) with relative seed yields derived from the field (Fig. 3.4), notwithstanding the differences in the severity of B deficiency between the two growth media. In *B. juncea*, no correlation between relative root length and relative seed yield was present.

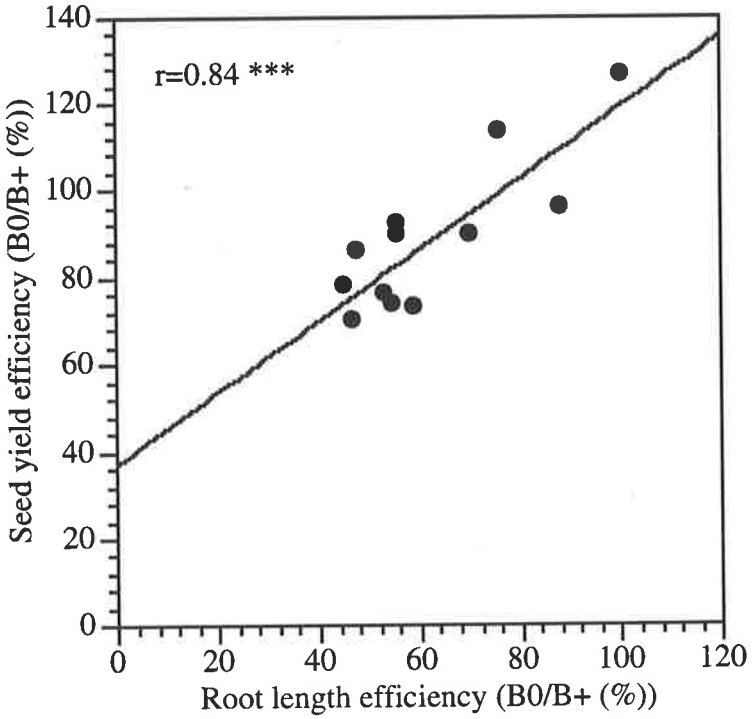


Fig. 3.4. The relationship between root length efficiency, as determined from solution culture screening (Chapter 2) of 10 d old *Brassica napus* genotypes, and seed yield efficiency from field evaluation of these same genotypes. Yield values were obtained from 3 replications, while root length data from 16 replications. Equation: Seed yield efficiency = 0.82 (root length ratio) + 37. Correlation coefficient, r, *** (P<0.001).

Experiment 3(b) Boron efficiency in three *Brassica napus* cultivars grown in pot culture

3.3.4 General Observations

Plants in adequately supplied treatments were all healthy in appearance and without any symptoms. At D28, cultivars grown in B0 treatments showed various visual symptoms to B deficiency. Barossa and Zhongyou 821 were chlorotic in the older leaf tissues which appeared to develop in some cases into necrotic lesions. Downward (adaxial) curvature of these leaves was also present, though not in Dunkeld. A 'puckered' appearance in the interveinal region of the leaf was also evident in Dunkeld, and in cultivars Barossa and Zhongyou 821, though to a less extent.

3.3.5 Shoot Growth and Net Boron Uptake

According to the B concentrations in the YOL+1, all B0 grown plants were B deficient, irrespective of variety although the lowest concentrations were in Barossa (Fig 3.5). Shoot growth increased with external B supply, but cultivars varied in the response to low B at both D14 and D28 (Fig. 3.6). At D14 shoot growth with B supplied was highest in Zhongyou 821 followed by Barossa and then Dunkeld, which corresponded to an increased net B uptake (net B accumulation) of B into the shoot of Zhongyou 821 more so than Barossa and Dunkeld. Relative shoot growth (B0/B+) ranged from 74-78 % for Zhongyou 821 and Barossa, while Dunkeld appeared more susceptible to B deficiency with a relative shoot growth of 56 %.

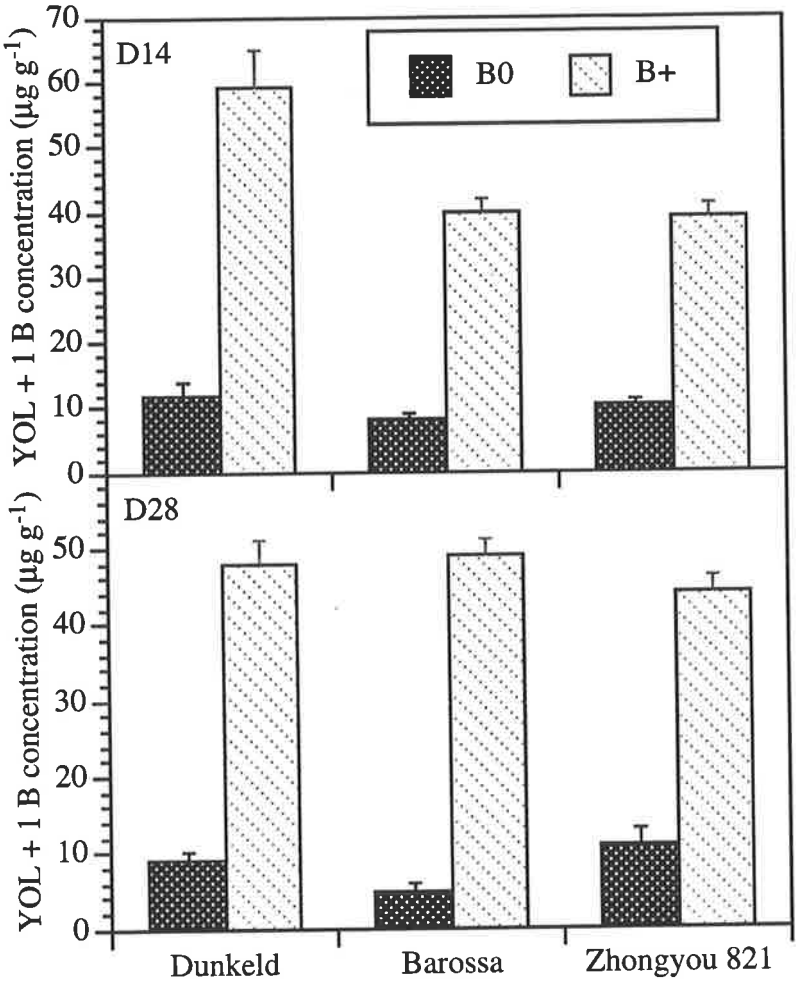


Fig. 3.5. Effect of low (B0: 0 mg B kg^{-1}) and adequate (B+: 0.25 mg B kg^{-1}) boron supply on B concentration of the YOL+1 leaf in three *Brassica napus* cultivars. Values represent means of 3 replications ± se.

In Zhongyou 821 relative shoot growth increased from D14 to D28 by 5%, while Dunkeld increased by 19 % and Barossa declined by 15 %. Zhongyou 821 demonstrated efficiency in accumulating B into the shoot, around 50 % more than in Barossa and 38 % more than Dunkeld.

Relative shoot growth at D28 correlated with relative seed yield, with Barossa most depressed by low B while Dunkeld and Zhongyou 821 maintained higher relative growth and seed yield in low B soil.

3.3.6 Yield Components

No reduction in branch number was observed in Dunkeld and Barossa, while a small reduction of around 17 % was observed in Zhongyou 821 (Fig. 3.7). Pod set was reduced in B0 plants by 23 and 21 % in both Barossa and Zhongyou 821 respectively, while in Dunkeld no response was seen. B deficiency depressed seed yield by 43, 21 and 17 % for Barossa, Dunkeld and Zhongyou 821, respectively. Responses of cultivars to low soil B in this pot study correspond to B responses from field trial, though the extent of B deficiency in Barossa was more severe in pots due to the level of B deficiency in the solution culture.

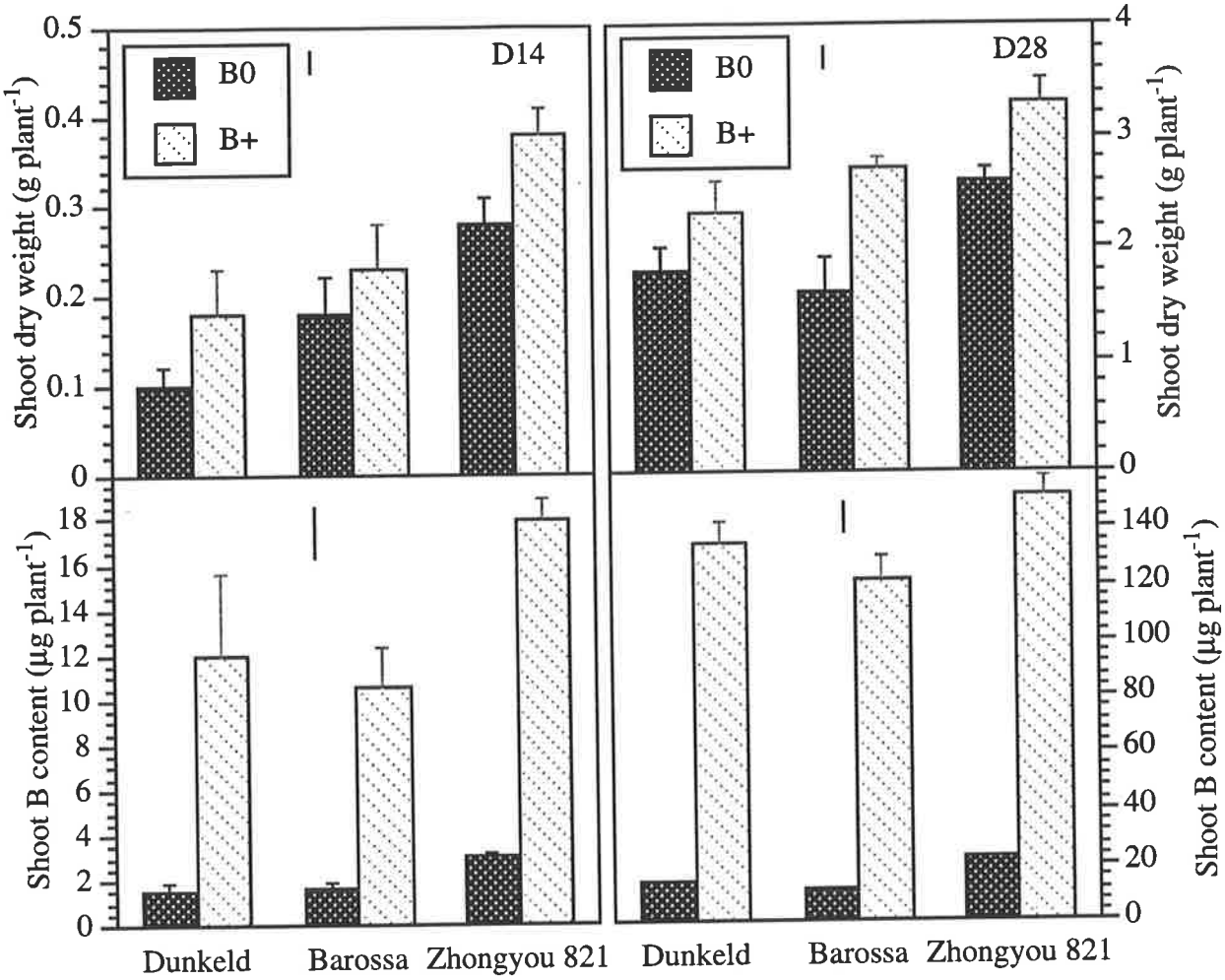


Fig. 3.6. Effect of low (B0: 0 mg B kg^{-1}) and adequate (B+: 0.25 mg B kg^{-1}) boron supply on shoot dry weight and B content at D14 and D28, in three *Brassica napus* cultivars. Values represent means of 3 replications ± se. Vertical lines depict LSD (P<0.05). D14: Shoot dry weight. Var, Boron. D14: Shoot B content. Var x Boron. D28: Shoot dry weight. Var x Boron D28: Shoot B content. Var x Boron.

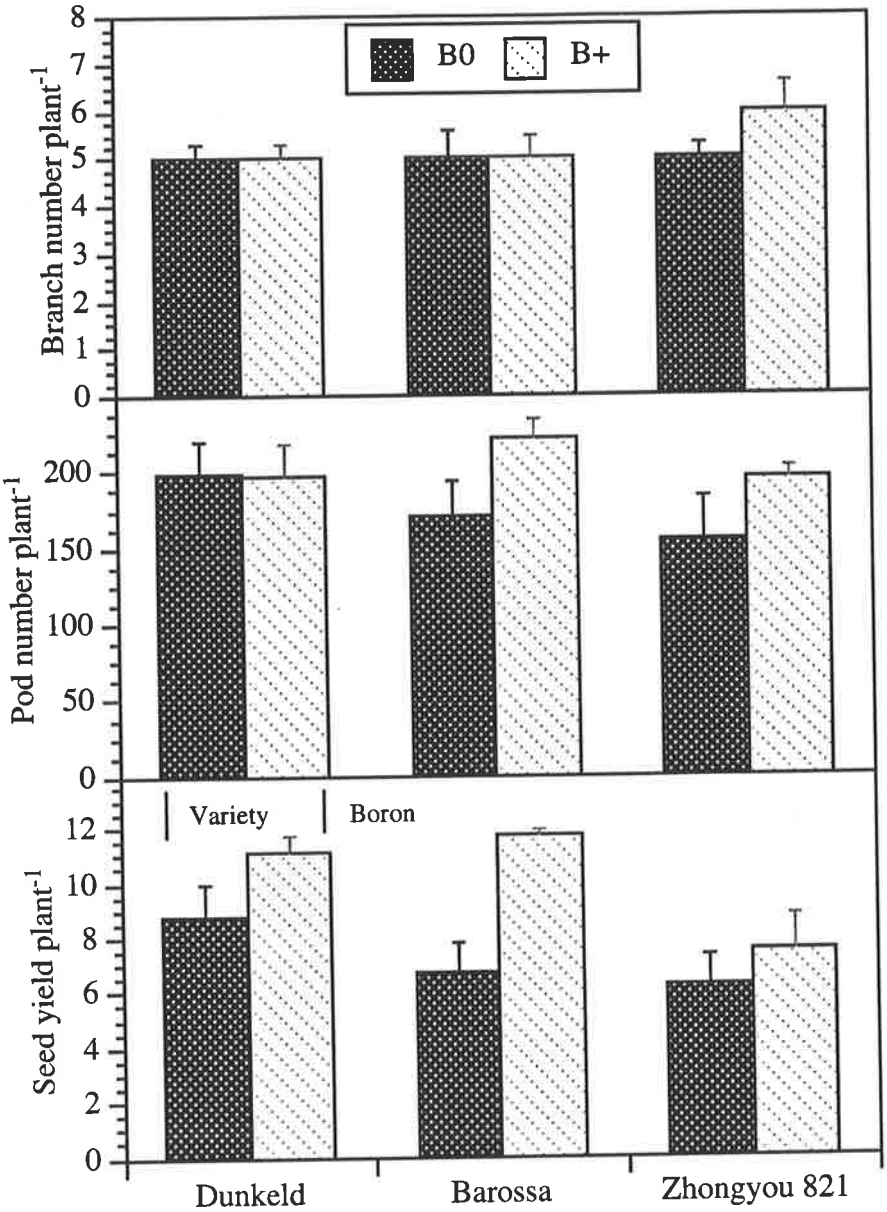


Fig 3.7. Yield components of three cultivars of Brassica napus grown under glasshouse conditions in low (0 mg B kg^{-1} soil) and adequate (0.25 mg B kg^{-1} soil) levels of B. Bars represent means of 3 replications ± se. Vertical lines depict LSD ($P<0.05$), Variety, Boron.

3.4 Discussion

The selection criterion for inclusion of a genotype into the field experiment was primarily on its performance in the solution culture screen (Chapter 2), with the aim of including both B efficient and inefficient genotypes. Where a genotype had previously been reported as B efficient (i.e 92-13: Yang *et al.*, 1993; Pusa Bold: Sakal *et al.*, 1991; Zhongyou 821: Yang *et al.*, 1994), these genotypes were also included in the field screen. Unfortunately, the availability of seed was a major problem which resulted in the omission of the hybrid canola cultivar Huashuang 2 (the most B efficient variety in solution culture screening, Chapter 2) and also resulted in a reduced sowing rate from 6 kg ha^{-1} (the normal seed rate used by farmers in South Australia when growing canola) down to 4 kg ha^{-1}. Following the initial field trial presented here, seed was multiplied under greenhouse conditions for subsequent field trials. In the two years that followed, field experiments were abandoned due to severe drought in the first year, and suspected residual herbicide effects in the second.

The decision to separate *B. juncea* from *B. napus* genotypes and to place them into individual field trials proved an appropriate decision in light of the exceptional growth and vigour of the *B. juncea*. *B. juncea* genotypes matured 2 weeks earlier than *B. napus* genotypes thereby reducing the adverse effects of increasing soil water deficit and its effect on B availability to the plants with increasingly dry weather as the season progressed.

In both *B. napus* and *B. juncea*, genotypic variation in B response was expressed in seed yield at maturity. B efficient *B. napus* genotypes were Zhongyou 821, Dunkeld, Zheyou 2 and 92-13, and B efficient *B. juncea* genotypes, Pusa Bold and CSIRO 6. The efficiency of Zhongyou 821, 92-13 and Pusa Bold corroborate previously published B efficiencies of these three genotypes (Sakal *et al.*, 1991; Yang *et al.*, 1993; Yang *et al.*, 1994).

The expression of B efficiency in Dunkeld is particularly significant, as unlike the remaining B efficient *B. napus* genotypes, it has been bred specifically for the Australian

canola growing regions. In areas of Australia where B deficiency has reduced oilseed rape yields (i.e. Southern Tablelands of NSW. Myers *et al.*, 1983), growing Dunkeld on these soils may help to maintain higher seed yields.

While a significant correlation ($r=0.84$; $P<0.05$) exists between B efficiency in root length (Chapter 2) and B efficiency from the field for *B. napus*, in *B. juncea*, no relationship exists. *B. juncea* varieties Pusa Bold and CSIRO 6 were B inefficient in the solution culture screening, yet very efficient in the field. The ambiguity in the present result means further field evaluation is required before the proposed screening technique in Chapter 2 can be endorsed for *B. juncea*; for *B. napus*, however, solution culture would appear to provide a suitable method for screening B efficient germplasm, the primary objective of the field experiment. This is a significant result which will enable plant breeders to screen a large number of *B. napus* genotypes for B efficiency, reducing the time and cost often associated with field screening.

In the pot study, B concentrations in the YOL+1 of B0 grown plants were more depressed (5-11 mg B kg^{-1} dry weight) compared to plants sampled in the field (18-19 mg B kg^{-1} dry weight for Dunkeld, Barossa and Zhongyou 821). For predicting final yield response, these B concentrations are outside of the adequate B range, and into the marginal (field trial) to deficient (pot study) range (for prognosis, adequate B: 20-50 mg B kg^{-1} DW; Weir and Cresswell, 1995), so loss of seed yield was expected. This yield loss did not occur in varieties Dunkeld and Zhongyou 821, and may indicate that the mechanism of B efficiency in these cultivars is associated with differences in external B requirement and/or uptake ability. An increased uptake ability is in line with observations made by Sakal *et al.* (1991) who showed grain yield and B uptake were highly correlated in sesame and mustard, with B efficient cultivars removing more B from the soil than susceptible cultivars. In contrast, the work of Rerkasem *et al.* (1993) demonstrated that vegetative response and reproductive success in wheat are not correlated. B efficiency in wheat may be due in part to differences in B supply to the germinating pollen in the stigma and style (Cheng and Rerkasem, 1993). From Experiment 3(b), the correlation between vegetative and final seed

yield response would indicate that unlike in wheat, the vegetative tissues contribute toward B efficiency in *B. napus*. Subsequent chapters will try to elucidate the mechanism behind B efficiency in oilseed rape.

Chapter 4

The Effects of Boron Supply on Rhizosphere pH in *Brassica napus* L.

4.1 Introduction

Changes in the rhizosphere through a release of organic acids, preferential cation uptake, and the release of photosynthetic substrates for rhizosphere micro-organism greatly affect nutrient uptake (Marschner, 1995).

In a recent review dealing with the mechanisms of B efficiency, Rerkasem and Jamjod (1997) proposed a potential role of root-soil interactions, including rhizosphere pH, in the efficiency mechanism. In such a situation, it is envisaged that an increased acidification of the rhizosphere by the B efficient genotype would mobilise soil adsorbed B, more so than in the B inefficient genotype. At this stage there is no direct evidence of such an effect occurring in any species, let alone oilseed rape, although the potential exists, with *Brassica napus* known to acidify its rhizosphere and enhance P availability under P deficiency (Grinsted *et al.*, 1982). With the ability to acidify its rhizosphere, there is potential for genotypic variation in the expression of this phenomenon.

The following study aims to observe the magnitude of rhizosphere acidification in varieties of *B. napus* which differ in B efficiency. Commensurate with observing rhizosphere acidification, changes in shoot B concentration and uptake (net B accumulation per unit of root length), will indicate whether there is enhanced B uptake that could be associated with rhizosphere acidification, leading toward the putative mechanism of B efficiency.

4.2 Materials and Methods

Experiment 4(a) The effects of boron supply on rhizosphere pH in oilseed rape grown in 'Mt. Compass' sand.

4.2.1 Design

The experiment used a factorial design: two *B. napus* varieties (Yickadee and Zhongyou 821) and two B treatments (0.4 mg B kg^{-1} (B0.4) and a treatment lacking B (B0)). The experiment was replicated four times.

4.2.2 Seed

Information on seed source and B content is given in Chapter 2, section 2.2.6 and Table 2.2 respectively.

4.2.3 Nutrient Solution and Growth Conditions

Prior to planting, seeds were surface sterilised in 70% EtOH for 1 min. and 1% NaOCl for 5 minutes, and then pregerminated at 22 °C for 24 h. Seeds were sown into PVC root boxes (12 x 25 x 1.5 cm; a photograph of a similar root box can be seen in Dinkelaker *et al.*, 1989) filled with a prewashed siliceous Mt. Compass sand (pH 5.4 in 0.01M $CaCl_2$; $CaCl_2$ extractable B, 0.1 - 0.2 mg B kg^{-1} soil) with the following nutrients added (mg kg^{-1} soil): $CaCO_3$, 1000 (mixed through before addition of other nutrients); $(NH_4)_2SO_4$, 497; $CaSO_4$, 652; $MgSO_4 \cdot 7H_2O$, 140; K_2SO_4, 114; KH_2PO_4, 72; $ZnSO_4 \cdot 7H_2O$, 6.6; NaCl, 3.2; $MnSO_4 \cdot 4H_2O$, 7.4; $CuSO_4 \cdot 5H_2O$, 2.3; $FeSO_4 \cdot 7H_2O$, 1.4; $CoSO_4 \cdot 5H_2O$, 0.23; H_2MoO_4, 0.2. After the addition of $CaCO_3$ and nutrient, pH of the soil was 8.0 in 0.01M $CaCl_2$. B treatments were 0.4 mg B kg^{-1} as boric acid (B0.4) and 0 mg B kg^{-1} (B0), imposed by omission. Root boxes were watered to 10% (w/w) with high purity water (18 MΩ. cm^{-1} conductivity, prepared by reverse osmosis and ion exchange) and placed in containers at an angle of 45 ° to enable plant roots to grow down the face of the transparent lid. Plants were placed in a growth chamber with 20°/15° C day/night temperatures, and a

10/14 h light/dark photoperiod supplied by high-pressure mixed-metal halide lamps delivering a photon flux density of 350 μmol m^{-2} s^{-1}.

4.2.4 Measurements

After 21 days growth, rhizosphere pH was measured by antimony microelectrode at various sites in the bulk and rhizosphere soil (Häussling *et al.*, 1985).

Following measurement of rhizosphere pH, shoots were rinsed for 30 s in high purity water and then oven dried at 80°C for at least 24 h. Samples were weighed and analysed for nutrient elements (except N) as previously reported in section 2.2.4.

Experiment 4(b) The effects of boron supply on rhizosphere pH in oilseed rape grown in Laffer sand.

4.2.5 Design

The experiment used a factorial design: two *B. napus* varieties (Yickadee and Dunkeld) and two B treatments (0.25 mg B kg^{-1} (B0.25) and a treatment lacking B (B0)). The experiment was replicated four times.

4.2.6 Seed

Information on seed source and B content is given in Chapter 2, section 2.2.6 and Table 2.2 respectively.

4.2.7 Nutrient Solution and Growth Conditions

Prior to planting, seeds were surface sterilised in 70% EtOH for 1 min. and 1% NaOCl for 5 minutes, and then pregerminated at 22 °C for 24 h. Seeds were sown in PVC root boxes with plexiglass lids (12 x 25 x 1.5 cm) filled with a prewashed siliceous sand (commonly known as Laffer sand; pH 6.4 in 0.01M $CaCl_2$; $CaCl_2$ extractable B, 0.2-0.3 mg B kg^{-1} soil) with the following nutrient composition added (mg kg^{-1} soil):

$(NH_4)_2SO_4$, 497; $CaSO_4$, 652; $MgSO_4 \cdot 7H_2O$, 140; K_2SO_4, 114; KH_2PO_4, 72; $ZnSO_4 \cdot 7H_2O$, 6.6; NaCl, 3.2; $MnSO_4 \cdot 4H_2O$, 7.4; $CuSO_4 \cdot 5H_2O$, 2.3; $FeSO_4 \cdot 7H_2O$, 1.4; $CoSO_4 \cdot 5H_2O$, 0.23; H_2MoO_4, 0.23. A nitrification inhibitor, N-Serve™ (Dow Chemicals, Michigan USA), was added at the rate of 1 ml of a 0.01% solution per kg soil. B treatments were 0.25 mg B kg^{-1} as boric acid (B0.25) and 0 mg B kg^{-1} (B0), imposed by omission. Root boxes were watered to 10% (w/w) with high purity water (18 MΩ. cm^{-1} conductivity, prepared by reverse osmosis and ion exchange) and placed in containers at an angle of 45 ° to enable plant roots to grow down the face of the transparent lid. Plants were placed in a growth chamber with 20°/15° C day/night temperatures, and a 10/14 h light/dark photoperiod supplied by high-pressure mixed-metal halide lamps delivering a photon flux density of 350 μmol m^{-2} s^{-1}.

4.2.8 Measurements

After 21 days growth, rhizosphere pH was determined by two non-destructive means; the use of an antimony microelectrode and the embedding of roots in agar, previously mixed with the pH indicator bromocresol purple (Marschner *et al.*, 1982; Häussling *et al.*, 1985). For the agar technique, the front side of one container was removed and a prepared agar sheet (1 % agarose, 3 mm thick) containing 0.01 % bromocresol purple as pH indicator was placed on the soil surface. In addition to the pH values indicated by the colour changes of the pH indicator, the pH was measured by an antimony microelectrode at different sites in the bulk soils and rhizosphere soil.

Following measurement of rhizosphere pH, shoots were rinsed for 30 s in high purity water and then oven dried at 80°C for at least 36 h. Samples were weighed and analysed for nutrient elements (except N) as previously reported in section 2.2.4.

4.2.9 Statistical Analyses

The data were subject to analysis of variance using the Genstat 5 statistical package. Significant mean separation is indicated by the use of the least significant difference (LSD) at the 5% level where the F value is significant.

4.3 Results

Experiment 4(a) The effects of boron supply on rhizosphere pH in oilseed rape grown in 'Mt. Compass' sand.

4.3.1 Rhizosphere pH

No significant B treatment effects on rhizosphere pH were measured in either variety, though Yickadee generally appeared to acidify the rhizosphere in B0 grown plants more than in B0.25 plants, especially at the tip of the tap root (Fig. 4.1).

4.3.2 Shoot Growth and Nutrient Concentration

No significant treatment effect on shoot dry weight was observed between the two varieties (Fig. 4.2), although addition of 0.4 mg B raised shoot B concentrations significantly ($p<0.001$) relative to B0 grown plants (Fig. 4.3). The B concentration in the YOL, YOL + 1 of plants grown in the B0 treatment, indicate that both Zhongyou 821 and Yickadee were within the B deficient range (6-13 mg B kg^{-1} soil; Reuter *et al.*, 1997) (Fig. 4.4), with Yickadee exhibiting a significantly lower B concentration in the young tissues (30 % reduction over Zhongyou 821) indicating it was more B deficient.

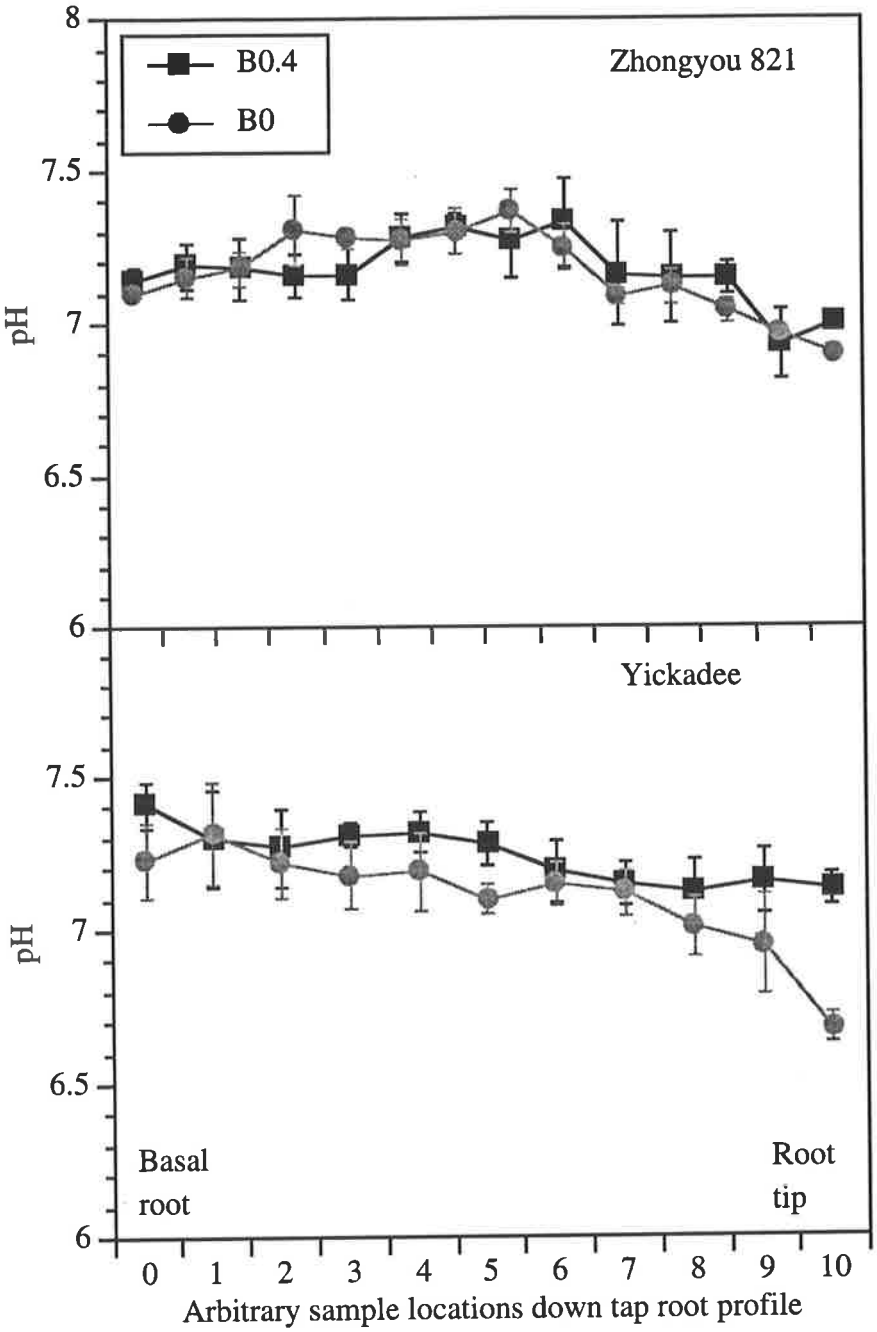


Fig. 4.1. The effects of B supply (B0.4, 0.40 mg B kg^{-1} soil; B0, B omitted) on the rhizosphere pH of two varieties of oilseed rape grown for 21 days in Mt. Compass sand. pH measurements were taken around every 2 cm down the tap root profile using an antimony micro-electrode. The pH of the bulk soil prior to growing plants was 8.0. Values represent means of 4 replications ± se.

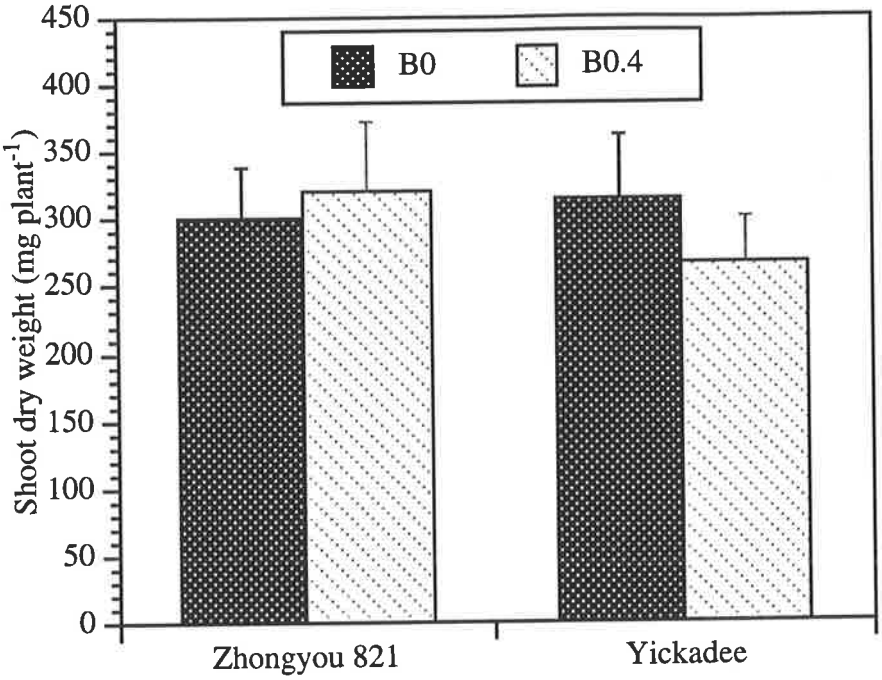


Fig. 4.2. The effect of B supply (B0.4, 0.4 mg B kg^{-1} soil; B0, B omitted) on 21 day old varieties of *B. napus* grown in Mt. Compass sand. Bars represent the mean of 4 replications ± se.

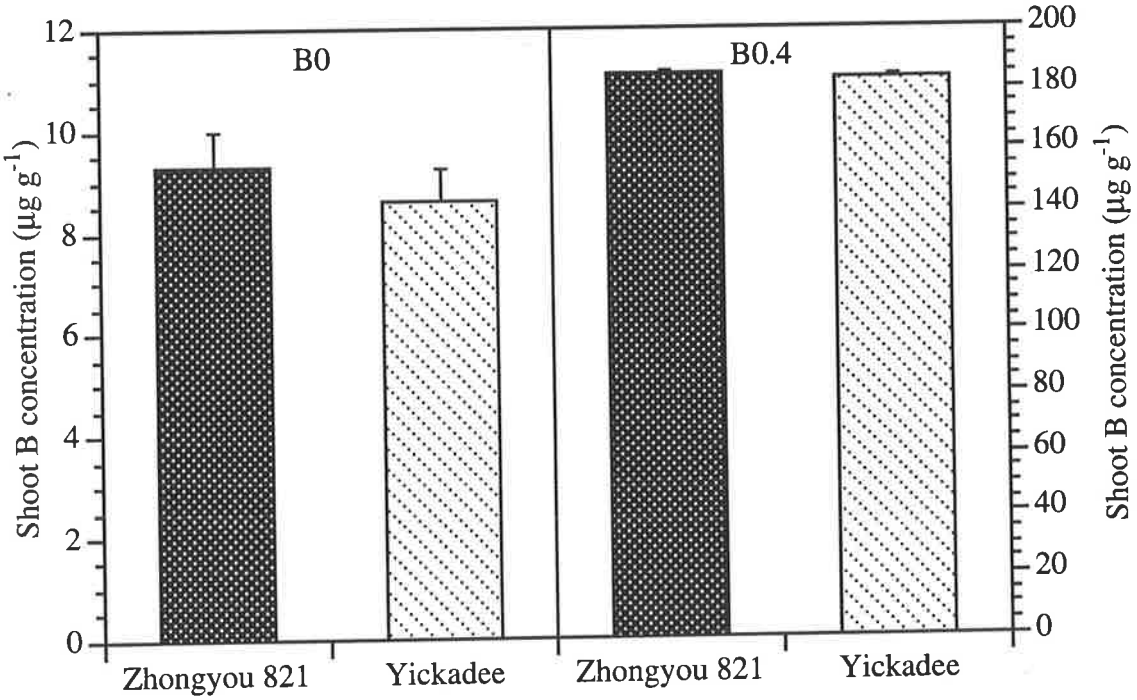


Fig. 4.3. Shoot B concentration in 21 day old varieties of *B. napus* grown at low (B0) and high (B0.4) B supply. Bars represent the mean of 4 replications ± se. LSD ($P<0.05$). Boron, 18

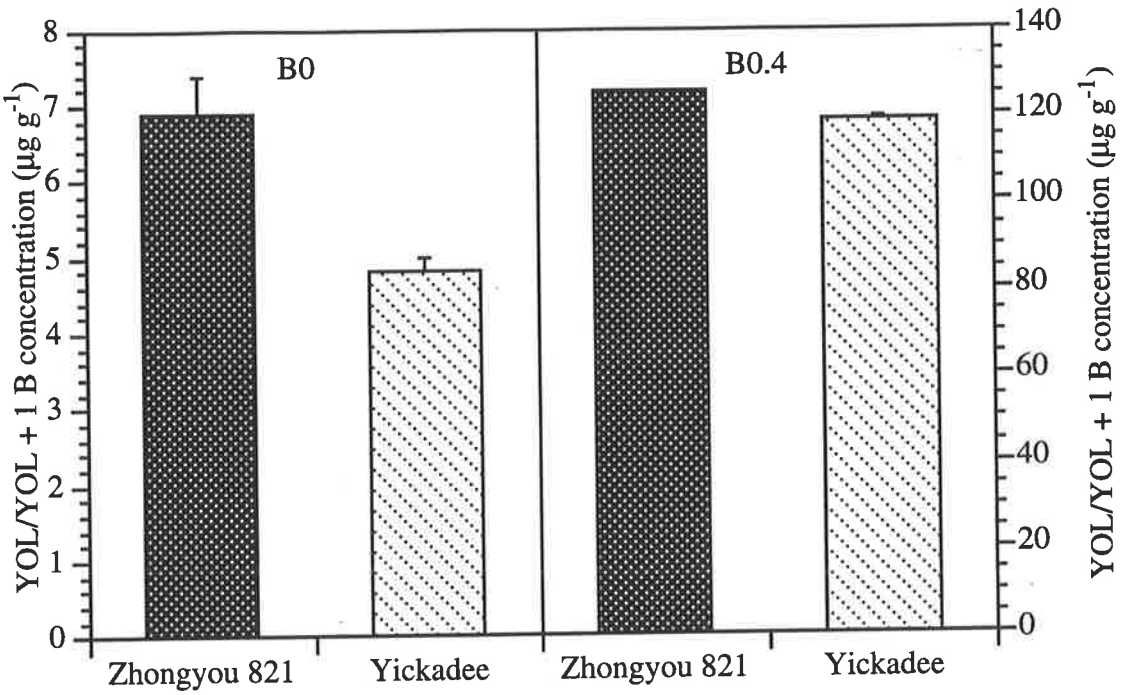


Fig. 4.4. The effect of low (B0) and high (B0.4) B supply on the YOL, YOL +1 (Youngest open leaf, youngest open leaf + 1) B concentration. Bars represent the mean of 4 replications ± se.

While the effect of B deficiency on shoot B concentration was severe, the majority of nutrients in the shoot were only marginally affected by low B supply (Table 4.1). Significant reductions in shoot nutrient concentration due to B deficiency were mainly observed in the variety Zhongyou 821, for the elements Mn, Cu, Ca and P. In contrast, a significant treatment effect in Yickadee was only present for S, while Mn and Zn concentrations in the shoot of B0 grown plants were also enhanced.

Table 4.1. Effect of B supply (B0, B omitted; B0.4, 0.4 mg B kg^{-1} soil) on shoot nutrient levels (mg kg^{-1}) in 21 day old oilseed rape varieties grown in Mt. Compass sand. Values are means of 4 replications.

		Boron Level			Boron Level	
Variety	Element	B0	B0.4	Variety	B0	B0.4
Zhongyou 821	Fe	67	78	Yickadee	72	80
	Mn	30	51		40	34
	B	9	189		7	183
	Cu	9	12		11	13
	Zn	53	58		63	52
	Ca (%)	2.6	3.4		2.8	2.9
	Mg (%)	0.66	0.61		0.73	0.83
	Na (%)	0.06	0.05		0.10	0.11
	K (%)	3.8	4.1		5.0	5.6
	P (%)	0.26	0.46		0.27	0.31
	S (%)	1.2	1.4		1.7	2.1

LSD ($P<0.05$). Variety (Var), Boron Treatment (B), Variety x Boron Treatment (Var x B). B: B, 18^{***}; Mn: Var x B, 9.3^{***}. Cu: B 1.5^{**}. Ca: Var x B, 0.5^{*}. Mg: Var, 0.09^{**}. Na: Var x B, 0.02^{*}. K: Var, 0.9^{**}. P: Var x B, 0.06^{**}. S: Var, 0.2^{***}; B, 0.1^{**}.

*** F prob. <0.001; * F prob. <0.01; * F prob. <0.05.

Experiment 4(b). The effects of boron supply on rhizosphere pH in oilseed rape grown in 'Laffer' sand.

4.3.3 Rhizosphere pH

In both B0 and B0.25 treatments, plants acidified their rhizosphere by up to 2.5 pH units (Fig 4.5 and 4.6), with little variation in pH observed down the tap root toward the root tip, or along the length of the root lateral (see Plate 4.1 for observed acidification). Varietal differences were seen not so much in the B0 treatment, but more so in the B0.25 treatment, where Yickadee acidified its rhizosphere more than Dunkeld. The treatment effect indicated that under B deficiency, rhizosphere pH fell more than in plants grown in adequate B. This treatment variation was around 1 pH unit in Dunkeld, with a smaller effect in Yickadee.

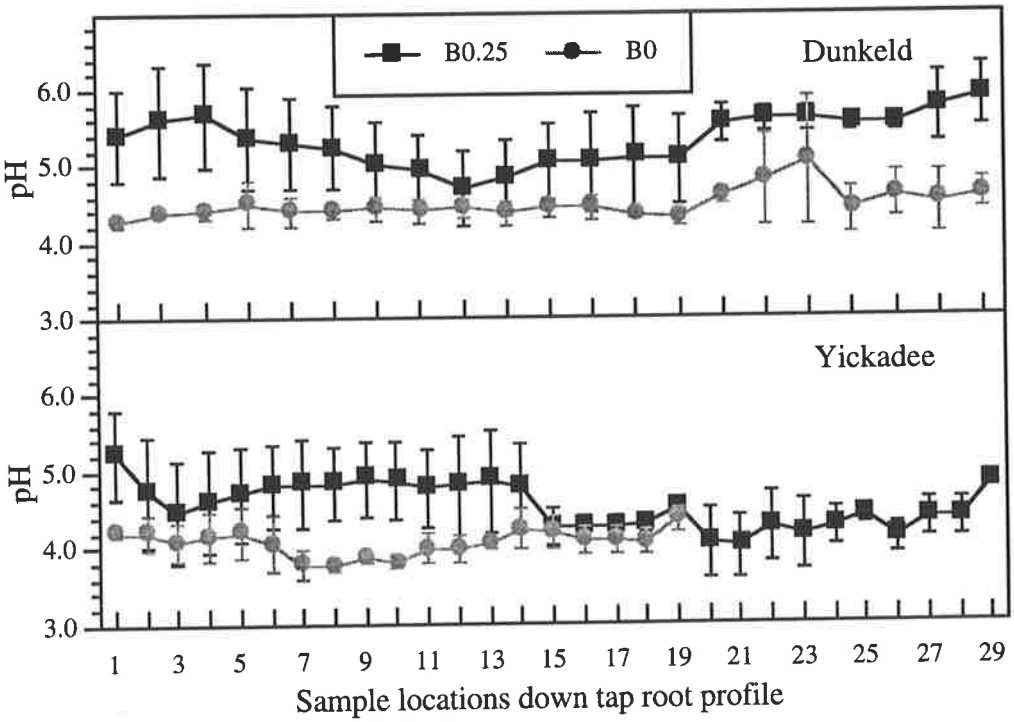


Fig. 4.5. The effect of B nutrition (B0.25, 0.25 mg B kg^{-1} soil; B0, B omitted) on the rhizosphere pH of two varieties of oilseed rape grown for 21 days in 'Laffer' sand. pH measurements were taken at 1 cm intervals on the tap root using an Antimony micro-electrode. The pH of the bulk soil prior to growing plants was 6.8. Values represent the mean of 4 replications ± se.

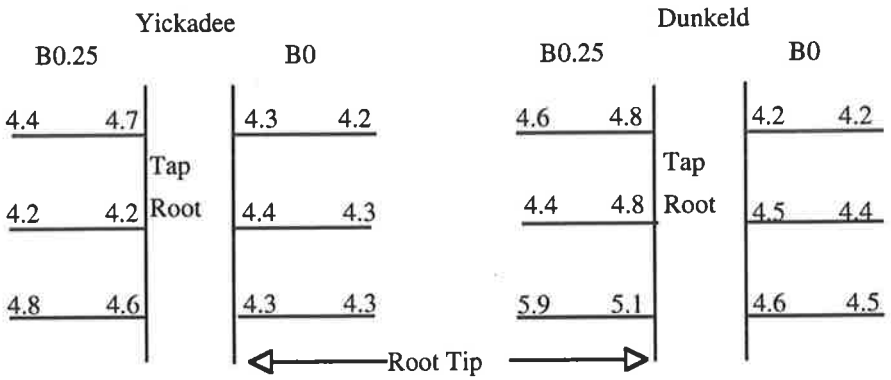


Fig. 4.6. The effect of B supply (B0.25, 0.25 mg B kg^{-1} soil; B0, B omitted) on lateral root rhizosphere pH of two canola varieties grown for 21 days in Laffer sand. pH measurements were taken using an Antimony micro-electrode approximately 1 cm from the tap root on the lateral (value closest to the tap root) and at the tip of the lateral root. The pH of the bulk soil prior to growing plants was 6.8. Values represent means of 4 replications. LSD ($P<0.05$). Variety, Boron: 0.2^{*}, 0.3^{**}. ** F prob. <0.01; * F prob. <0.05.

4.3.4 Plant Growth, Shoot B Concentration and Uptake

No visible signs of B deficiency on leaf tissue were observed in B0 grown plants, which corresponded to an absence of a response in shoot growth (Fig 4.7). In comparison, root dry weight and shoot B concentration were significantly ($P<0.001$) reduced in B0 grown plants (Fig. 4.7 and 4.8 respectively). Root dry weight declined by around 47 and 35 %, and shoot B concentration by 51 and 62 % for Dunkeld and Yickadee respectively. The effects of B deficiency were also observed in the primary root length of Yickadee with a relative (B0/B0.25) root length of 72 % , while in Dunkeld, root length was not significantly diminished (B0/B0.25, 98%) (Fig. 4.9).

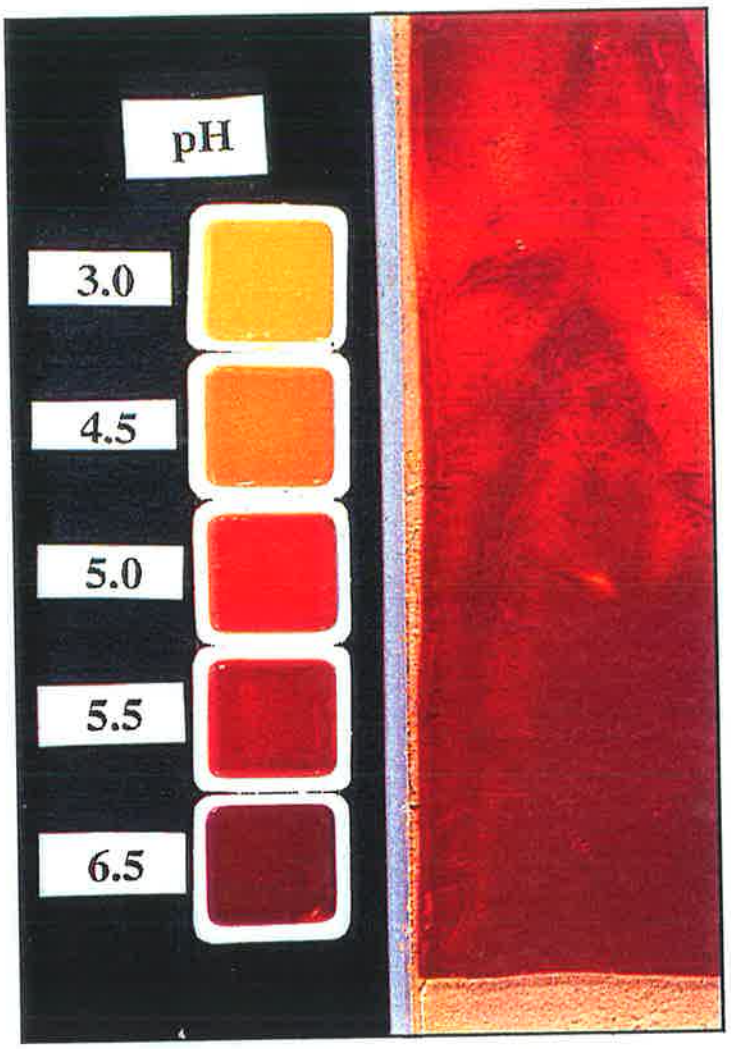


Plate 4.1. pH variation in the rhizosphere of Barossa grown in Mt. Compass sand with adequate B supply.

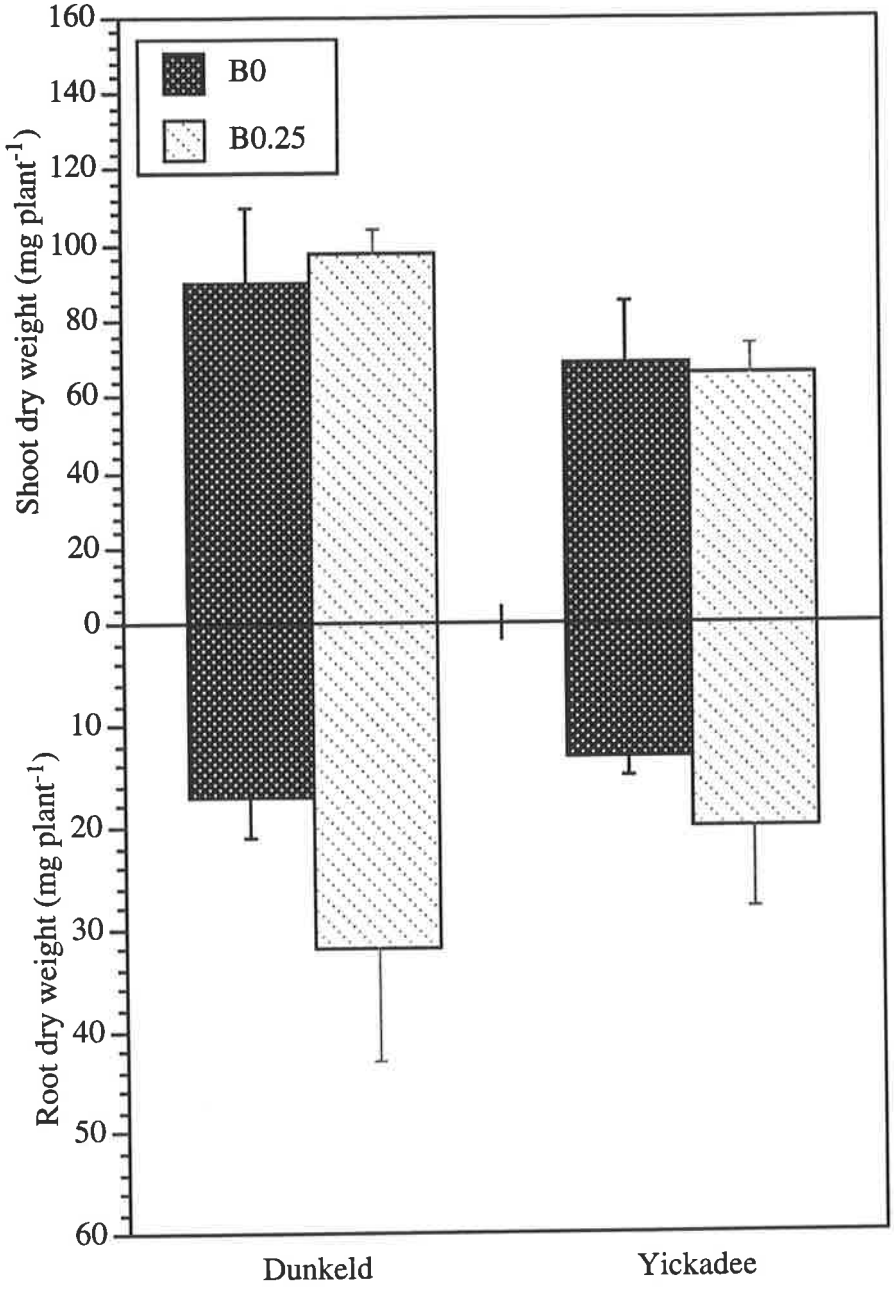


Fig. 4.7. The effect of B supply (B0.25, 0.25 mg B kg^{-1} soil; B0, B omitted) on the shoot and root dry weight of two canola varieties grown for 21 days in Laffer sand. Bars represent the mean of 4 replications ± se.

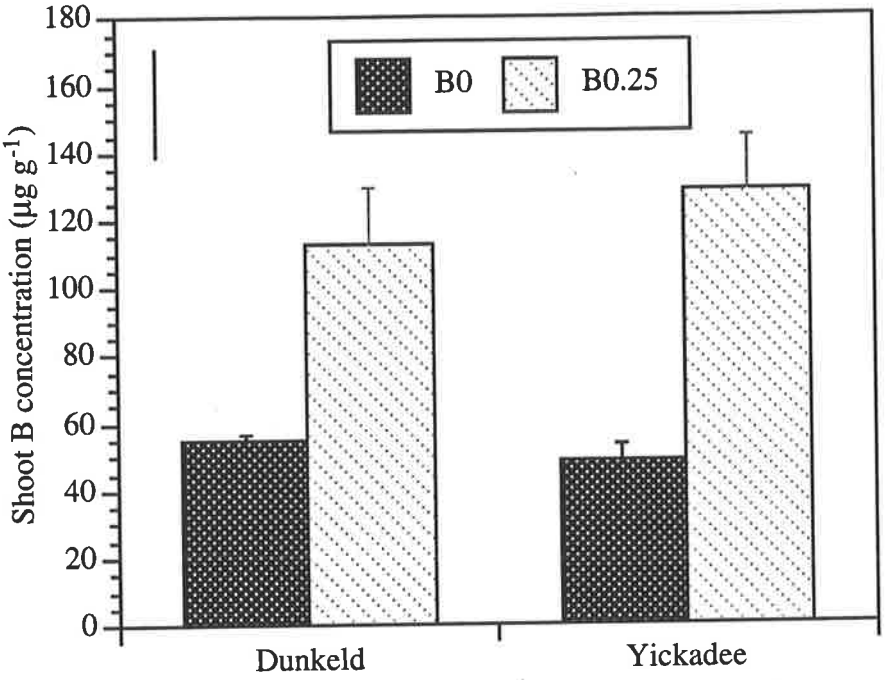


Fig. 4.8. The effect of B supply (B0.25, 0.25 mg B kg^{-1} soil; B0, B omitted) on the shoot B concentration of two canola varieties grown for 21 days in Laffer sand. Bars represent the mean of 4 replications ± se. Vertical line depicts LSD (P<0.05), Boron.

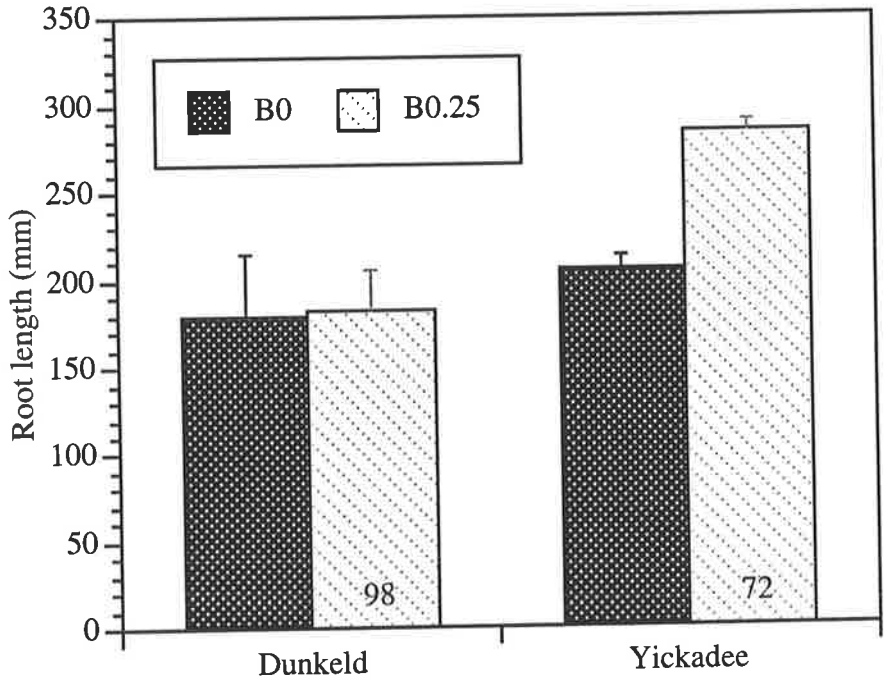


Fig. 4.9. The effect of B supply (B0.25, 0.25 mg B kg^{-1} soil; B0, B omitted) on the primary root length of two varieties of *B. napus* grown for 21 days in Laffer sand. Values placed in the base-line of the B0.25 treatment bars indicate relative root length (B0/B0.25 (%)). Bars represent the mean of 4 replications ± se.

A significant interaction ($p<0.05$) between variety and B for the uptake of B into the shoot (net B accumulation between D0-D21 per unit of root length), is indicative of a greater efficiency in the acquisition of B by the roots of the variety Dunkeld (Fig. 4.10). Dunkeld took up 56 % more B per mm of root length than the variety Yickadee when grown under adequate B supply, while under B deficiency, the uptake of B into the shoot of Dunkeld was reduced by around 53 %, and in Yickadee, by around 35 %. Uptake into the shoot of Dunkeld at low B supply was still greater than in Yickadee, by around 38 %.

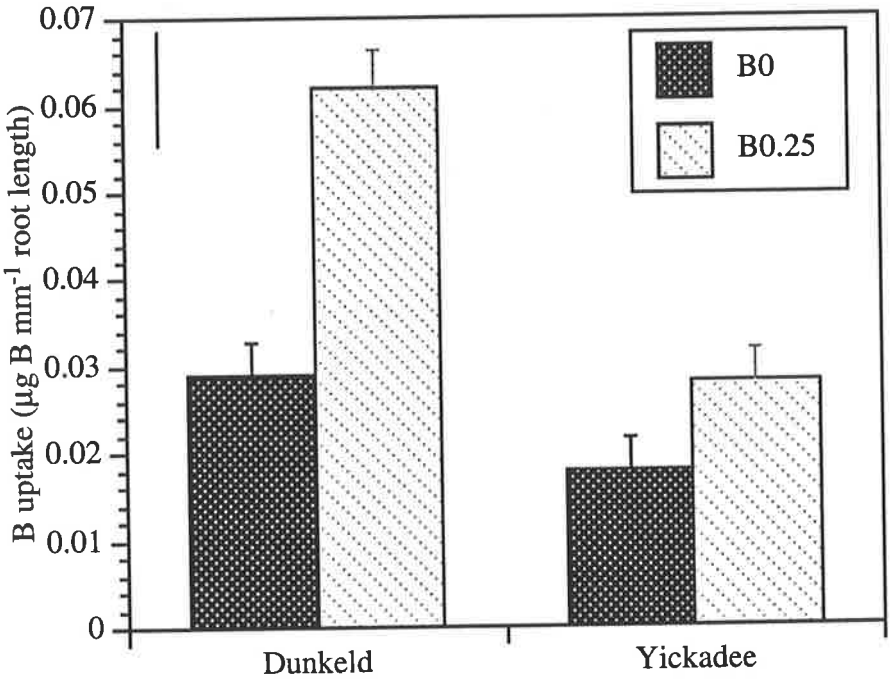


Fig. 4.10. The effect of B supply (B0.25, 0.25 mg B kg^{-1} soil; B0, B omitted) on B uptake (net B accumulation between D0 and D21) into the shoot per unit of primary root length in two varieties of canola grown for 21 days in Laffer sand. Bars represent the mean of 3 replications ± se. Vertical line depicts LSD ($P<0.05$), Var x Boron.

4.3.5 Shoot Nutrient Concentration

Shoot nutrient concentrations were generally maintained or enhanced (mainly for the metal ions) in B0 grown plants of Dunkeld, while in Yickadee, shoot nutrient concentrations were reduced (Table 4.2). Treatment effects were significant for only B and Ca, mainly as a result of the considerable variability within the data.

Table 4.2. Effect of B supply (B0, B omitted; B0.25, 0.25 mg B kg^{-1} soil) on shoot nutrient levels (mg kg^{-1}) in 21 day old oilseed rape varieties grown in Laffer sand. Values are means of 4 replications.

		Boron Level			Boron level	
Variety	Element	B0	B0.25	Variety	B0	B0.25
Dunkeld	Fe	225	219	Yickadee	127	206
	Mn	135	122		83	137
	B	55	113		49	129
	Cu	55	44		23	46
	Zn	276	255		179	301
	Ca (%)	1.4	1.7		0.9	1.9
	Mg (%)	0.4	0.4		0.3	0.4
	Na (%)	0.12	0.13		0.08	0.13
	K (%)	5.8	5.4		5.2	5.9
	P (%)	1.3	1.2		1.4	1.6
	S (%)	4.0	3.5		2.9	3.9

LSD ($P<0.05$). Boron Treatment (B). B: B, 32^{***}. Ca: B, 0.4^{*}. *** F prob. <0.001; * F prob. <0.05.

4.4 Discussion

An initial aim of this study was to observe the magnitude of rhizosphere acidification in varieties of *B. napus* which differed in B efficiency. Results suggest that both efficient and inefficient varieties are able to acidify their rhizosphere, with no indication of an enhanced acidification in the efficient variety. In fact the B inefficient variety, Yickadee, appeared to acidify its rhizosphere more than the efficient variety Zhongyou 821 in Experiment 4(a), and Dunkeld in Experiment 4(b). From this result it is highly unlikely that rhizosphere acidification contributes toward the differences in efficiency in oilseed rape.

A direct effect of rhizosphere acidification on B uptake is hard to determine given that both varieties acidified their rhizosphere by similar amounts and exhibited a similar depression in shoot B concentrations in B0 grown plants. In contrast, metal ion concentrations in the shoot of B0 grown Dunkeld were enhanced over B0.25 grown plants (i.e. for Fe, Mn, Cu and Zn), most likely as a direct effect of rhizosphere acidification and the mobilisation of nutrient. In Yickadee this enhanced metal ion concentration in B0 grown plants was not observed, even though it acidified its rhizosphere, and would indicate that the severe reduction in root length was the limiting factor to nutrient uptake.

A mechanism of B efficiency in Dunkeld would appear to be due to an increased capacity to uptake B. B uptake (net B accumulation) into the shoot per unit of root length was greater in Dunkeld in comparison to Yickadee. Jungk and Claasen (1986) report a similar effect for P and K uptake in *B. napus*, with P and K uptake per unit of root length positively correlated with the volume of the root hair cylinder. The increased B uptake in Dunkeld may also be due to such an occurrence, and requires continued research.

There were a number of anomalies in the results that require elucidation. For example, the smaller decline in rhizosphere pH in Experiment 4(a) when compared to Experiment 4(b). This can be explained by differences in the buffering capacity of the soil. For example, the extent to which roots acidify their rhizosphere depends on the rate of proton release by the roots and its diffusion into the soil, with a major factor affecting

diffusion being the soil buffering capacity for protons (Nye, 1972). In soils with high free $CaCO_3$ concentration, a lower pH decrease at the root surface can be explained by a strong buffering of $CaCO_3$ (Schaller, 1987). The smaller decline in rhizosphere pH in experiment 4(a) compared to experiment 4(b) may reflect such an occurrence. In experiment 4(a), $CaCO_3$ was added to the soil to raise the pH to eliminate a tendency in this soil to be manganese toxic for growing canola. While results indicate a substantial decrease in pH, irrespective of the high buffering capacity of the soil, the magnitude of the acidification may have been greater without the addition of excess $CaCO_3$. This effect was demonstrated in Laffer sand having no free lime.

The increase in rhizosphere acidification under B deficiency is another anomaly requiring elucidation. This may be explained in relation to maintaining cation-anion balance coupled with the effect of a reduced anion uptake due to B deficiency. With an excess cation uptake (as would be expected because NH_4^+ is used as the N source), the pH of the cytosol would decline. In this environment, cation-anion balance is maintained by an equivalent increase in the synthesis of organic acid anions, with subsequent transport of cations and anions into the vacuole or shoot (Marschner, 1995c). Hiatt and Hendricks (1967) reported a similar effect in barley roots supplied with a high level of K_2SO_4. Net efflux of H^+ led to a decrease in external pH from 5.60 to 5.12. Coupled with the onset of B deficiency, a reduced rate of anion uptake is envisaged (Pollard *et al.*, 1977; Parr and Loughman, 1983), further leading to an imbalance in the cation-anion uptake ratio, and a subsequent increased extrusion of H^+. The results presented in Experiment 4(b) may therefore reflect an effect of B on membrane function through a reduced anion uptake.

It is also feasible to suggest that this increased acidification arose from the leaking of intracellular contents into the rhizosphere. For example, Hedley *et al.* (1982) demonstrated a significant amount of non-volatile acidity extracted from oilseed rape rhizosphere soil originated from weakly acidic amino and phenolic groups of complex organic matter released into the rhizosphere. Recently, Cakmak *et al.* (1995) reported increased membrane leakage in boron deficient sunflower leaves. Leakage of phenolics,

amino acids and sucrose was enhanced by B deficiency. The increased acidification of the rhizosphere in B0 grown plants may be a reflection of increased leakage due to a reduced structural integrity of the membrane as a result of B deficiency.

While the rhizosphere effects are interesting in that they provide evidence for B nutritional effects at the root-soil interface, and most likely at the membrane level, the ability of the B inefficient variety to acidify its rhizosphere more so than the B efficient variety points to a limited (if any) effect of rhizosphere acidification in the efficiency mechanism. More importantly, shoot B uptake (net B accumulation) per unit of root length is greater in the B efficient variety Dunkeld, demonstrating a mechanism of B efficiency for this variety.

Chapter 5

Boron Uptake in Oilseed Rape

5.1 Introduction

Genotypic differences in nutrient efficiency occur as a result of variation in uptake, transport, and utilisation within plants (Marschner, 1995a). For example, Brown and Jones (1971) reported an increased uptake of boron (B) in the B efficient tomato cultivar Rutgers over the B inefficient T3238, with the former cultivar up to 15 times more efficient in absorbing B from the growth medium. Bellaloui and Brown (1998) confirmed these observations, citing a reduced rate of B uptake and translocation of B from root to shoot in the B inefficient T3238. These results indicate a mechanism of B efficiency localised to the root. Recently, Nable *et al.* (1988,1990) provided evidence for genotypic variation in the rate of B uptake into barley, with genotypes tolerant of B toxicity accumulating less B.

The study of B uptake in oilseed rape is warranted because there is conjecture about whether high B uptake can contribute to reproductive success (Rerkasem *et al.*, 1993). Uptake of B appears to control efficiencies in tomato and barley, in which efficient genotypes exhibit increased uptake efficiency. The following study aims to determine the rate of B uptake in oilseed rape varieties that differ in B efficiency (from field based evaluation, Chapter 3).

5.2 Materials and Methods

Experiment 5(a) B uptake (net B accumulation) rate in the canola variety Barossa

As this experiment was part of Chapter 2, Experiment 2(a), the research protocols including experimental design and growth conditions are found in section 2.2.1 to 2.2.3 of that chapter.

5.2.1 Calculation of B uptake and kinetics of B transport.

B uptake rates per unit of root dry weight (μg B g^{-1} root DW day^{-1}) were calculated according to Williams (1948).

$$\text{B uptake rate} = ((m1-m0)/(DW_{r1}-DW_{r0})) \times ((\ln DW_{r1}-\ln DW_{r0})/(t1-t0))$$

where m1 and m0 are the total B content, and DW_{r1} and DW_{r0} are the root dry weights at day t1 and t0, respectively.

To determine the kinetic parameters of B uptake (I_{max}, K_m and C_{min}), the following equation was used (Marschner, 1995d):

$$I = \frac{I_{max}\,(C_s-C_{min})}{K_m + (C_s-C_{min})}$$

where I is the net influx of B; C_{min} defines the concentration at which net B uptake ceases (net uptake zero; influx = efflux); I_{max} is defined as the maximum transport rate when all available carrier sites are loaded; K_m (the Michaelis constant) is defined as the substrate B concentration giving half the maximal transport rate. Kinetic parameters were derived using the graphical package Deltagraph®, which on iterating the data using the above equation, gave a curve of best fit statistically.

Experiment 5(b) Genetic variation in B uptake rate in oilseed rape

5.2.2 Design

The factorial experiment was modelled on a randomised complete block design, with two *B. napus* varieties (Dunkeld and Barossa), one *B. juncea* variety (Pusa Bold), and two B treatments (1 and 13 μM B). The experiment was replicated three times.

5.2.3 Seed

Information on seed source and B content for the two cultivars is given in Chapter 2, section 2.2.6 and Table 2.2 respectively.

5.2.4 Nutrient Solution and Growth Conditions

Nutrient solution and growth conditions were identical to those used in Chapter 2, section 2.2.3, with the following modifications. B treatments were applied at rates of 1 (B1) and 13 (B13) μM B (as boric acid), while plants were grown in a growth room at 20/15°C day/night temperature. At D19 and D22, plants were harvested and rinsed in high purity water for 30 s. After blotting dry with tissue paper, plants were separated into root and shoot, then oven dried at 80 °C for at least 24 h. Samples were digested according to previously reported procedures in section 2.2.4.

5.2.5 Calculation of B uptake and plant relative growth rate

The equation used to calculate the B uptake rate is reported in section 5.2.1. Plant relative growth rate (g g^{-1} day^{-1}) was calculated according to the formula: Plant relative growth rate (RGR) = $(\ln(DW_n) - \ln(DW_{n-1}))/T$

where DW_n = the average dry weight of whole plants at harvest (n), DW_{n-1} = the average dry weight of plants at the preceding harvest (n-1), and T = time (days) between the two harvests.

5.2.6 Statistical Analyses

The data were subject to analysis of variance using the Genstat 5 statistical package. Significant mean separation is indicated by the use of the least significant difference (LSD) at the 5% level where the F value is significant.

5.3 Results

Experiment 5(a) B uptake (net B accumulation) in the canola variety Barossa

5.3.1 Plant Growth

This experiment was initially conducted to determine the critical deficiency concentration for B (see Chapter 2, experiment 2(a)), while net B uptake (net B accumulation), was also calculated to give an insight into the uptake characteristics of oilseed rape. Details of plant growth response to B are given in Chapter 2, Fig. 2.2.

5.3.2 B uptake

Net B uptake (resultant of B influx and efflux) over a 10 day period showed a biphasic response to changes in the external B concentration (Fig. 5.1 A,B). B uptake was largely independent of concentration in the range 0.8 to 12.5 μM B, which may reflect the saturation of a high affinity transport system or saturation of internal B demand. Between 12.5 and 100 μM B, there was some effect of external B concentration that may indicate a low affinity transport system or simply concentration dependent passive permeation of the membrane.

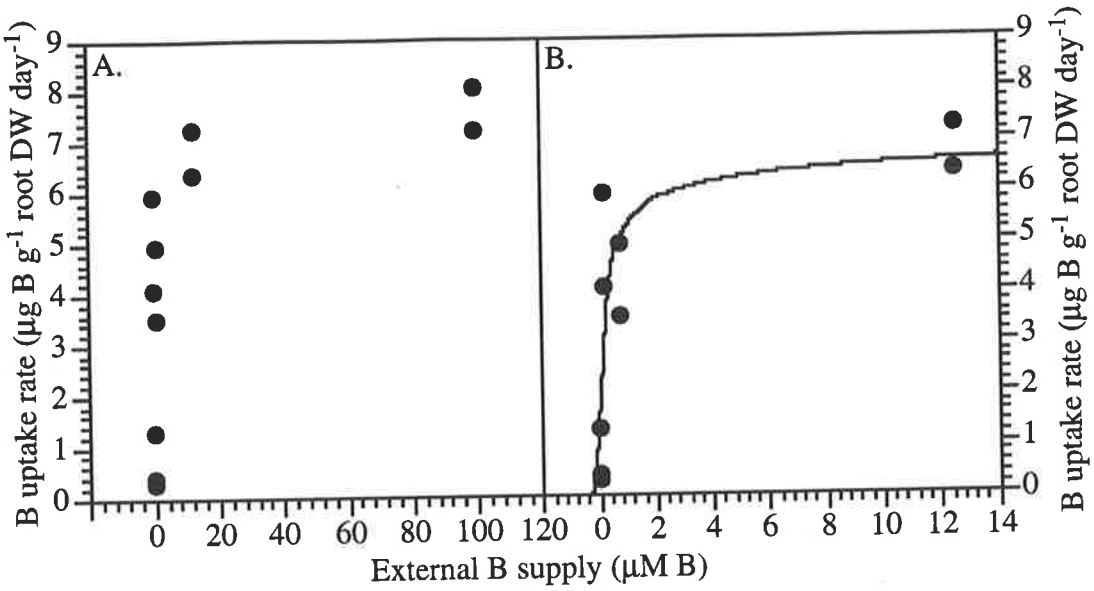


Fig. 5.1. The rate of B uptake in the variety Barossa, grown in solution culture. A. B uptake from 0 to 100 µM B. B. B uptake from 0 to 12.5 µM B.

Kinetic constants I_{max}, K_m and C_{min} were derived from the available data between the B concentrations of 0 to 12.5 µM B (Fig. 5.1 B). I_{max}, K_m and C_{min} values were 6.07 µg B g^{-1} DW day^{-1}, 0.26 µM B, and 0.07 µM B respectively.

Experiment 5(b) Genetic variation in B uptake rate in oilseed rape

5.3.3 Plant Growth

In all three varieties, plant growth was significantly reduced in B1 grown plants, with root growth more sensitive to B deficiency than shoot growth (Fig. 5.2). Barossa was most B inefficient, with a 54 % reduction in shoot dry weight, corresponding to a 77 % reduction in root dry weight. In contrast, the effects of B deficiency on shoot dry weights in Dunkeld (28% reduction) and Pusa Bold (24% reduction), were not as severe, though root growth was significantly reduced in both Dunkeld (60% reduction) and Pusa Bold (53% reduction).

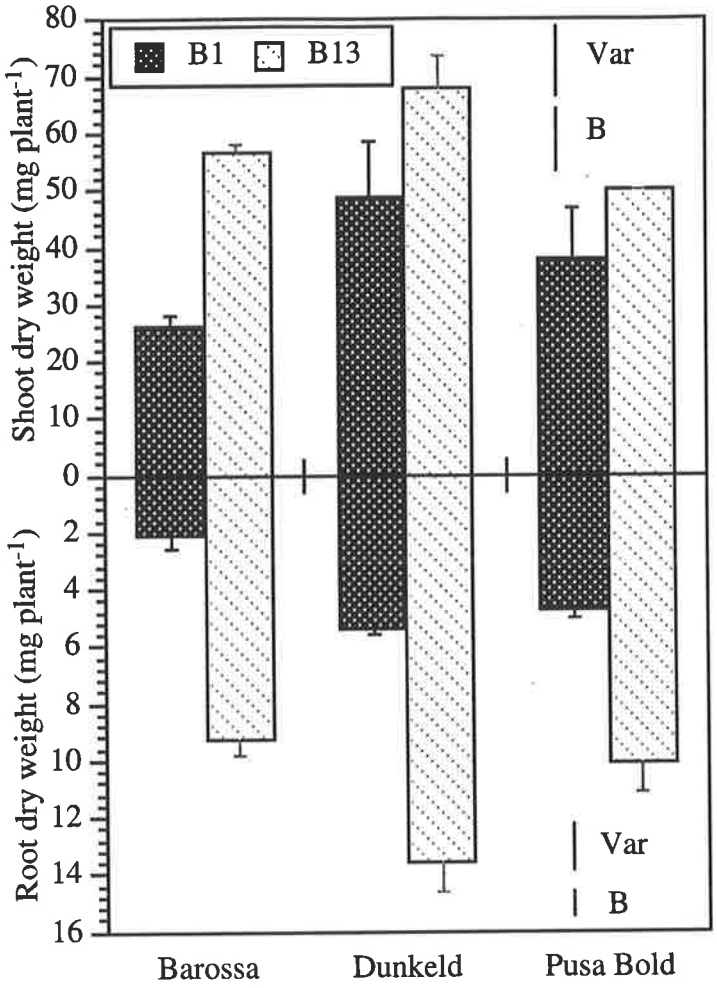


Fig 5.2. The effect of B nutrition (B1, 1 μM B; B13, 13 μM B) on shoot and root dry weight in 22 day old oilseed rape varieties grown in solution culture. Bars represent the mean of 3 replications ± se. Vertical lines depict LSD (P<0.05), Variety, Boron.

5.3.4 Boron Uptake

Net B uptake (net B accumulation from D0 to D22) was significantly reduced in B1 grown plants (Fig. 5.3), although at both low and adequate levels of B, B accumulation varied less than 2 fold between varieties. Of particular interest was a retention of B in the root at the expense of the shoot in B1 grown plants by around 6 to 11 percentage points (Table 5.1). Pusa Bold accumulated 11 % more B in the root, Barossa 8 %, and Dunkeld 6 %. This effect may indicate a reduced transpiration, with the magnitude of B redistribution bearing no correlation to a variety's B efficiency.

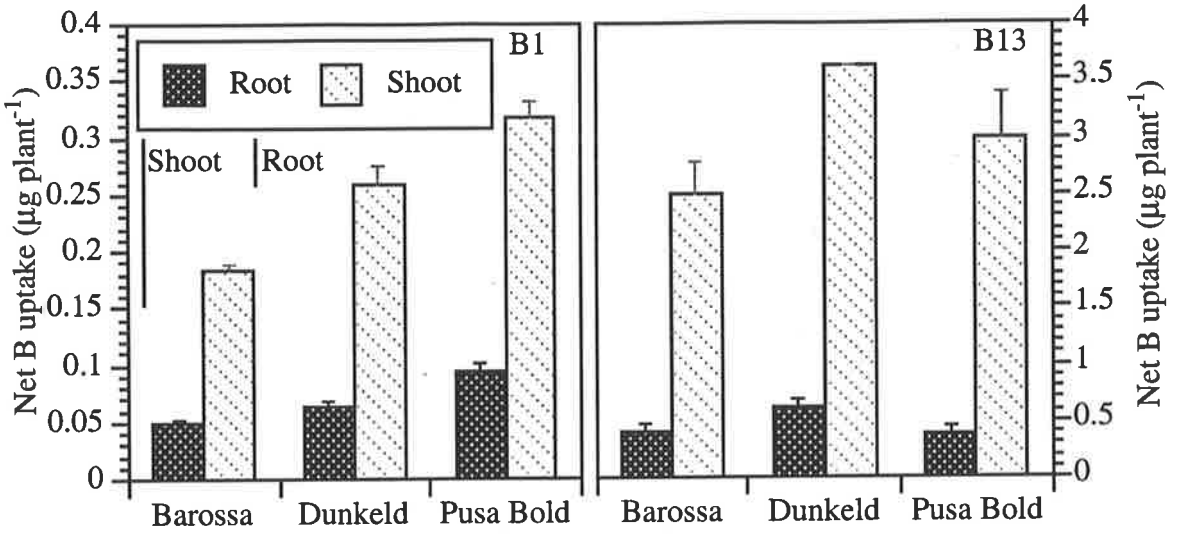


Fig. 5.3. The effect of B nutrition (B1, 1 µM B; B13, 13 µM B) on net B uptake (net B accumulation between D0-D22) into the root and shoot of 22 day old plants. Bars represent the means of three replications ± se. Vertical lines depict LSD ($P<0.05$), Variety x Boron.

Table 5.1. Boron content (% of total plant B) of root and shoot tissue in three oilseed rape varieties grown for 22 days at low (B1) and adequate (B13) levels of B.

	B content (% of total plant)			
	B1		B13	
Variety	Root	Shoot	Root	Shoot
Barossa	22	78	14	86
Dunkeld	20	80	14	86
Pusa Bold	23	77	12	88

Genotypic variation in the rate of B uptake (resultant of B influx and efflux) over a 3 day period was observed in B1 grown plants (Fig. 5.4). Dunkeld showed a 15 fold reduction in the rate of B uptake under B deficiency, while in Pusa Bold, the reduction was around 7 fold, and in Barossa, around 5 fold. B uptake corresponded with plant relative growth rates (RGR) at both low and adequate B supply (Fig. 5.5), though no statistically significant difference between variety or B treatment was observed, due to considerable variation between replications. The ranking of varieties according to uptake rates at low B supply (Barossa>Pusa Bold>Dunkeld) was not correlated with relative shoot growth (Pusa Bold>Dunkeld>Barossa). Net B uptake (B accumulation between D0 to D22) at low B supply (Fig 5.3) was a better indicator of relative shoot growth, indicating a mechanism of B efficiency in Dunkeld and Pusa Bold.

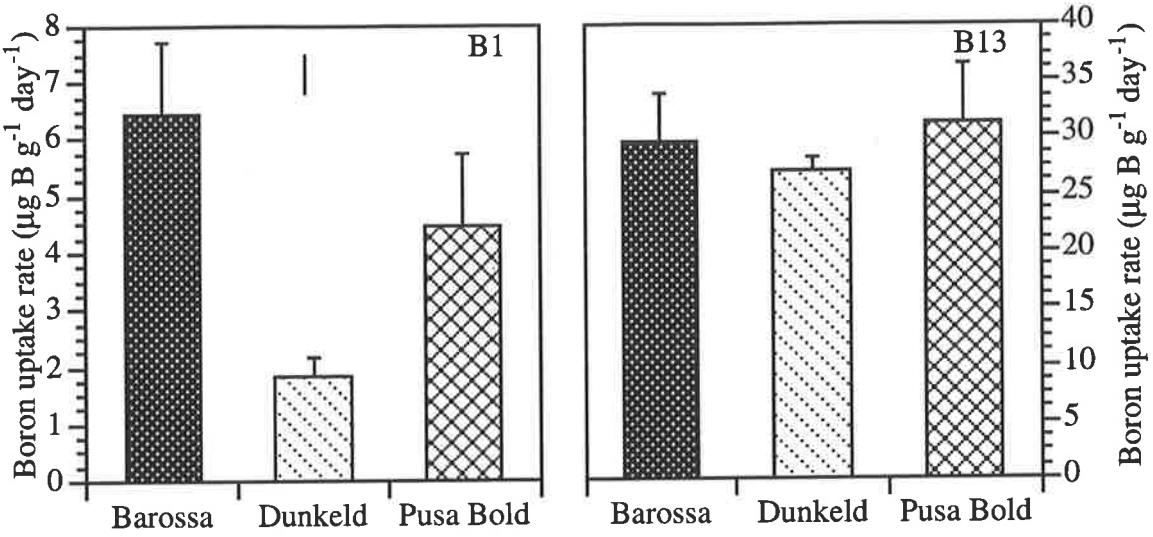


Fig 5.4. The effect of B nutrition (B1, 1 µM B; B13, 13 µM B) on the B uptake rate between D19 and D22 of three oilseed rape varieties grown in solution culture. Bars represent the mean of 3 replications ± se. Vertical line depicts LSD ($P<0.05$), Variety x Boron.

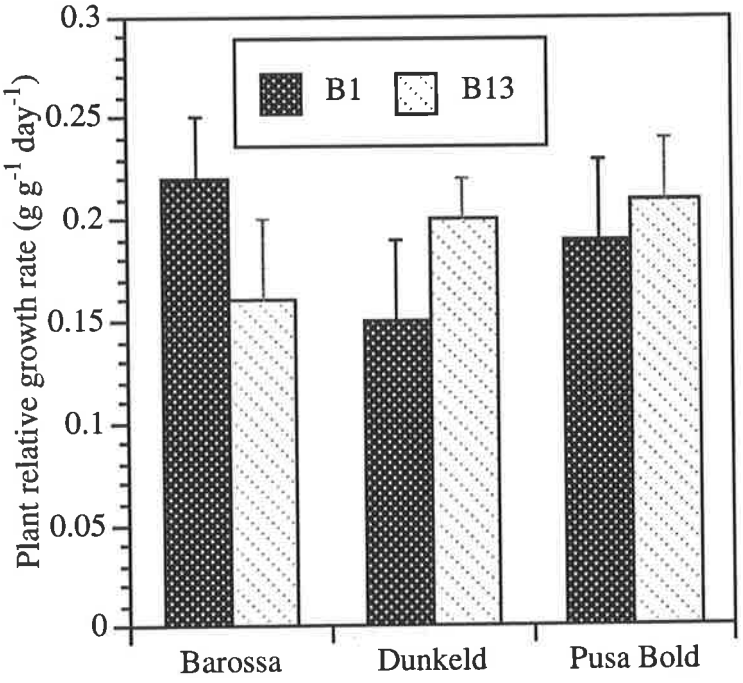


Fig 5.5. The effect of B nutrition (B1, 1 µM B; B13, 13 µM B) on plant relative growth rate between D19 and D22 in three oilseed rape varieties grown in solution culture. Bars represent the mean of 3 replications ± se.

5.4 Discussion

The aim of this study was to investigate the uptake of B into varieties of oilseed rape which differ in B efficiency. Clearly, varieties differed in their rate of B uptake, though there is no clear evidence to suggest rate of B uptake per se has a role in the efficiency mechanism. The B inefficient variety Barossa exhibited a higher rate of B uptake at low B supply than the B efficient variety Dunkeld. A similar result was also observed for Pusa Bold, which is B efficient in the field, yet exhibited a lower rate of B uptake than Barossa at low B supply. The rate of B uptake between D19 and D22 did not accord with the rate of shoot growth in Barossa, but net B uptake (the accumulation of B over a longer time (D0-D22)) did. Results therefore indicate that a mechanism of B efficiency in the varieties Dunkeld and Pusa Bold is related to an increased capacity to accumulate B. For Dunkeld, this is in keeping with results presented in Chapter 4, while for Pusa Bold, its efficiency in accumulating B is in agreement with published reports where its efficiency in yield response was associated with an increased B uptake (net B accumulation) (Sakal *et al.*, 1991).

Considering that B uptake is considered a passive process (Hu and Brown, 1997 and references therein), it is interesting to observe differences in the rate of B uptake between varieties grown in the same container. A similar result was observed with barley genotypes which differed in their tolerance to B toxicity (Nable *et al.*, 1990). Nable *et al.* (1990) reported B uptake to be a passive process, though genotypes differed in their rate of B uptake. Small differences in membrane permeability to boric acid have been postulated as the most likely reason for such a phenomenon (Hu and Brown, 1997). Likewise, one cannot exclude the effect of an active transporter for B at very low B supply. Data presented in this chapter are consistent with such a possibility.

Boron uptake may involve an active component in the variety Barossa. At this stage, the results have to be viewed with caution due to the absence of suitable external B concentrations in the range between 1 to 12.5 and 12.5 to 100 μM B, and this supports the need for further studies. Kinetic parameters I_{max} and K_m for a high affinity transporter were calculated at 6.07 μg B g^{-1} root DW day^{-1} and 0.26 μM B respectively. The K_m value is very low, though it is similar for the high affinity urea transporter (0.3 μM B),

which is sensitive to metabolic inhibition (Wilson *et al.*, 1988). Interestingly, urea is of similar molecular weight (60.06) to boric acid (Raven, 1980) and has a similar permeability coefficient (10^{-6} cm s^{-1}; Raven, 1980; Wilson and Walker, 1988).

The use of the Michaelis-Menten equation to describe root uptake processes in Experiment 5(a) may be questionable given that this equation should apply to unidirectional fluxes. In studying the rate of B uptake over a longer time period (days), as reported in Experiment 5(a), both B influx and efflux may be occurring simultaneously, although this well depend on intra-cellular complexation of B and the metabolism of B. For example, at sub-optimal B supply, internal complexation and metabolism might serve to maintain the free B concentration close to zero in which case efflux would be low and net uptake similar to unidirectional influx. The use of the Michaelis-Menten equation would therefore be appropriate in this case. Once saturation of B binding sites occurs, the measured uptake rate of B will include an efflux component. The results presented in Experiment 5(a) may indicate carrier mediated B uptake, or simply a regulation of B uptake by internal complexation of B. In the later example, once intracellular B binding sites are saturated, B efflux would increase resulting in what would appear as the saturation of a carrier.

In some respects, it would seem unnecessary to invoke an active transport system for B, with B concentrations in the apoplast exceeding cytoplasmic B concentrations (Matoh *et al.*, 1992; Martini and Thellier, 1993), and a high permeability coefficient for boric acid of around 10^{-6} cm s^{-1} (Raven, 1980). The use of active transport away from thermodynamic equilibrium would therefore appear energetically expensive. Yet, one could envisage a requirement for active B uptake in two B deficient situations. Firstly, active B transport may be required where free B (not total B) in the cytoplasm was higher than in the apoplast, and secondly where B uptake by diffusion was insufficient to meet intracellular demand, in which case a high affinity carrier or active transporter would be useful. The former situation would appear unlikely given the very small amount of B thought to exist in the cytoplasm compared to the apoplastic compartment (Martini and Thellier, 1993), while the latter explanation would appear the most likely reason, though it depends on an intracellular

demand for B which as yet has not been identified. A role for B in membrane function is well known (see recent reviews by Cakmak and Römheld, 1997; Goldbach, 1997; Blevins and Lukaszewski, 1998) and may provide the basis for an intracellular B demand. A possible role for B has recently been proposed by Heyes *et al.* (1991) and Goldbach (1997). Heyes *et al.* (1991) proposed that B may maintain the preferred conformation of the active protein in the membrane thereby affecting the transport capacity of cultured plant roots for a number of inorganic ions. Goldbach (1997) has proposed a role for B in sustaining the transport of cell wall precursors to the apoplast. Again, B through complexation with plasmalemma-bound glycoproteins and cell wall precursors would facilitate the supply of precursors to the apoplast. While these are only hypotheses, and are still unproven, an involvement of B in such processes would help to explain the expenditure of energy in an active uptake system, to alleviate or ameliorate B deficiency. Considering the significant difference in the rate of B uptake between Barossa and Dunkeld at low B supply, future research should use these two varieties to confirm the uptake isotherms in these varieties. If active uptake is confirmed, it is highly likely that these two varieties will exhibit differences in the affinity of the carrier for B.

A mechanism of B efficiency in celery is associated with an increased translocation of B from root to shoot (Wall and Andrus, 1962; Bellaloui and Brown, 1998). From data presented in this study, there is no evidence to suggest an increased translocation of B from root to shoot as a mechanism of B efficiency in oilseed rape. An increased relative retention of B in the root was observed in all varieties, with no trend in varietal variation (Table 5.1). In the following chapter (Chapter 6), evidence is presented to support a role for B retranslocation as a mechanism of B efficiency.

Chapter 6

Genotypic Variation in Boron Mobility in Oilseed Rape.

6.1 Introduction

The availability of boron (B) to floral tissues for sexual reproduction may depend on the mobility of B in the phloem (Dell and Huang, 1997). Historically, B is classified as phloem immobile as its distribution within plant tissues follows a pattern more aligned to that of an immobile element (i.e. B concentration gradients decreasing from old to young leaves). Meristematic tissue is usually the first affected when the availability of B is insufficient (Eaton, 1944; Odhnoff, 1957; MacIlrath, 1965; Oertli and Roth, 1969; Cohen and Lepper, 1977; Oertli, 1993). Oertli and Richardson (1970) proposed that phloem immobility was due to the high membrane permeability of B (Raven, 1980) which consequently results in phloem-xylem transfer and subsequent translocation to termini at the ends of the transpiration stream. While in most instances B is reported as immobile, in a number of species which translocate a sugar alcohol (polyol), B is mobile (Brown and Hu, 1996). To date, there are no reports in the literature of the presence or otherwise of a polyol in oilseed rape.

The redistribution of B to floral tissues via the phloem has been reported in *Brassica* species, with Shelp *et al.* (1992) reporting a higher floral B concentration in the broccoli (*B. oleracea* var. *italica*) cultivar Commander than in the B inefficient Premium Crop. Shelp and Shattuck (1987) also report the capacity for B redistribution in two rutabaga cultivars was correlated with their tolerance to B deficiency. Shelp *et al.* (1996) and Liu *et al.* (1993) showed that in *B. oleracea*, the phloem was able to supply the florets with sufficient B to enhance floret yield, and while the mechanism for B retranslocation in this species is unknown, the reported presence of mannitol in *B. oleracea* (Trip *et al.*, 1963) may explain

this phenomenon. However, there appear to be no reports of retranslocation of B in oilseed rape, which may possess similar phloem constituents to *B. oleracea*, namely, a polyol (i.e. mannitol) able to facilitate B translocation to floral tissues.

The aims of the following study are two-fold. Firstly, genotypic variation in B retranslocation is quantified in varieties known to differ in their B efficiency. The aim is to determine whether an association exists between B efficiency and B retranslocation. Secondly, the identification of soluble carbohydrates in leaf extracts will determine whether a polyol, such as mannitol, is present in oilseed rape. The aim is to corroborate recent reports of a polyol facilitating B remobilisation in related species, to provide a mechanism by which B retranslocation may occur.

6.2 Materials and Methods

Experiment 6 (a) The effect of B supply on the distribution of B in oilseed rape

6.2.1 Design

The trial was modelled on a factorial design with four *B. napus* varieties (Huashuang 2, Dunkeld, Zhongyou 821 and Barossa) and two B treatments (treatment lacking B (B0) imposed by omission, and 0.25 mg B kg^{-1} (B+)). Pots were placed in a greenhouse and grown under normal winter day temperatures and light. Plants were harvested at D14 and D28 for vegetative yield and B analyses.

6.2.2 Seed

Information on seed source and B content are given in Chapter 2, section 2.2.6 and Table 2.2 respectively.

6.2.3 Growth Conditions and Nutrient Additions

Growth conditions and basal nutrient additions are as reported in Chapter 3, Experiment 3(b).

6.2.4 Measurements

Three plants per pot were harvested at D14 and a further 2 plants at D28. Plants from each pot were bulked together, with the shoot washed for 5 s in high purity water and separated into the following plant components: Second youngest open leaf (YOL+1) blade and petiole; fourth youngest open leaf (YOL+3) blade and petiole; all remaining leaves, blade and petiole; stem. Plant material was dried at 80 °C for 48 h and dry weights recorded. Samples were analysed for all essential elements (except N) according to procedures in section 2.2.4, Chapter 2.

6.2.5 Statistical Analyses

The data were subject to ANOVA using the Genstat 5 statistical package. Significant mean separation is indicated by the use of the least significant difference (LSD) at the 5% level where the F value is significant.

Experiment 6 (b). Genotypic variation in B retranslocation

6.2.6 Design

Retranslocation of B was monitored using the stable isotope ^{10}B which has a natural abundance of 20 % compared to ^{11}B of 80 %. The experiment was modelled on a factorial design, with four *B. napus* varieties (Huashuang 2, Dunkeld, Zhongyou 821 and Barossa), two B treatments, (the application of ^{10}B isotope (B+), and the absence of ^{10}B isotope (B0)), and 8 plants per treatment (each plant treated as a replication).

6.2.7 Seed

Information on seed source and B content for the two cultivars is given in Chapter 2, section 2.2.6 and Table 2.2 respectively.

6.2.8 Growth Conditions

Varieties (16 plants per variety, with 2 plants grown per pot) of oilseed rape were grown in 9 kg of soil consisting of equal parts sand, peat and redwood chips. Plants were watered every third day with quarter strength (0.13 mg B litre^{-1}) Hoaglands solution (Hoagland and Arnon, 1950). Plants were maintained in a green house under natural lighting and day/night temperatures of approximately 30 °/20 °C. The experiment was conducted in the month of May in California, USA.

6.2.9 ^{10}B Application

At stem elongation, around 50 days from germination, eight plants from each variety were labelled with B isotope by immersing three mature leaf blades (classified old leaf) located in the same position on each plant (designated L1, L2, L3 with L1 the oldest and L3 the youngest leaf) for 10 s in 50 mM ^{10}B enriched boric acid solution (99.43 atom %; Eagle Picher. Quapaw, OK) with a surfactant L-77 (0.05 % (v/v)). The remaining 8 plants were treated as controls by withholding ^{10}B application, and applying only 0.05 % (v/v) L-77.

6.2.10 Measurements

Twenty four hours after labelling with ^{10}B isotope (designated D1), leaf blades from both old and young apical leaves (classified young leaf; designated L4, L5 and L6, with the oldest and youngest leaf L4 and L6 respectively) were collected from all treatments in the following manner. At D1, half of leaf L1 and L3 (leaf blade removed from one side of the midrib only) was harvested, washed for 30 s in RO water and blotted dry with a tissue. Following this, the same procedure was used to harvest from leaves L4 and L6. After

washing and blotting dry, sample material was dried for 48 h at 65 °C. Sequential harvests of these same plants occurred at day 5 (D5) and day 10 (D10) using the same procedures as reported for D1 with the following exceptions. At D5, leaf material was harvested from L2 and L3 (old leaf) and L5 and L6 (young leaf). At D10, leaf material was harvested from L1 and L2 (old leaf), and L4 and L5 (young leaf). Procedures used to analyse for B are similar to those reported in Brown and Hu (1994). Samples were dry-ashed at 500 °C and extracted at 100 °C for 20 min in 1 % HNO_3. Filtered extract was analysed for B using Inductively Coupled Plasma-Mass Spectrometer (ICP-MS, Elan 500, Perkin Elmer, Norwalk, Conn.) with Be as the internal standard. Calibration was performed against NIST isotopic reference (National Institute of Standards and Technology, Gaithersburg, VA, USA) and tissue standards.

Experiment 6 (c) Determining Soluble Carbohydrate Content in Oilseed Rape

6.2.11 Design and Growth Conditions

Two oilseed rape varieties (6 plants per variety) (Huashuang 2 and Barossa) were grown for 35 days in 9 kg of soil consisting of equal parts sand, peat and redwood chips. Plants were watered every third day with quarter strength Hoaglands solution (Hoagland and Arnon, 1950). Plants were maintained in a green house under natural lighting and day/night temperatures of approximately 30 °/20 °C.

6.2.12 Measurements

6.2.12.1 Soluble Carbohydrate Determination by HPLC

35 days after sowing (D35), plant tissue was sampled and extracted for soluble carbohydrate composition using a modified method of Madore *et al.* (1988). At 9.30 am in the morning, six quarter inch leaf disks were excised from the fifth youngest leaf (YOL + 4) on each plant. Leaf disks were immediately placed in centrifuge tubes and plunged into liquid nitrogen (within 20 s of excising) to minimise enzymatic activity. Leaf disks were

lyophilized for 48 h in a Virtis® automatic freeze drier, then weighed to obtain dry weights, and extracted in hot 80% EtOH (EtOH: water (80:20 v/v) at 80 °C). After 3 extractions and subsequent collection of supernatant, the extract was filtered through filter paper (Whatman No. 1), taken to dryness under nitrogen gas, and resuspended in 2 ml of high purity water. The extract was then passed through 2 micron HPLC filters and frozen ready for analysis by HPLC.

Soluble sugars were separated by HPLC on a Sarasep CAR-Ca carbohydrate column maintained at 80 °C using water as the mobile phase at a flow rate of 0.6 ml min^{-1}. Sugars were identified by comparison of retention times of known sugars and quantified by refractive index monitoring.

To give an indication of the validity of using the above protocols for the extraction and analyses of leaf tissue, celery leaf tissue was also extracted for soluble carbohydrate analyses using the same methods used for oilseed rape. Celery was used because it is known to transport mannitol as a carbohydrate in the phloem (Hu *et al.*, 1997).

6.2.12.2 Soluble Carbohydrate Determination by GC-MS

The same procedures as reported in section 6.2.12.1, were used for collection of six leaf disks and their lyophilising. Methylation analysis for carbohydrate composition by GC-MS was conducted according to a modified method of Anumula and Taylor (1992). Leaf disks were weighed and placed in a 9 cm test tube with the following additions: 500 µl of 1 mg ml^{-1} Quebrachitol (internal standard); 150 µl acetic anhydride containing 5 mg ml^{-1} 4-N,N'-dimethylaminopyridine; 50 µl pyridine and 150 µl acetic anhydride. Samples were left at room temperature for 4 h for acetylation, then the reaction was stopped by adding 2 ml of water. Samples were vortexed and left for 10 min, and an equal volume of dichloromethane was added to each sample. After centrifuging at 5000 rpm for 5 min, the upper phase was discarded, and samples were then washed in 500 µl water, with subsequent removal of water. Samples were dried under a stream of nitrogen gas, and

dissolved in dichloromethane (20 µl) for GC-MS analyses. For analyses, 2 µl of sample was injected into the GC-MS, at a sample flow rate of 0.6 ml min^{-1}.

6.3 Results

Experiment 6 (a). The effect of B supply on the redistribution of B in oilseed rape

6.3.1 Distribution of Boron and Dry Matter Within the Shoot

Boron concentrations in leaf tissue were significantly depressed ($P<0.001$) in plants grown at low B supply (B0), and were in the deficiency range for oilseed rape (YOL+1: 6-13 µg g^{-1}; Reuter *et al.*, 1997), while concentrations in plants adequately supplied with B (B+) were in the B adequate range (YOL+1: 22-50 µg g^{-1}; Reuter *et al.*, 1997) (Fig 6.1).

Plants grown with adequately supplied B exhibited concentration gradients between old leaf tissue (YOL + 3) and young leaf tissue (YOL + 1) the latter lower, though the magnitude of this effect was reduced in older plants (D14-D28). In contrast, concentration in the younger leaf tissue of B0 grown plants was higher than in older leaf tissue in all four varieties, and was not correlated with leaf age which may indicate B supply to the young leaf by secondary translocation. Between the four varieties, B concentrations in the YOL + 1 at D14 ranged from around 8-12 µg g^{-1}, while at D28, this differential was less, around 7-9 µg g^{-1}, with Barossa showing a marginally lower B concentration.

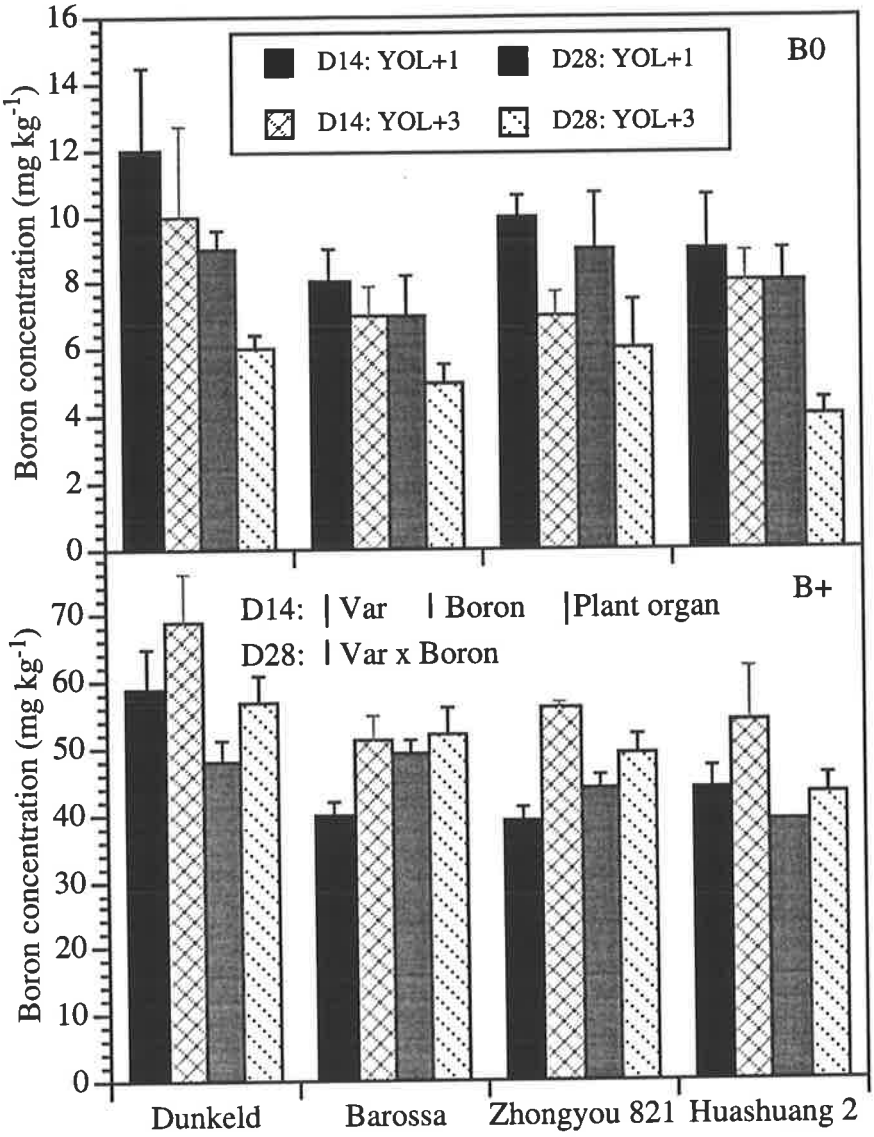


Fig 6.1. The effect of low (B0; 0 mg B kg^{-1} soil) and adequate (B+; 0.25 mg B kg^{-1} soil) B supply on YOL +1 and YOL + 3 blade B concentrations in four cultivars of oilseed rape grown for 14 (D14) and 28 (D28) days in low B Mt. Compass sand. Bars represent means of 3 replications ± se. Vertical bar depicts LSD ($P<0.05$).

The effect of B supply on concentrations in the blade, petiole and stem, indicate a redistribution of B under the influence of B deficiency (Fig 6.2). B concentrations between the petiole and stem differed minimally in B+ grown plants, while the blade retained higher B concentrations. The variety Dunkeld exhibited a higher blade concentration over the remaining three varieties; around 26, 18 and 15 μg g^{-1} more than Huashuang 2, Barossa and Zhongyou 821 respectively. In B deficient tissue, the concentration gradients were the

opposite. Huashuang 2 differed to other cultivars by exhibited a relatively lower B concentration in the petiole, possibly a dilution effect of its very high petiole dry weight (Fig 6.3).

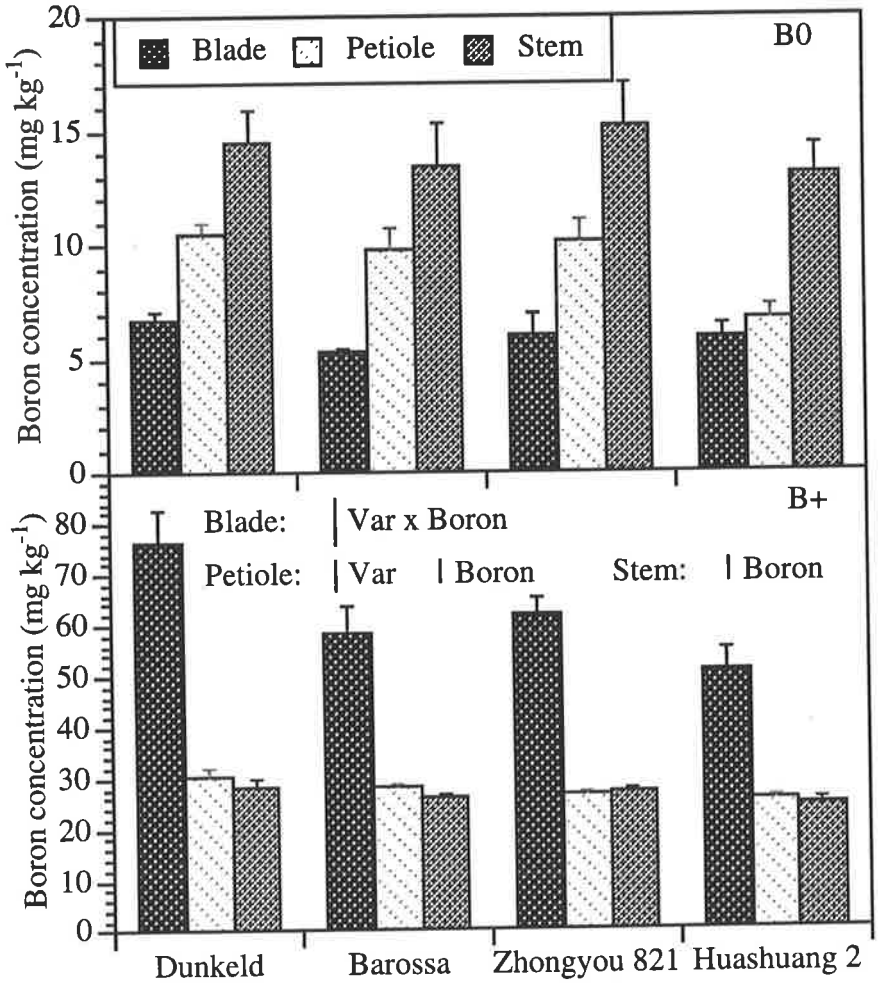


Fig 6.2. The effect of low (B0; 0 mg B kg^{-1} soil) and adequate (B+; 0.25 mg B kg^{-1} soil) B supply on B concentrations of plant parts in four cultivars of oilseed rape grown for 28 days in Mt. Compass sand. Bars represent means of 3 replications ± se. Vertical bar depicts LSD ($P<0.05$).

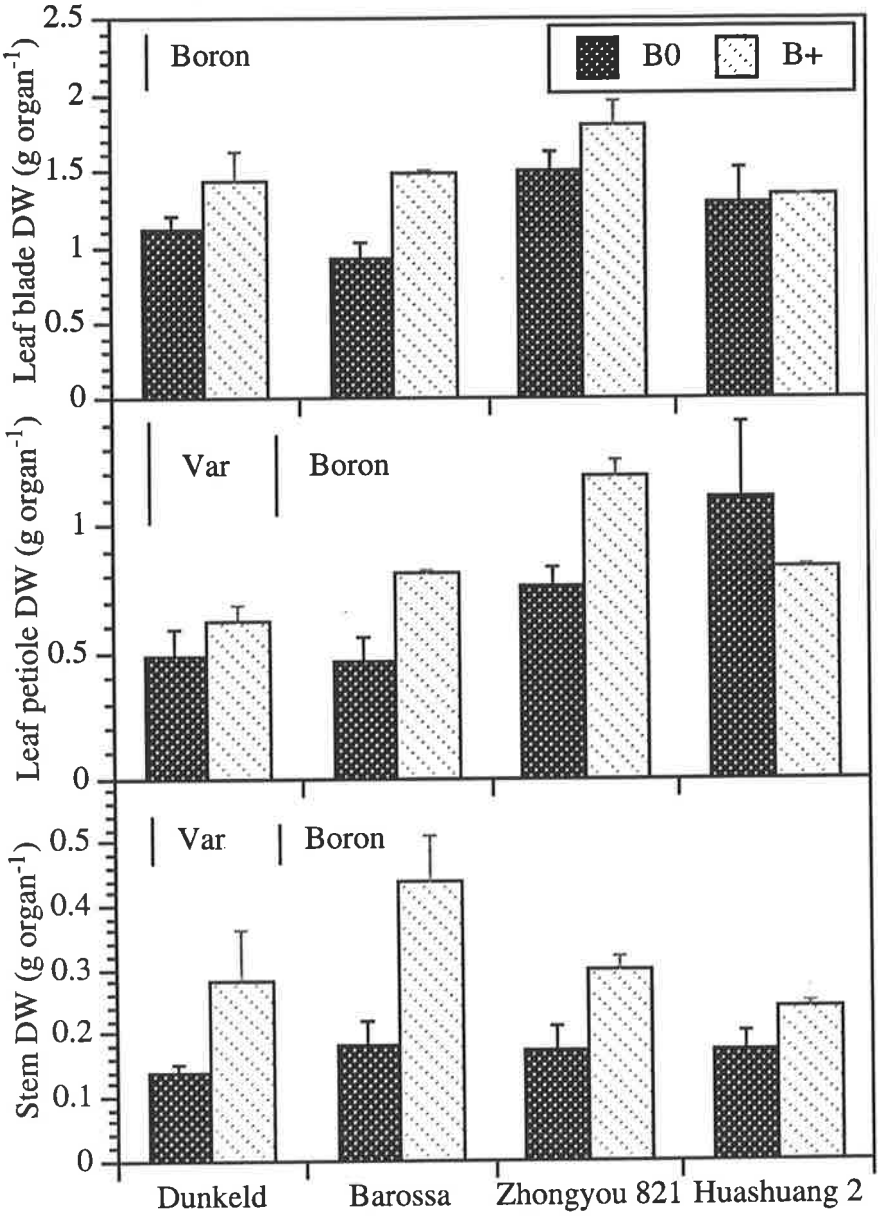


Fig 6.3. The effect of low (B0; 0 mg B kg^{-1} soil) and adequate (B+; 0.25 mg B kg^{-1} soil) B supply on dry weights of plant parts in four cultivars of oilseed rape grown for 28 days in Mt. Compass sand. Bars represent means of 3 replications ± se. Vertical bar depict LSD ($P<0.05$). Leaf blade and petiole dry weight were best described on log transformation.

Genotypic variation in the partitioning of biomass (as measured by tissue dry weight) between various shoot organs was also present in B+ grown plants (Fig 6.3). Zhongyou 821 partitioned more biomass to the leaf blade and petiole than the remaining three varieties, yet its stem dry weight was similar to Dunkeld and Huashuang 2. In contrast, Barossa, partitioned more biomass to the stem than any other variety, while having a similar blade and petiole dry weight to Dunkeld and Huashuang 2.

In terms of relative growth (B0/B+), Barossa appeared less efficient for every plant part measured, consistent with its sensitivity to B deficiency, whereas Huashuang 2, appeared the most efficient. Zhongyou 821 maintained a greater leaf blade dry weight under B deficiency, at the expense of the petiole, which was significantly reduced. This is in contrast to Dunkeld which exhibited a low blade dry weight and, relative to leaf blade, a high petiole dry weight under B deficiency. Efficiency in maintaining leaf blade dry weight was 60, 78, 88 and 96 % for Barossa, Dunkeld, Zhongyou 821 and Huashuang 2 respectively, while for the petiole, around 60, 81, 65 and 134 %, and for the stem, 41, 50, 57 and 72 % respectively.

Experiment 6 (b). Genotypic variation in B retranslocation

6.3.2 B retranslocation

Genotypic variation in B retranslocation was observed following application of ^{10}B isotope (Fig 6.4). Net B movement out of mature leaves over 10 days occurred in all varieties; however, for Barossa, retranslocation was minimal (Fig 6.4 A). 80, 67, 53, and 10 μg B $leaf^{-1}$ was retranslocated out of the mature leaf for Dunkeld, Huashuang 2, Zhongyou 821 and Barossa respectively. Genotypic variation in the concentration of ^{10}B isotope present in the labelled leaf at D1 was mainly due to differences in tissue B concentration when isotope was first applied. Also, on application of isotope, the leaves chosen were of similar size, though the leaf surface area was not quantified, nor was the amount of ^{10}B applied, which would affect the amount of ^{10}B isotope initially present in the leaf. In leaf material without application of ^{10}B isotope (Fig 6.4 B), significant B retranslocation occurred in Huashuang 2 and to a much smaller extent in Zhongyou 821, while the remaining two varieties showed an increase in ^{10}B over the 10 day period. This would indicate that apart from Huashuang 2, net B remobilisation in the remaining three varieties is minimal; however, the total uptake of B into the leaf may have exceeded the B remobilised out of the leaf during the 10 day period.

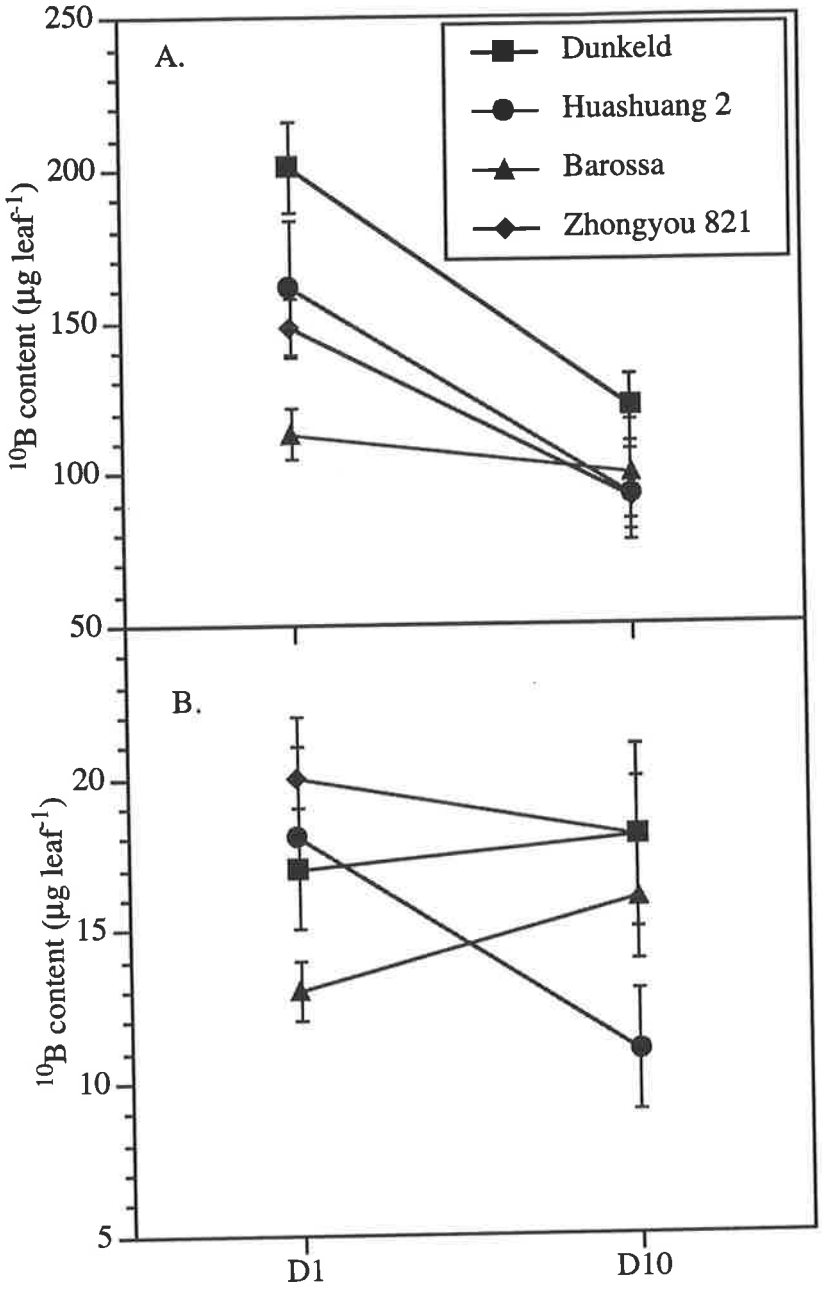


Fig 6.4. ^{10}B concentration in mature leaves of plants labelled with 50 mM ^{10}B enriched boric acid (A), and leaf tissue without application of ^{10}B isotope (B). Leaves were sampled at D1, D5 and D10, though D1 and D10 data are only presented as little deviation away from the trend between D1 and D10 occurred. Data points represent the mean of 8 replications ± se.

B isotopic ratios (^{10}B:^{11}B) are not only useful in observing the movement of B out of plant tissues, but also into plant tissues (Fig 6.5). Naturally occurring ^{10}B:^{11}B ratio in plant tissues is 0.24, with values above this indicating enrichment of ^{10}B isotope. In plants labelled with ^{10}B, enrichment in young leaves was observed more so in Huashuang 2, with

young leaves enriched by 72 %, 24 h after labelling, while after 10 days this was reduced to 40 %. The decline by day 10 may indicate retranslocation of ^{10}B out of the younger leaves which over the 10 day period had matured and become a source instead of a sink for B. In comparison, enrichment in the remaining three varieties was minimal, with an increase in ^{10}B into the young leaves of around 16 , 11 and 8 % for Dunkeld, Zhongyou 821 and Barossa respectively.

A characteristic of the movement of ^{10}B out of the mature leaves, was a continuous decline in the ^{10}B:^{11}B ratio in Huashuang 2 over ten days, while in the remaining three varieties, ^{10}B:^{11}B ratios plateaued off after 5 days indicating that the majority of the applied ^{10}B was not available for further retranslocation. Taken together with results from the net movement of ^{10}B out of mature leaf tissue (Fig 6.4), the majority of ^{10}B label applied to Barossa, Dunkeld and Zhongyou 821 was unavailable for retranslocation, whereas in Huashuang 2, it was highly mobile.

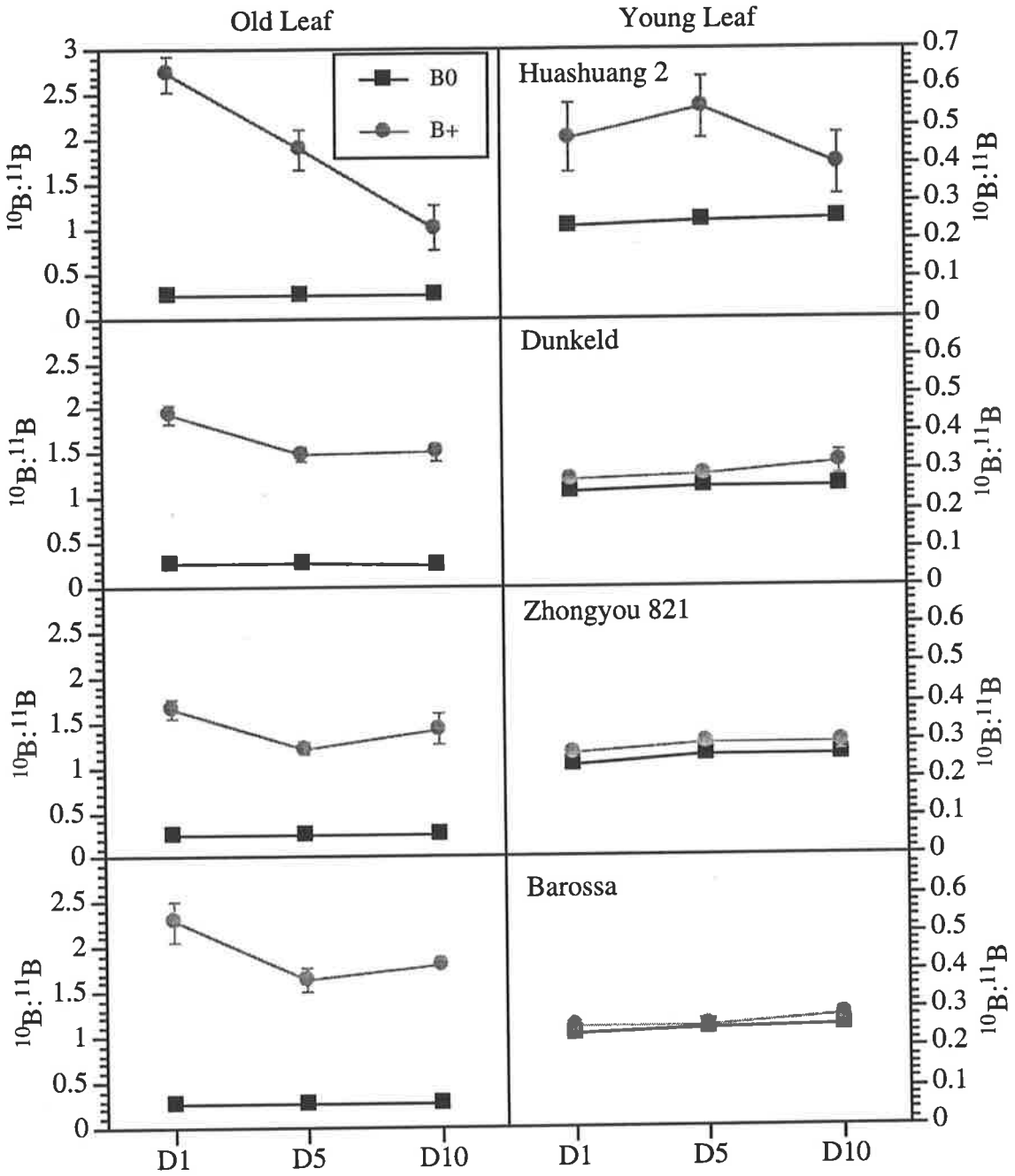


Fig. 6.5. Boron isotopic ratios in mature and young leaf tissue of four varieties of oilseed rape on the addition (B+) of 50 mM ^{10}B enriched boric acid to the mature leaf. Control treatments (B0) did not receive ^{10}B. D1, D5 and D10 are sampling dates taken 1, 5 and 10 days after application of ^{10}B isotopc. Points represent the mean of 8 replications ± se.

Experiment 6 (c) Determining the Soluble Carbohydrate Content in Oilseed Rape

6.3.3 Soluble Carbohydrate Content

Soluble carbohydrates were identified by HPLC and verified by GC-MS. A characteristic HPLC analysis is presented in Fig 6.6 for the variety Huashuang 2, and indicates the absence of the polyol mannitol in this variety. Sorbitol was used as an internal standard after GC-MS analyses verified its absence. Due to the overlap of fructose and myo-inositol in the HPLC spectra, GC-MS was used to quantify these compounds, using material isolated from the same plants as used for the HPLC analyses. Soluble carbohydrates contained in leaf extracts were predominantly sucrose, glucose, fructose and myo-inositol, with Huashuang 2 exhibiting increased concentrations approximately 20, 28, 21 and 80 %, respectively, higher than in the B inefficient variety Barossa (Fig 6.7). It should be noted that there were a number of carbohydrates present in the HPLC which were not identifiable, and were eluted before sucrose (Fig 6.6). On slowing the flow rate to 0.4 ml min^{-1}, this large peak consisted of three compounds, one of which was stachyose. The two remaining compounds could not be identified, though from literature produced from the manufacturer of the carbohydrate column, they were not polyols. To verify the absence of a polyol, GC-MS analysis was conducted on leaf material isolated from the same leaves of those used in the HPLC analyses. No polyol was identified.

As further confirmation of the extraction procedures, plant extracts from celery were analysed. The concentration of mannitol in celery was very high (Fig 6.8), and confirmed that the isolation, digestion and analysis procedures were adequate to identify a polyol if it was present in oilseed rape leaf tissue.

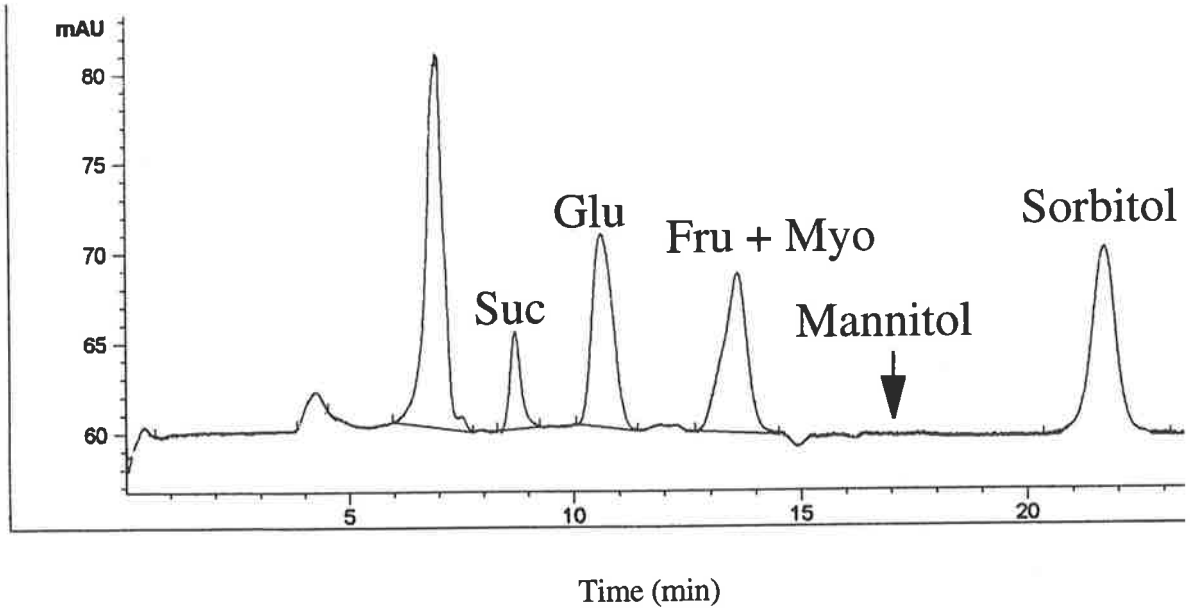


Fig 6.6. Soluble carbohydrate analyses from leaf tissue extract of Huashuang 2. The arrow points to where Mannitol is expected to elute. Sorbitol was added as an internal standard prior to extraction. Soluble carbohydrates were separated by HPLC on a Serasep CAR-Ca carbohydrate column maintained at 80 °C using water as the mobile phase at a flow rate of 0.6 ml min^{-1}. Suc: Sucrose; Glu: Glucose; Fru+Myo: Fructose+Myo-inositol.

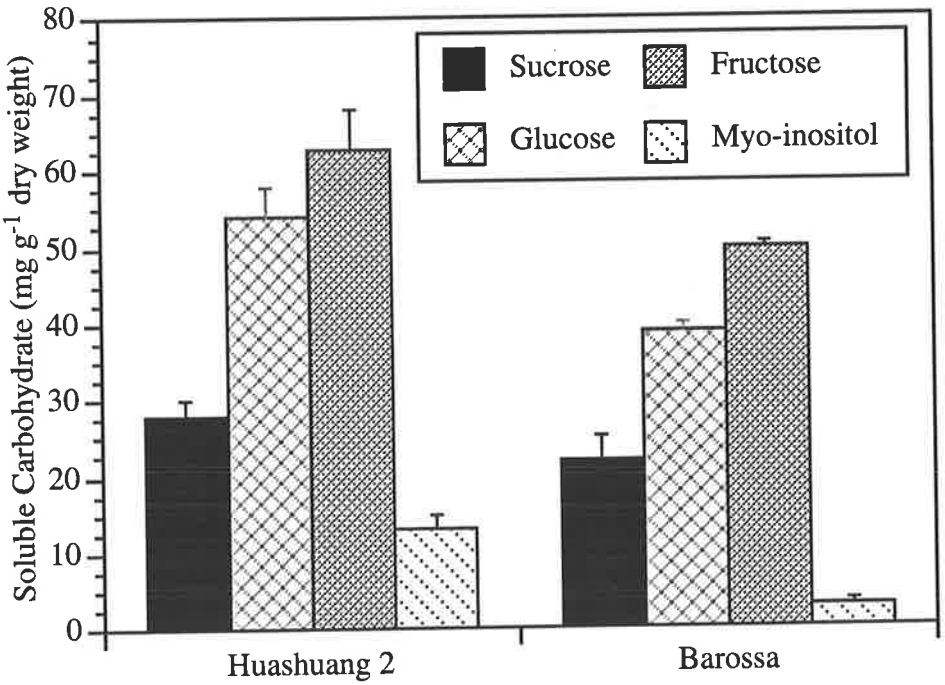


Fig 6.7. Soluble carbohydrate isolated from mature leaf tissue in two varieties of oilseed rape. Bars represent means of 6 plants ± se. Due to the elution of fructose and myo-inositol at a similar time using the HPLC, these two carbohydrates were quantified using GC-MS, while sucrose and glucose were quantified by HPLC.

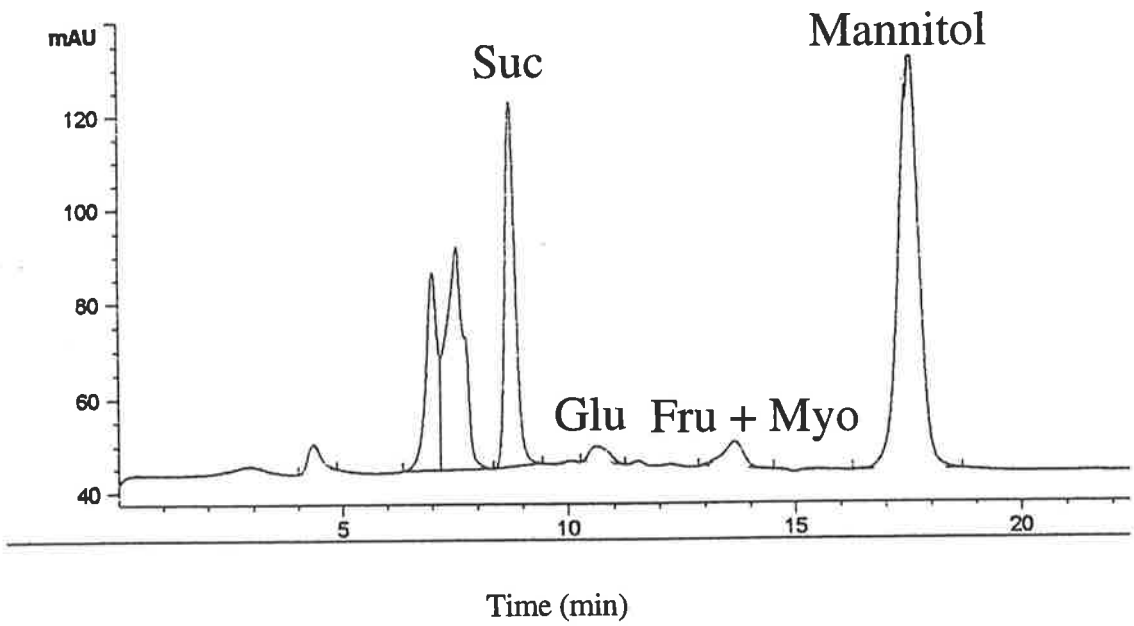


Fig 6.8. Soluble carbohydrate analysis from celery leaf tissue indicating the presence of the polyol mannitol. Soluble carbohydrates were separated by HPLC on a Serasep CAR-Ca carbohydrate column maintained at 80 °C using water as the mobile phase at a flow rate of 0.6 ml min^{-1}. Suc: Sucrose; Glu: Glucose; Fru+Myo: Fructose+Myo-inositol.

6.4 Discussion

The present study aimed at providing evidence to support a role for B retranslocation as a mechanism of B efficiency in oilseed rape. In a related species, *B. oleracea*, B retranslocation has been reported (Shelp and Shattuck, 1987; Shelp *et al.*, 1992; Shelp *et al.*, 1996; Liu *et al.*, 1993) and is eluded to as a potential mechanism for B efficiency in this species (Shelp and Shattuck, 1987). In the study presented here, B retranslocation was potentially indicated in all varieties through B concentration gradients between young and old leaves not correlating with the age of the plant part, which may support B supply by secondary translocation (i.e. retranslocation via the phloem). On application of ^{10}B isotope, the degree to which remobilisation occurred differed between varieties. The B efficient varieties Huashuang 2, Dunkeld and Zhongyou 821 retranslocated more B out of labelled leaves when compared to the B inefficient Barossa. These results correlate with the B efficiency of the individual varieties (Chapter 2 and 3), and give an indication of a potential mechanism for B efficiency in oilseed rape.

The remobilisation result using ^{10}B isotope was not reproduced in its absence, with small amounts of net ^{10}B movement out of unlabelled mature leaves only observed in Huashuang 2 and to a less extent Zhongyou 821. This discrepancy may be explained by recent observations made by Liu *et al.* (1993) who reported B accumulation in source leaves of broccoli only when supply exceeded the requirements of the sink regions, perhaps suggesting regulation of the extent to which B undergoes retranslocation. Alternatively, B retranslocation in plants supplied with luxury levels of B (as seen in Experiment 6(b)) may in effect be masked by a high net movement of B into the leaf.

The ability of Huashuang 2 to enrich young leaf tissue just 24 h after application of isotope may indicate a higher concentration of the borate chelator in the phloem. Since no polyol was found in any of the varieties, an alternative mechanism is required for the remobilisation of B in oilseed rape. Two possible explanations are: firstly the presence of a non-polyol chelator with *cis*-diol groups able to form a stable bond with borate. Higher

soluble carbohydrate concentrations in Huashuang 2 may point to a higher concentration of the chelator. Interestingly, Cao *et al.* (1997) also reported a higher sugar content in B efficient oilseed rape, which may be an inherent feature of B efficient genotypes. From the analyses of leaf tissue extract, a likely chelator is fructose, which is known to complex strongly borate (Makkee *et al.*, 1985; Loomis and Durst, 1992) and has been reported in the phloem of plant species which exhibit enhanced B mobility (Hu and Brown, 1996). Like any free reducing sugar, fructose undergoes Maillard reactions which produce toxic products (Loomis and Durst, 1992) such as free radicals that cause cellular disruptions such as induced DNA strand breakage (Hiramoto *et al.*, 1995). Thus Hu and Brown (1996) concluded that their identification of fructose in extra floral nectar was most likely as a result of post-phloem metabolism, and one cannot therefore assume the presence of fructose in the phloem of oilseed rape without collection and identification of phloem sap constituents. This must be an aim of future research. The second possibility is that due to boric acid being highly permeable across plant membranes, active transport of B is generally regarded as energetically expensive and therefore unlikely to occur (Raven, 1980). The potential may exist for active B transport into the phloem of oilseed rape. Van Goor and van Lune (1980) conclude that differences in phloem mobility may occur at different stages of transport (i.e. the loading of the sieve tubes, the rate of the translocation itself, loss during transport and absorption in the sink-organ), though differences are assumed especially in the loading step and considerably less in the transport by the sieve tubes. Further studies are required to determine whether B is actively transported into the phloem, and if so, whether this may explain the genotypic variation in remobilisation in oilseed rape.

Chapter 7

General Discussion

7.1 General conclusions

The following general conclusions can be made regarding the genotypic variation, and mechanism of boron (B) efficiency in oilseed rape.

1. From both field and solution culture screening, the following genotypes of *Brassica napus* exhibit some level of B efficiency: Huashuang 2, Nangchang rape, Huashuang 1, Zhongyou 821, Zheyou 2, Dunkeld, Xinza 2, Nangjin 2051, 92-58, 92-13 and Awassa 115. From field based evaluation, B efficient *B. juncea* include Pusa Bold and TM18.

2. Root length is a suitable selection criterion for identifying B efficient *B. napus* genotypes.

3. When B deficiency is severe, pre-reproductive growth in *B. napus* closely reflects reproductive success, indicating its contribution to the efficiency of a genotype.

4. Rhizosphere acidification did not contribute toward the mechanism of B efficiency in the *B. napus* varieties Dunkeld and Zhongyou 821.

5. Boron deficiency enhances rhizosphere acidification in *B. napus* roots, which may indicate an effect of B on membrane function and structure.

6. A mechanism of B efficiency in the *B. napus* variety, Dunkeld, and *B. juncea* variety, Pusa Bold, is associated with an increased capacity to uptake (net B accumulation) B.

7. B efficiency is not associated with an increased translocation of B from root to shoot.

8. Genotypic variation in B retranslocation is observed in *B. napus*, and may contribute toward the mechanism of B efficiency in the variety Huashuang 2.

9. A polyol was not identified in leaf tissue extract of *B. napus*, therefore it is unlikely that B retranslocation is occurring by association with a polyol. B retranslocation is facilitated by some other, as yet unknown means.

10. Soluble carbohydrate concentrations in the B efficient Huashuang 2 are higher than in the B inefficient Barossa, which may indicate that it is worth looking for the borate-chelator among the *cis*-hydroxy-containing soluble carbohydrates.

7.2 The need for breeding B efficient oilseed rape

The data presented in this thesis will ultimately contribute toward facilitating the introduction of B efficient oilseed rape into areas where B deficiency is a threat to maintaining yield potential. For China in particular, the majority of its oilseed rape crop is grown on soils predominantly low in B (Liu and Chen, 1986). Recent estimates of oilseed rape production in China for the 98/99 season are 8.3 million tonnes (1998 Australian Oilseeds Federation estimate. Issue 48), with a large part of this yield maintained with the addition of boron fertilisers. In Australia, oilseed rape, and in particular, canola, is a major crop in the rotations of Southern Australia. Recent figures have shown an increase in production from 641,000 ton in the 96/97 season, up to 1.6 million tonne for the 98/99 season (1998 Australian Oilseeds Federation. Issue 48). With this rapid expansion, the incidence of B deficiency is likely to increase, more so in areas previously reporting the effects of B deficiency in this crop (Myers *et al.*, 1983). Introducing B efficient oilseed rape into these areas is one strategy that will contribute to the long-term sustainability of agriculture in these B deficient regions.

7.3 Genotypic variation in B efficiency

There are numerous reports of genotypic variation in B efficiency (Rerkasem and Jamjod, 1997 and references therein), yet for oilseed rape, published accounts are few (Sakal *et al.*, 1991; Yang *et al.*, 1993; Yang *et al.*, 1994). In this thesis, a relatively large number (up to 65) of oilseed rape varieties and breeder's lines were screened for their response to B (Chapter 2 and 3). The efficiency of a genotype was defined according to the definition of Graham (1984), as the ability of a cultivar to grow and yield well in soils too deficient in a nutrient for a standard cultivar. B efficiency was observed in *Brassica napus* (Huashuang 2, Nangchang rape, Huashuang 1, Zhongyou 821, Zheyou 2, Dunkeld, Xinza 2, Nangjin 2051, 92-58, 92-13 and Awassa 115) and *B. juncea* (Pusa Bold and TM18). Efficient genotypes provide the genetic material required for a successful breeding strategy to alleviate the effects of B deficiency.

A major outcome of this thesis was the identification of a suitable screening technique for B efficiency in *B. napus* (Chapter 2) which will reduce the time and cost of screening germplasm in the field. For a screening technique to be successful, it should 'either indirectly correlate with the economic yield or be involved in the first (in the chronological order of development) physiological process most sensitive to B deficiency that is limiting yield' (Rerkasem and Jamjod, 1997). For example, Chantachume *et al.* (1995) demonstrated the sensitivity of root length to B toxicity, which culminated in a suitable screening technique for wheat genotypes tolerant to B toxicity. From results presented in Chapter 2, root length was highly sensitive to B deficiency, impacting on B uptake (net B accumulation) and growth of the shoot (Chapter's 2, 4 and 5), and final yield response (Chapter 3). At this stage, the technique developed in Chapter 2 is suitable for screening *B. napus* germplasm only, and appears unsuitable for *B. juncea.*

Of the *B. napus* varieties identified as having some form of B efficiency, Huashuang 2, Nangjin 2051, Zheyou 2 and Dunkeld are of particular interest as they have recently been bred for their canola quality, and therefore exhibit the best alleles at many loci (Allard, 1960). In the interim, before high quality B efficient varieties are developed, the above varieties may have immediate use in B deficient regions.

While the results of Chapters 2 and 3 provide a small number of B efficient varieties and breeder's lines which exhibit some form of B efficiency, the identification of more efficient germplasm is likely given the limited number of lines screened, and these efforts should be pursued further. Access to germplasm from the breeding institutions that bred the efficient varieties identified in this thesis would most likely give access to more efficient germplasm.

7.4 Mechanisms of B efficiency

From data presented in this thesis, there is evidence to suggest two mechanisms of B efficiency in oilseed rape. In Dunkeld, the mechanism of its B efficiency is associated with an increased B uptake (net B accumulation) per unit of root length (Chapter 4), while there is an indication that it also retranslocates B. In the variety Huashuang 2, B efficiency appears related to an efficiency in the utilisation of B due to enhanced B retranslocation (Chapter 6). The potential now exists for breeding a cultivar which expresses both of these enhanced traits. This task may be achieved by back-crossing efficiency traits into a canola quality cultivar agronomically suited to a region. As this is a time-consuming task, B efficient progeny can be identified with the use of the rapid screening technique developed in Chapter 2. Alternatively, when the borate-chelator is eventually identified in Huashuang 2, efficient genotypes with the B retranslocation trait may be identified by analysing phloem or leaf tissue extract by HPLC or GC-MS. In effect the chelator will become a biochemical marker.

7.5 Implications of B retranslocation in fertiliser management

Knowing that a variety retranslocates significant amounts of B has implications in the fertiliser management of the crop during the growing season. Shelp *et al.* (1996) recently demonstrated the benefits of foliar versus soil applied B in a cultivar of broccoli which retranslocates B. Foliar-fertilisation was more effective in mitigating the effects of B deficiency than soil applied fertilisation. Developing a cultivar which can retranslocate B to a very high level, such as in Huashuang 2, will greatly enhance the effectiveness of foliar applied applications of B during the growing season and contribute toward a more judicious use of boron fertilisers.

7.6 Future molecular approaches derived from this research

With the identification of significant genotypic variation in B retranslocation (Chapter 6), the identification of the borate chelator in the phloem deserves research effort. This will not only provide a biochemical marker for future selection purposes, but also provide a biochemical target for genetic manipulation which has implications for breeding efficiency into other genotypes and species of oilseed rape. For example, Tao *et al.* (1995) recently integrated and expressed the apple cDNA encoding NADP-dependent sorbitol-6-phosphate dehydrogenase (S6PDH) in transgenic tobacco, which was sufficient for the synthesis of sorbitol. Sorbitol-borate complexation facilitates the remobilisation of B in the *Malus*, *Prunus* and *Pyrus* and species (Brown and Hu, 1996). Likewise, in the tobacco encoding for S6PDH, B remobilisation now occurs and has recently been shown to mitigate the effects of B deficiency (P H Brown personal communications). The identification of the borate-chelator in Huashuang 2 will allow for a similar approach to be taken as reported by Tao *et al.* (1995); to transform other genotypes and species for expression of the borate-chelator.

7.7 Physiological implications of this research

While trying to identify the mechanism of B efficiency, a number of potential efficiency mechanisms were investigated, including the mobilisation of B through rhizosphere acidification (Chapter 4), B uptake (Chapter 4 and 5), enhanced root to shoot B translocation (Chapter 5) and B retranslocation (Chapter 6). As previously stated in this discussion, mechanisms of B efficiency were found to include an increased B uptake (net B accumulation) per unit of root length in Dunkeld and B retranslocation in Huashuang 2. The remaining studies did not provide any indication of a B efficiency mechanism, though information derived from these studies provide a basis for future research into the physiology of B. These include:

1. An increased acidification of the rhizosphere in plants grown under sub-optimal B supply (Chapter 4). To my knowledge this is the first report of a measured nutritional effect of B on rhizosphere pH, which may indicate an effect of B on membrane function and structure.

2. B uptake (net B accumulation) per unit of root length was greater in the B efficient variety Dunkeld (Chapter 4), which may indicate genotypic variation in the volume of the root cylinder.

3. The rate of B uptake was greater in the B inefficient variety Barossa when compared to the B efficient Dunkeld (Chapter 5). This may simply indicate differences in the membrane permeability of boric acid (Hu and Brown, 1997), or alternatively may result from variation in the uptake kinetics of a carrier at very low B supply. There is evidence in Chapter 5 to suggest B uptake is an active process in Barossa. If an active component of uptake is confirmed, this may provide the impetus for gaining an understanding of a primary function for B.

4. While genotypic variation in B retranslocation was identified in a number of *B. napus* varieties (Chapter 6), the mechanism facilitating this retranslocation remains unclear. Polyols, sorbitol and mannitol, are associated with B retranslocation in *Prunus*, *Malus* and *Pyrus* species (Brown and Hu, 1996; Hu *et al.*, 1997), though in this thesis (Chapter 6) no polyol was identified which could explain B retranslocation. This most likely indicates the presence of a non-polyol chelator for borate in this species, or alternatively, genotypic variation in the loading of B into the phloem.

7.8 Suggestions for future research

The outcomes of this thesis can be viewed as an initial step in the development of B efficient varieties of oilseed rape. The scientific community is encouraged to consider the following suggestions for future research.

1. Further corroborate the solution culture screening technique with field based evaluation of *B. napus* and *B. juncea* genotypes. Include other related *Brassica* species such as *B. campestris.*

2. Determine the mechanism of B efficiency in the *B. juncea* varieties Pusa Bold and TM 18.

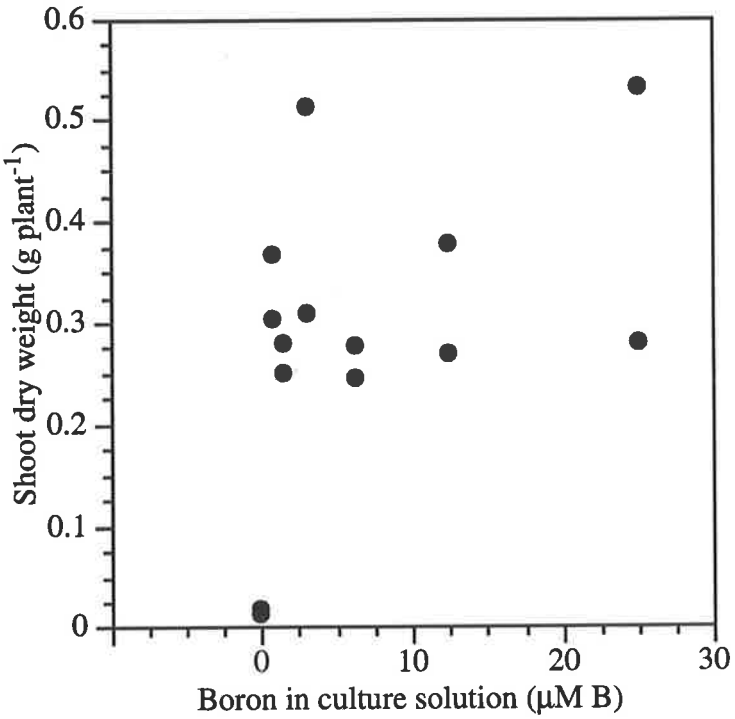
3. Quantify the benefits of soil and foliar applied B on seed yield in genotypes which retranslocate significant amounts of B (Huashuang 2), and exhibit an increased capacity to take up B (Dunkeld).

4. Collect phloem sap and identify the borate-chelator responsible for facilitating B retranslocation. This will provide a biochemical marker for future selection of the retranslocation trait in oilseed rape, as well as provide a target site for further genetic manipulation.

5. Determine, with more appropriate levels of external B supply and the use of metabolic inhibitors (i.e. DNP), the rate of B uptake in Barossa, to corroborate, or otherwise, B uptake in this variety as an active or passive process.

6. Breed a variety with an increased capacity to uptake (i.e. as in Dunkeld) and retranslocate (i.e. as in Huashuang 2) B, and quantify the benefits, or otherwise, of a dual efficiency mechanism.

Appendix I.



Effect of external B supply on shoot dry weight in 10 day old Barossa. B rates are: 0, 0.8, 1.6, 3.1, 6.3, 12.5 and 25 µM B.

References

Agarwala S C and Sharma C P, Chatterjee C and Nautiyal B D (1984). Annual progress report of all-India coordinated scheme of micronutrients in soils and plants. Department of Botany, University of Lucknow, India. 127 p

Allard R W (1960). Principles of plant breeding. John Wiley, New York.

Anderson A J (1952). Responses of subterranean clover and lucerne to boron. Journal of Australian Institute of Agricultural Science 18, 159-162.

Anumula K R and Taylor P B (1992). A comprehensive procedure for preparation of partially methylated alditol acetates from glycoprotein carbohydrates. Analytical Chemistry 203, 101-108.

Asher C J and Edwards D G (1983). Modern solution culture techniques. *In* Encyclopedia of Plant Physiology. New Series. Vol. 15A. Inorganic Plant Nutrition. Eds. Lauchli A and Bieleski R L. Springer-Verlag, Berlin, Germany. pp 94-119.

Aubert H and Pinta M (1977). Trace Elements in Soils. Developments in Soil Science 7. Elsevier, New York.

Barr R, Böttger M and Crane F L (1993). The effect of boron on the plasma membrane electron transport and associated proton secretion by cultured carrot cells. Biochemistry and Molecular Biology International 31(1), 31-39.

Barrow N J and Mendoza R E (1990). Equations for describing sigmoid yield responses and their application to some phosphate responses by lupins and by subterranean clover. Fertiliser Research 22, 181-188.

Bate N, Orr J, Ni W, Meromi A, Nadler-Hassar T, Doerner P W, Dixon R A, Lamb C J and Elkind Y (1994). Quantitative relationship between phenylalanine ammonia-lyase levels and phenylpropanoid accumulation in transgenic tobacco identifies a rate-determining step in natural product synthesis. *Proceedings of the National Academy of Sciences USA* 91, 7608-7612.

Bell R W (1997). Diagnosis and prediction of boron deficiency for plant production. Plant and Soil 193, 149-168.

Bell R W and Huang L (1994). A field survey of nutrient levels in canola plants (*Brassica napus*). *In* Management of Boron and Zinc in Oilseed Crops in China. ACIAR research project 9120, annual report. pp 164-167.

Bell R W, McLay L, Plaskett D, Dell B and Loneragan J F (1990). Internal boron requirements of green gram (*Vigna radiata*). *In* ed. van Beusichem M L. Plant Nutrition-Physiology and Application. Kluwer Academic Publishers, Dordrecht, The Netherlands. pp 275-280.

Bellaloui N and Brown P H (1998). Cultivar differences in boron uptake and distribution in celery (*Apium graveolens*), tomato (*Lycopersicon esculentum*) and wheat (*Triticum aestivum*). Plant and Soil 198, 153-158.

Benson N R, Lindner R C and Bullock R M (1966). Plum, prunes and apricot. *In* Nutrition of Fruit Crops. Ed. Childers N F. Horticultural Publications. Rutgers University, New Brunswick, New Jersey. pp 504-517.

Berger K C (1949). Boron in soils and crops. Advances in Agronomy 1, 321-351.

Berger K C and Truog E (1945). Boron availability in relation to soil reaction and organic matter content. Soil Science Society of America. Proceedings 10, 113-116.

Bergmann W (1983). Ernährungsstörungen bei kulturpflanzen, entstehung und diagnose. Fischer Verlag, Jena.

Biggar J W and Fireman M (1960). Boron adsorption and release by soils. Soil Science Society of America. Proceedings 24, 115-120.

Bingham F T (1973). Boron in cultivated soils and irrigation waters. *In* Trace Elements in the Environment. Ed. Kothny E L. American Chemical Society, Washington D C. pp 130-138.

Bingham F T, Elseewi A and Oertli J J (1970). Characteristics of boron absorption by excised barley roots. Soil Science Society of America. Proceedings 34, 613-617.

Bingham F T, Page A L, Coleman N T and Flach K (1971). Boron adsorption characteristics of selected soils from Mexico and Hawaii. Soil Science Society of America. Journal 35, 546-550.

Birnbaum E H, Beasley C A and Dugger W M (1974). Boron deficiency in unfertilised cotton (*Gossypium hirsutum*) ovules grown *in vitro*. Plant Physiology 54, 931-935.

Blamey F P C, Mould D and Chapman J (1979). Critical boron concentration in plant tissues of two sunflower cultivars. Agronomy Journal 71, 243-247.

Blaser-Grill J, Knoppik D, Amberger A and Goldbach H (1989). Influence of B on the membrane potential in *Elodea densa* and *Helianthus annuus* roots and H^+ extrusion of suspension cultured *Daucus carota* cells. Plant Physiology 90, 280-284.

Blevins D G and Lukaszewski K M (1998). Boron in plant structure and function. Annual Review of Plant Physiology and Plant Molecular Biology 49, 481-500.

Bloesch P M, Bell L C and Hughes J D (1987). Adsorption and desorption of boron by goethite. Australian Journal of Soil Research 25, 377-390.

Böeseken J (1949). The use of boric acid for the determination of the configuration of carbohydrates. *In* Advances in Carbohydrate Chemistry. Vol. 4. Eds. Pigman W W and Wolfrom M L. Academic Press, New York. pp. 189-210.

Bowen J E (1981). Kinetics of active uptake of boron, zinc, copper, and manganese in barley and sugarcane. Journal of Plant Nutrition 3(1-4), 215-223.

Bowen J E (1972). Effect of environmental factors on water utilisation and boron accumulation and translocation in sugarcane. Plant and Cell Physiology 13, 703-714.

Bowen J E (1968). Borate absorption in excised sugarcane leaves. Plant and Cell Physiology 9, 467-478.

Bowen J E and Nissen P (1977). Boron uptake by excised barley roots. II. Characteristics and kinetics of active uptake. Physiologia Plantarum 41, 109-115.

Bowen J E and Nissen P (1976). Boron uptake by excised barley roots. I. Uptake into the free space. Plant Physiology 57, 353-357.

Boyce P J and Blevins D G (1993). Carbon transport in boron-deficient cotton. Plant Physiology 102 (1) Suppl 163.

Brown J C and Ambler J E 1972. Genetic control of uptake and a role of boron in tomato. Soil Science Society of America. Proceedings 37, 63-66.

Brown J C and Jones W E (1971). Differential transport of boron in tomato (*Lycopersicon esculentum* Mill.). Physiologia Plantarum 25, 279-282.

Brown P H and Hu H (1996). Phloem mobility of boron is species dependant: evidence for phloem mobility in sorbitol-rich species. Annals of Botany 77, 497-505.

Brown P H and Hu H (1994). Boron uptake by sunflower, squash and cultured tobacco cells. Physiologia Plantarum 91, 435-441.

Brown P H and Shelp B J (1997). Boron Mobility in Plants. Plant and Soil 193, 85-101.

Bussler W (1962). Boron deficiency in maize. Mitteilungen der Deutschen Landwirtschafts-Gesellschaft 77, 1110-1112.

Cakmak I and Römheld V (1997). Boron deficiency-induced impairments of cellular functions in plants. Plant and Soil 193, 71-83.

Cakmak I, Hengeler C and Marschner H (1994). Changes in phloem export of sucrose in leaves in response to phosphorous, potassium and magnesium deficiency in bean plants. Journal of Experimental Botany 45 (278), 1251-1257.

Cakmak I, Kurz H and Marschner H (1995). Short-term effects of boron, germanium and high light intensity on membrane permeability in boron deficient leaves of sunflower. Physiologia Plantarum 95, 11-18.

Campbell L C, Miller M H and Loneragan J F (1975). Translocation of boron to plant fruits. Australian Journal of Plant Physiology 2, 481-487.

Cao X, Wuding L and Pi M (1997). Composition of root exudates and root bleeding sap of a boron-efficient and inefficient rape genotype. *In* Boron in Soils and Plants. Ed Bell R W and Rerkasem B. pp 171-174. Kluwer Academic Publishers, Netherlands.

Chalmers A G, Darby R J, Evans E J, Sinclair A H and Withers P J A (1992). Crop nutrition and fertiliser requirements for the double low oilseed rape crop. HGCA Oilseeds Research Review. No. OS5. Publishers: Home-grown cereals Authority, Research and Development. pp 96-103.

Chantachume Y, Smith D, Hollamby G J, Paull J G and Rathjen A J (1995). Screening for boron tolerance in wheat (*T. aestivum*) by solution culture in filter paper. Plant and Soil 177, 249-254.

Cheng C and Rerkasem B (1993). Effects of boron on pollen viability in wheat. Plant and Soil 156, 313-315.

Cohen M S and Lepper R Jr 1977. Effect of boron on cell elongation and division in squash roots. Plant Physiology 59, 884-887.

Cotton F A and Wilkinson G (1976). Basic Inorganic Chemistry. John Wiley and Sons, Inc. pp 231.

Dannel F, Pfeffer H and Römheld V (1998). Compartmentation of boron in roots and leaves of sunflower as affected by boron supply. Journal of Plant Physiology. In press.

Dean J A (1979). Lange's Handbook of Chemistry. 12th Edition. McGraw Hill Book Company, New York.

Dear B S and Lipsett J (1987). The effect of boron supply on the growth and seed production of subterranean clover (*Trifolium subterraneum* L.). Australian Journal of Agricultural Research 38, 537-546.

Dell B and Huang L (1997). Physiological response of plants to low boron. Plant and Soil 193, 103-120.

Dinkelaker B, Römheld V and Marschner H (1989). Citric acid excretion and precipitation of calcium citrate in the rhizosphere of white lupin (*Lupinus albus* L.). Plant Cell and Environment 12, 285-292.

Dugger W M and Palmer R L (1985). Effect of boron on the incorporation of glucose from by cotton fibers grown *in vitro*. Journal of Plant Nutrition 8, 311-325.

Dugger W M and Palmer R L (1980). Effect of boron on the incorporation of glucose from UDP-glucose into fibers grown *in vitro*. Plant Physiology 65, 266-273.

Dugger, W.M., (1983). Boron in plant metabolism. *In* Encyclopedia of Plant Physiology, New Series, Vol 15. Inorganic Plant Nutrition. Ed. Lauchli, A. and Bieleski, R.L. Springer -Verlag, Berlin. pp 627-650

Eaton F M (1944). Deficiency, toxicity and accumulation of boron in plants. Journal of Agricultural Research 69, 237-277.

Eaton F M and Blair G Y (1935). Accumulation of boron by reciprocally grafted plants. Plant Physiology 10, 411-422.

Eaton F M and Wilcox L V (1939). The behaviour of boron in soils. US Department of Agronomy Technical Bulletin 696.

Elkind Y, Edwards R, Mavandad M, Hedrick S A, Ribak O, Dixon R A and Lamb C J (1990). Abnormal plant development and down-regulation of phenylpropanoid biosynthesis in transgenic tobacco containing a heterologous phenylalanine ammonia-lyase gene. *Proceedings of the National Academy of Sciences USA* 87, 9057-9061.

Elrashidi M A and O'Connor G A (1982). Boron sorption and desorption in soils. Soil Science Society of America. Journal 46, 27-31.

Elseewi A A and Elmalky A E (1979). Boron distribution in soils and waters of Egypt. Soil Science Society of America. Journal 43, 297-300.

Evans C M and Sparks D L (1983). On the chemistry and mineralogy of boron in pure and in mixed systems: A review. Communications in Soil Science and Plant Analysis 14, 827-846.

Findeklee P and Goldbach H E (1996). Rapid effects of boron deficiency on cell wall elasticity modulus in *Cucurbita pepo* roots. Botanica Acta 109, 463-465.

Fleming G A (1980). Essential micronutrients. I. Boron and molybdenum. *In* Applied Soil Trace Elements. Ed. Davies B E. John Wiley and Sons, New York. pp 155-197.

Forno D A, Asher C J and Edwards D G (1979). Boron nutrition of cassava, and the boron x temperature interaction. Field Crops Research 2, 265-279.

Gauch H G and Dugger W M Jr (1953). The role of boron in the translocation of sucrose. Plant Physiology 28, 457-466.

Goldbach H E (1997). A critical review on current hypotheses concerning the role of boron in higher plants: suggestions for further research and methodological requirements. Journal of Trace and Microprobe Techniques. 15(1), 51-91.

Goldbach H E, Hartmann D and Rötzer T (1990). Boron is required for the ferricyanide-induced proton release by auxins in suspension-cultured cells of *Daucus carota* and *Lycopersicon esculentum*. Physiologia Plantarum 80, 114-118.

Goldberg S (1997). Reactions of boron with soils. Plant and Soil 193, 35-48.

Goldberg S and Forster H S (1991). Boron sorption on calcareous soils and reference calcites. Soil Science 152, 304-310.

Goldberg S and Glaubig R A (1985). Boron adsorption on aluminum and iron oxide minerals. Soil Science Society of America. Journal 49, 1374-1379.

Graham R D (1984). Breeding for nutritional characteristics in cereals. Advances in Plant Nutrition 1, 57-102.

Greenwood H N (1973). Boron. *In* Comprehensive Inorganic Chemistry. Volume 1. Ed. Bailan J C *et al.* Pergamon Press, Oxford. pp 665-991.

Grinsted M J, Hedley M J, White R E and Nye P H (1982). Plant-induced changes in the rhizosphere of rape (*Brassica napus* var. Emerald) seedlings. I. pH change and the increase in P concentration in the soil solution. New Phytologist 91, 19-29.

Gupta U C (1979). Boron nutrition of crops. Advances in Agronomy 31, 273-307.

Gupta U C (1968). Relationship of total and hot-water soluble boron, and fixation of added boron, to properties of Podzol soils. Soil Science Society of America. Proceedings 32, 45-48.

Gupta U C and Cutcliffe (1975). Boron deficiency in cole crops under field and greenhouse conditions. Communications in Soil Science and Plant Analysis 6 (2), 181-188.

Gupta U C, Jame Y W, Campbell C A, Leyshon A J and Nicholaichuk W (1985). Boron Toxicity and Deficiency: A Review. Canadian Journal of Soil Science 65, 381-409.

Haneklaus S and Schnug E (1993). Genetic variability and pattern of mineral nutrients in varieties of *Triticum aestivum* and *Brassica napus*. Aspects of Applied Biology 34, 211-218.

Harada T and Tamai M (1968). Some factors affecting behaviour of boron in soil. I. Some soil properties affecting boron adsorption of soil. Soil Science and Plant Nutrition 14, 215-224.

Harris H C and Brolman J B (1966). Comparison of calcium and boron deficiencies of the peanut. I. Physiological and yield differences. Agronomy Journal 58, 575-578.

Häussling M, Leisen E, Marschner H and Römheld V (1985). An improved method for non-destructive measurements of the pH at the root-soil interface (rhizosphere). Journal of Plant Physiology 117, 371-375.

Hedley M J, Nye P H and White R E (1982). Plant-induced changes in the rhizosphere of rape (*Brassica napus* var. Emerald) seedlings. II. Origin of the pH change. New Phytologist 91, 31-44.

Heslop-Harrison J S (1986). Ion localisation in the stigma and pollen tube of cereals. *In* Biotechnology and Ecology of Pollen. Eds. Mulcahy D L, Gergamini Mulcahy, G and Ottaviano E. Springer-Verlag.

Heslop-Harrison J S (1979). Aspects of the structure, cytochemistry and germination of the pollen of rye. Annals of Botany 44: Suppl 1, 1-47.

Heyes J A, White P J and Loughman B C (1991). The role of boron in some membrane characteristics of plant cells and protoplasts. *In* Current Topics in Plant Biochemistry and Physiology 10. Randall D D, Blevins D G and Miles C D eds., University of Missouri-Columbia. pp 179-194.

Hiatt A J and Hendricks S B (1967). The role of CO_2 - fixation in accumulation of ions by barley roots. Zeitschrift für Pflanzenphysiologie 56, 220-232.

Hiramoto K, Sekiguchi K, Asp-o R, Ayuha K, Ni-iyama H, Kato T and Kikugawa K (1995). DNA strand breaks induced through active oxygen radicals by fragrant component 4-hydroxy-2-hydroxymethyl-5methyl-3(2H)-furanone in Maillard reaction of hexose/amino acid. Food and Chemical Toxicology 33,(10), 803-814.

Hoagland D R and Arnon D I (1950). The water culture method for growing plants without soil. *California Experiment Station Circular 347.* Berkeley, CA: The College of Agriculture, University of California.

Hoddinott J (1993). The influence of toxic and deficient boron levels on carbon partitioning in *Phaseolus vulgaris*. Plant Physiology 102 (1) Suppl 164.

Hu H and Brown P H (1994). Localisation of boron in cell walls of squash and tobacco and its association with pectin. Evidence for a structural role of boron in the cell wall. Plant Physiology 105, 681-689.

Hu H and Brown P H (1996). Phloem mobility of boron is species dependent: evidence for phloem mobility in sorbitol-rich species. Annals of Botany 77, 497-505.

Hu H and Brown P H (1997). Absorption of boron by plant roots. Plant and Soil 193, 49-58.

Hu H, Brown P H and Labavitch J M (1996). Species variability in boron requirement is correlated with cell wall pectin. Journal of Experimental Botany 47, 227-232.

Hu H, Penn S, Lebrillo C and Brown P H (1997). Isolation and characterization of soluble boron complexes in higher plants. Plant Physiology 113, 649-655.

Huang C and Graham R D (1990). Resistance of wheat genotypes to boron toxicity is expressed at the cellular level. Plant and Soil 126, 295-300.

Huang L, Ye Z Q and Bell R W (1996). The importance of sampling young leaves for the diagnosis of boron deficiency in canola (*Brassica napus* L.). Plant and Soil 183, 187-198.

Ishii T and Matsunaga T (1996). Isolation and characterisation of a boron-rhamnogalacturonan-II complex from cell walls of sugar beet pulp. Carbohydrate Research 284, 1-9.

Jackson J F (1991). Borate control of energy-driven protein secretion from pollen and interaction of borate with auxin or herbicide-a possible role for boron in membrane events. *In* Current Topics in Plant Biochemistry and Physiology, volume 10. Eds. Randall D D, Blevins D G and Miles C D. University of Missouri, Columbus. pp 221-229.

Jarvis B C, Yasmin S, Ali A H N and Hunt R (1984). The interaction between auxin and boron in adventitious root development. New Phytologist 97, 197-204.

Jin J Y, Martens D C and Zelazny L W (1988). Plant availability of applied and native boron in soils with diverse properties. Plant and Soil 105, 127-132.

Johnson S L and Smith K W (1976). The interaction of borate and sulfite with pyradine nucleotides. Biochemistry 15, 553-559.

Jones J B jr, Wolf B and Mills H A (1991). Plant Analysis Handbook. Micro-Macro Publishing. Athens, Georgia. 213 p.

Jungk A and Claassen N (1986). Availability of phosphate and potassium as the result of interactions between root and soil in the rhizosphere. Zeitschrift für Pflanzenernährung und Bodenkunde 149, 411-427.

Kabata-Pendias A and Pendias H (1992). Trace Elements in Soils and Plants. 2nd Edition. CRC Press, Boca Raton, Florida. pp 154.

Kelly J F and Gabelman W H (1960). Variability in the tolerance of varieties and strains of red beet (*Beta vulgaris* L.) to boron deficiency. American Society of Horticultural Science. Proceedings 76, 409-415.

Keren R, Bingham F T and Rhoades J D (1985). Effect of clay content in soil on boron uptake and yield of wheat. Soil Science Society of America. Journal 49, 1466-1470.

Kirk G J and Loneragan J F (1988). Functional boron requirement for leaf expansion and its use as a critical value for diagnosis of boron deficiency in soybean. Agronony Journal 80, 758-762.

Kobayashi M, Matoh T and Azuma J (1996). Two chains of rhamnogalacturonan II are cross-linked by borate-diol ester bonds in higher plant cell walls. Plant Physiology 110, 1017-1020.

Kohl H C Jr and Oertli J (1961). Distribution of boron in leaves. Plant Physiology. 36, 420-424.

Kouchi H and Kumazawa K 1976. Anatomical responses of root tips to boron deficiency. III. Effect of boron deficiency on subcellular structure of root tips, particularly on morphology of cell wall and its related organelles. Soil Science and Plant Nutrition 22, 53-71.

Lambert M J and Turner J (1977). Dieback in high site quality *Pinus radiata* stands-the role of sulphur and boron deficiencies. New Zealand Journal of Forest Science 8, 351-368.

Lane P, Galway N and Alvey N (1988). Genstat 5: An introduction. Oxford University Press.

Lee S G and Aronoff S (1967). Boron in plants: A biochemical role. Science 158, 798-799.

Lee S G and Aronoff S (1966). Investigations on the role of boron in plants. III. Anatomical observations. Plant Physiology 41, 1570-1577.

Liu C and Chen Z (1986). Boron nutrition of oilseed crops. *In* Research and Application of Micronutrient Fertilisers. Ed. Agriculture Bureau of the Ministry of Agriculture. Hubei Science and Technology Press, Wuhan, China. pp112-134.

Liu C, Dean J F D, Friedman W E and Eriksson K E (1994). A laccase-like phenoloxidase is correlated with lignin biosynthesis in *Zinnia elegans* stem tissue. Plant Journal 6, 213-224.

Liu L, Shelp B J and Spiers G A (1993). Boron distribution and retranslocation in field-grown broccoli (*Brassica oleracea* var. *italica*). Canadian Journal of Plant Science 73, 587-600.

Liu Z H and Ger M J (1997). Changes of enzyme activity during pollen germination in maize, and possible evidence of lignin synthesis. Australian Journal of Plant Physiology 24, 329-335.

Liu Z, Zhu Q and Tang L (1981). Boron deficient soils and their distribution in China. Soil research report no. 5. Institute of Soil Science, Academia Sinica, Nanjing, China. pp 1-13.

Löhnis M P (1940). Meded. Landbouwhogesch. Wangeningen 44, 3-36.

Löhnis M P (1937). Meded. Landbouwhogesch. Wangeningen 41, 3.

Loomis W D and Durst R W (1992). Chemistry and biology of boron. Biofactors 3, 229-239.

Lukaszewski K M and Blevins D G (1996). Root growth inhibition in boron-deficient or aluminum-stressed squash may be a result of impaired ascorbate metabolism. Plant Physiology 112, 1135-1140.

Madore M A, Mitchell D E and Boyd C M (1988). Stachyose synthesis in source leaf tissues of the CAM plant *Xerosicyos danguyi* H. Humb. Plant Physiology 87, 588-591.

Makkee M, Kieboom A P G and van Bekkum H (1985). Studies on borate esters III. Borate esters of D-mannitol, D-glucitol, D-fructose and D-glucose in water. Reclueil des Travaux Chimiques Pays-Bas 104, 230-235.

Marschner H (1995a). Mineral Nutrition of Higher Plants. 2nd Edition. Academic Press, London. pp.600-601.

Marschner H (1995b). Mineral Nutrition of Higher Plants. 2nd Edition. Academic Press, London. pp. 379-396.

Marschner H (1995c). Mineral Nutrition of Higher Plants. 2nd Edition. Academic Press, London. pp. 45-50.

Marschner H (1995d). Mineral Nutrition of Higher Plants. 2nd Edition. Academic Press, London. pp.28.

Marschner H, Kirkby E A and Cakmak I (1996). Effect of mineral nutritional status on shoot-root partitioning of photoassimilates and cycling of mineral nutrients. Journal of Experimental Botany 47, 1255-1263.

Marschner H, Römheld V and Ossenberg-Neuhaus H (1982). Rapid method for measuring changes in pH and reducing processes along roots of intact plants. Zeitschrift für Pflanzenphysiologie 105, 407-416.

Martini F and Thellier M (1993). Boron distribution in parenchyma cells of clover leaves. Plant Physiology and Biochemistry 31, 777-786.

Matoh T (1997). Boron in plant cell walls. Plant and Soil 193, 59-70.

Matoh T, Ishigaki K, Mizutani M, Matsunaga W and Takabe K (1992). Boron nutrition of cultured tobacco BY-2 cells. I. Requirement for and intracellular localisation of boron and selection of cells that tolerate low levels of boron. Plant Cell Physiology 33, 1135-1141.

Matoh T, Ishigaki K, Ohno K and Azuma J (1993). Isolation and characterization of a boron-polysaccharide complex from radish roots. Plant Cell Physiology 34, 639-642.

Matoh T, Kawaguchi S and Kobayashi M (1996). Ubiquity of a borate-rhamnogalacturonan II complex in the cell walls of higher plants. Plant Cell Physiology 34, 639-642.

Matsunaga T and Nagata T (1995). *In vivo* 11B NMR observation of plant tissue. Analytical Science 11, 889-892.

Maud, R. R. (1972). Geology, geomorphology, and soils of central County Hindmarsh (Mount Compass-Milang), South Australia. C.S.I.R.O., Division of Soils, Soil Publication No. 29.

McDougall G J, Stewart D and Morrison I M (1994). Oxidation of coniferyl alcohol to lignin-like products by tobacco xylem cell walls. Phytochemistry 37, 683-688.

McIlrath W J (1965). Mobility of boron in several dicotyledonous species. Botanical Gazette 126, 27-30.

McIlrath W J and Palser B F (1956). Responses of tomato, turnip, and cotton to variations in boron nutrition. I. Physiological responses. Botanical Gazette 118, 43-52.

Mezuman U and Keren R (1981). Boron adsorption by soils using a phenomenological adsorption equation. Soil Science Society of America. Journal 45, 722-726.

Moewus F (1950). Zur physiologie und biochemie der selbsterilität bei *Forsythia*. Biologisches Zentralblatt 69, 181-197.

Morgan V (1980). Boron geochemistry. *In* Supplement to Mellor's Comprehensive Treatise on Inorganic and Theoretical Chemistry, volume V, Part A. Boron-Oxygen Compounds. Longman, New York.

Mozafar A (1993). Role of boron in seed production. *In* Boron and its Role in Crop Production. Ed. Gupta U C. CRC Press, Boca Raton, Florida. pp 186-206.

Mozafar A, Schreiber P and Oertli J J (1993). Photoperiod and root-zone temperature: Interacting effects on growth and mineral nutrients of maize. Plant and Soil 153, 71-78.

Myers L F, Lipsett J and Kirchner R (1983). Response of rapeseed (*Brassica napus* L.) to phosphorus, boron and lime on an acid soil near Canberra. Australian Journal of Agricultural Animal Husbandry 23, 172-177.

Nable R O (1988). Resistance to boron toxicity amongst several barley and wheat cultivars - A preliminary examination of the resistance mechanism. Plant and Soil 112, 45-52.

Nable R O, Cartwright B and Lance R C M (1990). Genotypic differences in boron accumulation in barley: Relative susceptibilities to boron deficiency and toxicity. *In.* Genetic aspects of plant mineral nutrition. Ed. El Bassam *et al.* Kluwer Academic Publishers, Netherlands. pp. 243-251.

Nable R O, Lance R C M and Cartwright B (1990). Uptake of boron and silicon by barley genotypes with differing susceptibilities to boron toxicity. Annals of Botany 66, 83-90.

Neales T F (1960). Some effects of boron on root growth. Australian Journal of Biological Science 13 (3), 232-249.

Northcote, K. H. (1971). A factual key for the recognition of Australian soils. Third edition. (Rellim Technical Publications: Glenside).

Norvell W A and Welch R M (1993). Growth and nutrient uptake by barley (*Hordeum vulgare* L cv Herta): Studies using an-(2-Hydroxyethyl) ethylenedinitrilotrilotriacetic acid-buffered nutrient solution technique. I. Zinc ion requirements. Plant Physiology 101, 619-625

Odhnoff C (1957). Boron deficiency and growth. Physiologia Plantarum 10, 984-1000.

Oertli J J (1993). The mobility of boron in plants. Plant and soil 155/156, 301-304.

Oertli J J (1969). Characteristics of boron uptake and loss in barley leaves. Agrochimica 13, 212-219.

Oertli J J (1963). The influence of certain environmental conditions on water and nutrient uptake and nutrient distribution in barley seedlings with special reference to boron. Advancing Frontiers of Plant Sciences 6, 55-85.

Oertli J J and Grgurevic E (1975). Effect of pH on the absorption of boron by excised barley roots. Agronomy Journal 67, 278-280.

Oertli J J and Kohl H C (1961). Some considerations about the tolerance of various plant species to excessive supplies of boron. Soil Science 92, 243-247.

Oertli J J and Richardson W F (1970). The mechanism of boron immobility in plants. Physiologia Plantarum 23, 108-116.

Oertli J J and Roth J A (1969). Boron supply of sugar beet, cotton and soybean. Agronomy Journal 61, 191-195.

O'Malley D M, Whetten R, Bao W, Chen C L and Sederoff R (1993). The role of laccase in lignification. Plant Journal 4, 751-757.

O'Neill M A, Warrenfeltz D, Kates K, Pellerin P, Doco T, Darvill A G and Albersheim P (1996). Rhamnogalacturonan-II, a pectic polysaccharide in the walls of growing plant cells, forms a dimer that is covalently cross-linked by a borate ester. Journal of Biological Chemistry 271, 22923-22930.

Palser B F and McIlrath W J (1956). Responses of tomato, turnip, and cotton to variations in boron nutrition. II. Anatomical responses. Botanical Gazette 118, 53-71.

Parks W L and White J L (1952). Boron retention by clay and humus systems saturated with various cations. Soil Science Society of America. Proceedings 16, 298-300.

Parr A J and Loughman B C (1983). Boron and membrane function in plants. *In* Metals and Micronutrients. Uptake and Utilisation by Plants. Ed. Robb D A and Pierpoint W S. Academic Press, New York. pp. 87-107.

Pollard A S, Parr A J and Loughman B C (1977). Boron in relation to membrane function in higher plants. Journal of Experimental Botany 28, 831-841.

Pope D R and Munger H M (1953). The inheritance of susceptibility to boron deficiency in celery. Proceedings of the American Society of Horticultural Science 61, 481-486.

Porter P M (1993). Canola response to boron and nitrogen grown on the southeastern coastal plain. Journal of Plant Nutrition 16 (12), 2371-2381.

Power P P and Woods W G (1997). The chemistry of boron and its speciation in plants. Plant and Soil 193, 1-13.

Raven J A (1980). Short and long-distance transport of boric acid in plants. New Phytologist 84, 231-249.

Rawson H M (1996). The developmental stage during which boron limitation causes sterility in wheat genotypes and the recovery of fertility. Australian Journal of Plant Physiology 23, 709-717.

Rerkasem B and Cheng C (1993). Effects of boron on pollen viability in wheat. Plant and Soil 156, 313-315.

Rerkasem B and Jamjod S (1997). Genotypic variation in plant response to low boron and implications for plant breeding. Plant and Soil 193, 169-180.

Rerkasem B, Bell R W and Loneragan J F (1990). Effect of seed and soil boron on early seedling growth of black and green gram. *In* Plant Nutrition-Physiology and Application. Ed. van Beusichem. M L.Kluwer Academic Publishers, Dordrecht, The Netherlands. pp 281-285.

Rerkasem B, Bell R W and Lordkaew S and Loneragan J F (1993). Boron deficiency in soybean (*Glycine max* (L.) Merr.). peanut (*Arachis hypogea* L.) and black gram (*Vigna mungo* (L.) hepper): Symptoms in seeds and differences among soybean cultivars in susceptibility to boron deficiency. Plant and Soil 150, 289-294.

Rerkasem B, Netsangtip R, Bell R W and Loneragan J F (1988). Comparative species responses to boron on a Typic Tropaqualf in Northern Thailand. Plant and Soil 106, 15-21.

Rerkasem B, Netsangtip R, Lordkaew S and Cheng C (1993). Grain set failure in boron deficient wheat. Plant and Soil 155/156, 309-312.

Reuter D J, Edwards D G and Wilhelm N S (1997). Temperate and tropical crops. *In* Plant Analysis, an Interpretation Manual. Ed. Reuter D J and Robinson J B. 2nd edition. CSIRO publications, Australia. pg 105.

Robbertse P J, Lock J J, Stoffberg E and Coetzer L A (1990). Effect of boron on directionality of pollen tube growth in *Petunia* and *Agapanthus*. South African Journal of Botany 56, 487-492.

Robertson G A and Loughman B C (1974). Response to B deficiency: A comparison with responses produced by chemical methods of retarding root elongation. New Phytologist 73, 821-832.

Roldán M, Belver A, Rodríguez-Rosales P, Ferrol N and Donaire J P (1992). *In vivo* and *in vitro* effects of boron on the plasma membrane proton pump of sunflower roots. Physiologia Plantarum 84, 49-54.

Römheld V (1986). pH changes in the rhizosphere of various crop plants, in relation to the supply of plant nutrients. Potash Review, Subject 6, No. 12. pp 1-8.

Saarela, I. (1985). Plant available boron in soils and boron requirements of spring oilseed rape. Annales Agriculturae Fenniae 24:183-265.

Sakal R, Singh A P, Singh R B snd Bhogal N S (1991). Relative susceptibility of some important varieties of sesamum and mustard to boron deficiency in calcareous soils. Fertilizer News 36, 43-50.

Schaller G (1987). pH changes in the rhizosphere in relation to the pH-buffering of soils. Plant and Soil 97, 439-444.

Schmucker T (1933). Zur Blutenbiologie Tropischer *Nymphaea* Arten. II. Bor, alsentscheidener Faktor. Planta 18, 641-650.

Schmucker T (1934). Über den Einfluss von Borsäure auf Pflanzen, insbesondere keimende Pollekörner. Planta 23, 264-283.

Schon M K, Novacky A and Blevins D G (1990). Boron induces hyperpolarization of sunflower root cell membranes and increases membrane permeability to K^+. Plant Physiology 93, 566-571.

Scott L E (1944). Boron nutrition of grapes. Soil Science 57, 55-65.

Scott H D, Beasley S D and Thompson L F (1975). Effect of lime on boron transport to and uptake by cotton. Soil Science Society of America. Proceedings 39, 1116-1121.

Scott L E and Schrader A L (1947). Effect of alternating conditions of boron nutrition upon the growth and boron content of grape vines in sand culture. Plant Physiology 22, 526-537.

Seresinhe P S J W and Oertli J J (1991). Effects of boron on growth of tomato cell suspensions. Physiologia Plantarum 81, 31-36.

Shelp B J (1993). Physiology and biochemistry of boron in plants. *In* Boron and its Role in Crop Production. Ed. Gupta U C. CRC Press, Boca Raton, Florida. pp 53-85.

Shelp B J (1988). Boron mobility and nutrition in broccoli (*Brassica oleracea* var. *italica*). Annals of Botany 61, 83-91.

Shelp B J, Marentes E, Kitheka A M and Vivekanandan P (1995). Boron mobility in plants. Physiologia Plantarum 94, 356-361.

Shelp B J, Penner R and Zhu Z (1992). Broccoli (*Brassica oleracea* var. *italica*) cultivar response to boron deficiency. Canadian Journal of Plant Science 72, 883-888.

Shelp B J and Shattuck V I (1987). Boron nutrition and mobility, and its relation to hollow stem and the elemental composition of greenhouse grown cauliflower. Journal of Plant Nutrition 10(2), 143-162

Shelp B J, Vivekanandan P, Vanderpool R A and Kitheka A M (1996). Translocation and effectiveness of foliar-applied boron in broccoli plants of varying boron status. Plant and Soil 183, 309-313.

Shkolnik M Y (1984). Boron. *In* Trace Elements in Plants. Elsevier, Amsterdam, Oxford, New York, Tokyo. pp 68-109.

Shorrocks V M (1997). The occurrence and correction of boron deficiency. Plant and Soil 193, 121-148.

Sidhu R J K and Malik C P (1986). Metabolic role of boron in germinating pollen and growing pollen tubes. *In* Biothechnology and Ecology of Pollen. Eds. Mulcahy D L, Mulcahy B and Ottaviano G. Springer-Verlag. pp 373-378.

Sillanpää M (1982). Micronutrients and the nutrient status of soils: a global study. Food and Agriculture Organization of the United Nations soil bulletin 48. pg 46-54.

Singh H M, Sinha S D and Prasad R B (1976). Effect of boron on seed setting in wheat under North Bihar conditions. Indian Journal of Agronomy 21, 100-101.

Singh R P, Sharma Y P, Awasti R P (1990). Influence of different cultural practices on premature cracking of pomegranate. Progressive Horticulture 22, 92-96.

Singh S S (1964). Boron adsorption equilibrium in soils. Soil Science 98, 383-387.

Sisler E C, Dugger W M Jr., and Gauch H G (1956). The role of boron in the translocation of organic compounds in plants. Plant Physiology 31, 11-17.

Smith F W and Loneragan J F (1997). Interpretation of plant analysis: concepts and principles. *In* Plant Analysis. An Interpretation manual. Eds. Reuter D J and Robinson J B. Inkata Press, Melbourne. pp 1-12.

Smith P E (1966). Citrus nutrition. *In* Nutrition of Fruit Crops. Ed. Childers N F. Horticultural Publications. Rutgers University, New Brunswick, New Jersey. pp 174-207.

Smithson J B and Heathcote R G (1974). Boron deficiency on cotton in northern Nigeria. II. The effect of variety. Experimental Agriculture 10, 209-218.

Spurr A R (1957). The effect of boron on cell-wall structure in celery. American Journal of Botany 44, 637-650.

Stanley R G (1971). Pollen chemistry and tube growth. *In* Pollen-development and physiology. Ed. Heslop-Harrison J S. Butterworth and Company, London. pp 131-155.

Su C and Suarez D L (1995). Coordination of adsorbed boron: A FTIR spectroscopic study. Environment and Science Technology 29, 302-311.

Sylvester-Bradley R and Makepeace R J (1984). A code for stages of development in oilseed rape. Aspects of Applied biology 6, 399-419.

Tagliavini M, Scudellari D, Marangoni B, Bastianel A, Franzin F and Zamborlini M (1992). Leaf mineral composition of apple trees: sampling date and effects of cultivar and rootstock. Journal of Plant Nutrition 15, 605-619.

Tanada T (1983). Localization of boron in membranes. Journal of Plant Nutrition 6, 743-749.

Tanaka H (1967a). Boron absorption by plant roots. Plant and Soil 27, 300-302.

Tanaka H (1967b). Boron absorption by crop plants as affected by other nutrients of the medium. Journal of Soil Science and Plant Nutrition 13, 41-44.

Tao R, Uratsu S L and Dendekar A M (1995). Sorbitol synthesis in transgenic tobacco with apple cDNA encoding NADP-dependent sorbitol-6-phosphate dehydrogenase. Plant and Cell Physiology 36(3), 525-532.

Tehrani G, Munger H M, Robinson R W and Shannon S (1971). Inheritance and physiology of response to low boron in red beet (*Beta vulgaris* L.). Journal of the American Society of Horticultural Science 96, 226-230.

Thellier M and Ayadi A (1967). Contribution à l'étude d'interactions ioniques phosphate-borate lors de l'absorption par la *Lemna minor* L. Academie des Sciences (Paris). Comptes Rendus Hebdomadaires des Seances. Serie D. Sciences Naturelles 265, 1940-1943.

Thellier M, Dubal Y and Demarty M (1979). Borate exchanges of *Lemna minor* L. as studied with the help of the enriched stable isotopes of a (n, a) nuclear reaction. Plant Physiology 63, 283-288.

Thellier M and le Guiel J (1967). Etude, grace à l'isotope stable ^{10}B de l'absorption du borate par la *Lemna minor* L. Academie des Sciences (Paris). Comptes Rendus Hebdomadaires des Seances. Serie D. Sciences Naturelles 264, 294-295.

Tollenaar D (1969). Boron deficiency in sugar cane, oil palm and other monocotyledons on volcanic soils of Ecuador. Netherlands Journal of Agricultural Science 17, 81-91.

Trip P, Krotkov G and Nelson C D (1963). Biosynthesis of mannitol-C^{14} from $C^{14}O_2$ by detached leaves of white ash and lilac. Canadian Journal of Botany 41, 1005-1010.

Ulrich A (1952). Physiological bases for assessing the nutritional requirements of plants. Annual Review of Plant Physiology 3, 207-228.

van de Venter and Currier H B (1977). The effect of boron deficiency on callose formation and ^{14}C translocation in bean (*Phaseolus vulgaris* L.) and cotton (*Gossypium hirsutum* L). American Journal of Botany 64 (7), 861-865.

van Goor B J and van Lune P (1980). Redistribution of potassium, boron, magnesium and calcium in apple trees determined by an indirect method. Physiologia Plantarum 48, 21-26.

Vasil I K (1963). Effect of boron on pollen germination and pollen tube growth. *In* Pollen Physiology and Fertilization. Ed. Linskens H F. North-Holland Publishing Company, Amsterdam. pp 107-119.

Vaughan A K F (1977). The relation between the concentration of boron in the reproductive and vegetative organs of maize plants and their development. Rhodesian Journal of Agricultural Research 15, 163-170.

Visser T (1955). Meded. Landbouwshogesch. Wageningen 55, 1-68.

Walker J C, Jolivette J P and Hare W W (1945). Varietal susceptibility in garden beet to boron deficiency. Soil Science 59, 461-464.

Wall J R and Andrus C F (1962). The inheritance and physiology of boron response in the tomato. American Journal of Botany 49, 758-762.

Warington K (1923). The effect of boric acid and borax on the broad bean and certain other plants. Annals of botany 37, 629-672.

Wear J I and Patterson R M (1962). Effect of soil pH and texture on the availability of water-soluble boron in the soil. Soil Science Society of America. Proceedings 26, 344-346.

Wei Y, Bell R W, Yang Y, Ye Z and Wang K (1998). Prognosis of boron deficiency in oilseed rape (*Brassica napus*) by plant analysis. Australian Journal of Agricultural Research 49, 867-874.

Weinberger P and Yee D (1984). The influence of nitrogen sources on root-mediated changes in substrate pH. Canadian Journal of Botany 62, 161-162.

Weir R G and Cresswell G C (1995). Plant nutrient disorders. IV. Pasture and field crops. Inkata Press, Melbourne.

Welch R M, Allaway W H, House W A and Kubota J (1991). Geographic distribution of trace element problems. *In* SSSA Book series, no. 4. Eds. Mortveldt *et al.* Micronutrients in Agriculture. 2nd ed. pp 36.

Whittington W J (1958). The role of boron in plant growth. II. The effect on growth of the radicle. Journal of Experimental Botany 10 (28), 93-103.

Whittington W J (1957). The role of boron in plant growth. I. The effect on general growth, seed production and cytological behaviour. Journal of Experimental Botany 8, 353-367.

Wild A and Mazaheri A (1979). Prediction of the leaching rate of boric acid under field conditions. Geoderma 22, 127-136.

Wildes R A and Neales T F (1971). The absorption of boron by disks of plant storage tissues. Australian Journal of Biological Science 24, 873-884.

Williams R F (1948). The effect of phosphorous supply on the rate of intake of phosphorous and nitrogen and upon certain aspects of phosphorous metabolism in gramineous plants. Australian Journal of Science Research. B1, 333-361.

Willis L G (1945). Defective strawberry fruit corrected by borax. Better Crops with Plant Food 29, 39-40.

Wilson C M, Lovvorn R L and Woodhouse W W Jr (1951). Movement and accumulation of water-soluble boron within the soil profile. Agronomy Journal 43, 363-367.

Wilson M R and Walker N A (1988). The transport and metabolism of urea in *Chara australis*. I. Passive diffusion, specific transport and metabolism of urea, thiourea and methylurea. Journal of Experimental Botany 39 (203), 739-751.

Wilson M R, O'Donohue S I and Walker N A (1988). The transport and metabolism of urea in *Chara australis*. III. Two specific transport systems. Journal of Experimental Botany 39 (203), 763-774.

Wright J W, Lemmien W A and Bright J N (1969). Early growth of ponderosa pine ecotypes in Michigan. Forest Science 15, 121-129.

Yamauchi T, Hara T and Sonoda Y (1986). Distribution of calcium and boron in the pectin fraction of tomato leaf cell wall. Plant Cell Physiology 27, 729-732.

Yang Y, Xue J, Ye Z and Wang K (1993). Responses of rape genotypes to boron application. Plant and Soil 156, 321-324.

Yang Y, Xue J, Ye Z and Wang K (1994). Management of B and Zn in oilseed crops in China. Annual Report of ACIAR project 9120. pp 231-237.

Yermiyahu U, Keren R and Chen Y (1995). Boron sorption on composted organic matter. Soil Science Society of America. Journal 52, 1309-1313.

Zhang X, Shen Z and Shen K (1994). The effect of boron on the development of floral organs and seed yield of rape. Acta Pedologica Sinica 31, 146-151.

Zarcinas B A, Cartwright B and Spouncer L R (1987). Nitric acid digestion and multi-element analysis of plant material by inductively coupled plasma spectrometry. Communications in Soil Science and Plant Analyses 18, 131-146.