Chapter 1

Literature review

1.1 Absorption of mineral nutrients by plant roots

Plants absorb inorganic nutrients from the soil via their roots. Ions and other low-molecular-weight solutes in soil solution are generally moved to the root surface by diffusion or mass flow. Nutrients arrive at the root surface and are taken up into the roots. There are two parallel pathways of ions and water across the root. One is passing through the apoplasm (cell walls and intercellular spaces) and another is passing from cell to cell in the symplasm (Marschner, 1995). The apoplasmic pathway of ions is restricted by the Casparian band in the walls of the endodermal cells (Peterson *et al.*, 1993), exodermis or suberization of rhizodermis depending on the plant species and the root zone (Enstone and Peterson, 1992). In the symplasmic pathway, radial transport across cell walls that connect the cytoplasm of neighbouring cells occurs via plasmodesmata (Marschner, 1995). Nevertheless, mineral ion transport across the roots may involve a system of membrane transport proteins in the plasmalemma. The function of nutrient transporters is associated with proton pumps through cotransporter or antiporter activities. The specificity of these transporter proteins controls the entry of ions into the symplasm of plant roots (Clarkson and Lüttge, 1991).

The rate of nutrient uptake depends on the quantity of root surface and the uptake properties of this surface (Marschner, 1995). The effective absorbing root surface can be enlarged by root hairs (Bates and Lynch, 1996) which have greatest effect on absorption of ions that diffuse slowly in soil.

1.2 Nutrient transport within plants

Once taken up into the roots, nutrient ions are transported to different parts of the plant via two transport systems, the xylem and the phloem.

1.2.1 Xylem transport

Xylem, principal water-conducting tissue, is a complex tissue, consists of several different types of cells, living and nonliving (Esau, 1898). The most characteristic components are dead tracheary elements (Thompson and Schulz, 1999) which act both in transport and support. Further support is provided by fibers, and parenchyma, the latter is required for vital function, such as the storage of starch (Esau, 1898). Sap flow in the xylem is driven by the gradient in hydrostatic pressure (root pressure) and by the gradient in the water potential (Burgess *et al.*, 2000), created by water loss at the leaf surface by evapotranspiration. Primary xylem translocation is directed mainly to the sites of highest transpiration, which are not usually the sites of highest nutrient requirements (Pate *et al.*, 1975). Therefore, solute flow in the xylem from the roots to the shoots is unidirectional (Marschner, 1995). Xylem plays the dominant role in the flow of water through the plant and in nutrient delivery from the root to the shoot (Schurr, 1998).

Ions and organic solutes are released into fully differentiated non-living xylem vessels or tracheid after radial transport in the symplasm into the stele. The release of ions and organic solutes for xylem loading, requires a respiratory-dependent proton pump at the plasma membrane of the living parenchyma cells. This proton pump transfers protons into the apoplasm of xylem vessels and may act indirectly by reabsorption as a driving force for the secretion of cations. For anions, they may be secreted by cotransport with the protons or along the electrical potential gradient formed by the proton pump (DeBoer *et al.*, 1983). Wegner and Raschke (1994) suggested that xylem loading is similar to the ion flux that occurs when guard cells at closing. Ions are released into the xylem sap through ion channels in a process which is thermodynamically passive. So, there are at least two regulated membrane transport processes involve in radial transport of mineral nutrients from the external solution into the xylem (Marschner, 1995). The mechanism of xylem loading is regulated separately which provides the possibility of controlling selectivity and the rate of long-distance transport to the shoot, for example as a feedback regulation depending on shoot demand (Marschner, 1995).

1.2.2 Phloem transport

Phloem is the principal food-conducting tissue of vascular plants. The basic components of the phloem are the sieve elements, transfer cells, parenchyma cells, fibers, and sclereids (Esua, 1898). The role of phloem is to deliver photosynthates, mineral nutrients and diverse macromolecules to heterotrophic plant tissues (Kulikova *et al.*, 2003). Phloem transport is an important component in cycling of mineral nutrients between shoots and roots, particularly the transport to parts receiving large

amounts of assimilates e.g. young growing tissues, fruits, seeds and storage organs (Van Goor and Van Lune, 1980) because phloem translocation is independent of transpiration (Pate *et al.*, 1975). In contrast to the xylem, long-distance transport in the phloem is bidirectional probably in adjacent sieve tube cells. However, mineral elements and organic solutes are transferred between the xylem and phloem by exchange processes (Pate and Gunning, 1972) facilitated by metabolic active transfer cells with extended wall and enhanced plasmalemma surface (Lampinen and Noponen, 2003).

Phloem mobility consists of different stages of transport: the loading of the sieve tubes in the source organ, the translocation itself, and unloading in the sink organ (Van Goor and Van Lune, 1980). Phloem transport mechanism was proposed by Münch (Münch, 1930 cited by Marschner, 1995) in a pressure flow hypothesis. Solutes are moved long-distance through the sieve tubes by mass flow and driven by a pressure gradient between source and sink regions of the plant (Münch, 1930 cited by Oparka and Santa Cruz, 2000). Due to the concentrated of solutes in the phloem, water is sucked into the phloem and increased hydrostatic pressure, whereas utilization of assimilates in growth, storage, and respiration results in lowered concentration and hence lowered hydrostatic pressure where these activities are going on. Because of the gradients of hydrostatic pressure so created, solution moves from regions of synthesis to regions of utilization through the sieve tubes of the phloem (Crafts, 1956 cited by Zimmermann, 1960).

1.2.3 Retranslocation of mineral nutrients

Retranslocation of mineral nutrients must take place in the phloem because of the limitation of the gradient in xylem water potential (Marschner, 1995). So, nutrient retranslocation can be named phloem mobility, the extent of which may vary between elements. The three categories of phloem mobility are shown in Table 1.1. Mobility may differ between plant species as discussed later for B.

Table 1.1 Characteristic differences in mobility of mineral nutrients in the phloem.

High Mobility	Intermediate Mobility	Low Mobility
Potassium	Iron	Calcium
Magnesium	Zinc	Manganese
Phosphorus	Copper	
Sulfur	Boron	
Nitrogen (amino-N)	Molybdenum	
Chlorine		
Sodium		

Source: Marschner (1995)

Retranslocation is based on a range of different physiological and biochemical processes: utilization of mineral nutrients stored in vacuoles, break down of storage proteins, or, finally, transforming structurally bound mineral nutrients into a mobile form (Marschner, 1995). The pattern of distribution and the rate and the extent of re-cycling and remobilization of each nutrient can vary widely with the nutrient, environmental conditions, and plant nutrient status, species, and stage of

development. For example, partitioning and remobilization of nutrients are involved in the rapid developmental changes occurring during the plant's life cycle of annual species (Loneragan *et al.*, 1980) while in perennial species are related to particular phenological stages (Conradie, 1991; Nambiar and Fife, 1991; Switzer and Nelson, 1972; Miller, 1986; Turner and Lambert, 1986). So, partitioning and remobilization of mineral nutrients is important for plant growth, especially in the following stages: seed germination, periods of insufficient supply to the roots during vegetative growth, reproductive growth, and the period before leaf drop of perennials (Marschner, 1995).

1.3 Boron

1.3.1 Role of boron in plants

Boron is an essential element for higher plants (Warrington, 1922, cited by Marschner, 1995). Boron deficiency affects many processes in plants, including anatomical, physiological and biochemical alterations which influence plant growth and development (Shelp *et al.*, 1995).

From recent reports, specific roles of boron in plants have been identified but speculation remains as to some functions that have been proposed. Detailed studies on cell wall fractions show that B as borate is a cross-linking molecule with pectin polysaccharides, forming a B-rhamnogalacturonan-II (RG-II-B) complex (Hu and Brown, 1994; Kobayashi *et al.*, 1996; Matoh *et al.*, 1996; O'Neill *et al.*, 1996; O'Neill *et al.*, 2004). So, the first B deficiency symptoms are often abnormalities in the cell wall and middle lamella arrangement due to a loss in borate cross-linking of pectin (Matoh *et al.*, 1992; Brown and Hu, 1994; Hu and Brown, 1994; Brown *et al.*, 2002). Most cellular B is localized as RG-II-B in the cell wall [up to 98% in tobacco (Matoh

et al., 1992) or 96-97% in squash (Hu and Brown, 1994)]. However, B may be present in plant cells as free boric acid and borate, or more loosely bound to other ligands (Goldbach *et al.*, 2000).

Turning to less clearly understood functions, evidence from the inhibition and recovery of proton release on B withdrawal and restitution in plant culture medium suggests that B is involved in membrane processes (Blevins and Lukaszewski, 1998). Moreover, B–complexing by reaction with hydroxyl-rich compounds was proposed as the membrane constituent that was exhibited to maintain the structural integrity of plasma membrane (Goldbach *et al.*, 1990; Cakmak *et al.*, 1995; Brown *et al.*, 2002). Boron deficiency appeared to affect membrane potential (hyperpolarisation) (Schon *et* al., 1990) and induced membrane damage resulting in increased K leakage and sugars (Cakmak *et al.*, 1995), stimulated H^+ efflux and reduced ATPase (Goldbach *et al.*, 1990). Boron may also affect the stability of the plasma membrane. In addition, B may be involved in metabolic pathways by binding apoplastic proteins to *cis*-hydroxyl groups of cell walls and membranes (Blevins and Lukaszewski, 1998).

1.3.2 Boron deficiency symptoms in plants

The occurrence of B deficiency is prevalent among crop species. Incidences of B deficiency is common, especially in the tropics where the soils have very low B contents or are highly alkaline so that B is only sparingly available to plants (Shorrocks, 1997). Deficiency symptoms may be observed from vegetative until reproductive growth of plants. These include stem crack in celery, hollow stem disorder in broccoli (Shelp, 1988), and grain set failure in wheat (*Triticum aestivum*) (Cheng and Rerkasem, 1993). Boron deficiency is also widespread in tropical food

legumes. Boron deficiency can occur during vegetative growth e.g. by reducing leaf blade elongation of green gram (Bell *et al.*, 1990a), but is particularly severe in reproductive growth affecting seed yield and quality of black gram, green gram, soybean, sunflower, peanut (Rerkasem *et al.*, 1988; Bell *et al.*, 1990b) and many other crop species. As the result of B deficiency, crop yield may be lost or crop quality is ruined or both. Boron application can increase yield and quality of crops growing on low B soils (Shorrocks, 1997). Reproductive stage is generally more susceptible to low B supply than vegetative growth so yield of crops may be depressed without visible B deficiency symptoms during the vegetative stage. For example, B deficiency was reported to depress seed yield of sunflower (*Helianthus annuus*) and black gram (*Vigna mungo*) and seed quality of green gram (*Vigna radiata*), soybean (*Glycine max*) and peanut (*Arachis hypogaea*), which were grown in northern Thailand (Rerkasem *et al.*, 1988). Dell and Huang (1997) concluded that shortage of B reduced male fertility of flowering plants by damaging microsporogenesis and pollen tube growth. Moreover, low B can affect embryogenesis leading to seed abortion or incomplete embryo formation and malformed fruit (Dell and Huang, 1997).

The correction of B deficiency can be done by applying B fertilizer. An understanding of phloem B movement within plants is useful in crop production for more precision in diagnosis as well as for fertilizer management. The idea that deficiency symptom should be looked for in young tissues and growing points is applicable only in B immobile species, and may be less appropriate in B mobile species. Sampling for tissue analysis should also be similarly discriminating. In species where B has been reported to be immobile, e.g. corn, wheat, alfafa and some

vegetable crops, B fertilizer should be applied in such a way that supply is available at all stages of plant growth. Foliar application to these plants can correct B deficiency only in the current tissue thus it has minimal effect on new growth. Consequently, B must be directly applied to specific organs every time B is required. Foliar B application, however, has been successful for B deficiency management in many species of fruit trees which now been shown to translocate B in their phloem (Brown and Hu, 1998). Moreover, understanding the mechanism for B mobility may lead to new ways to improved B efficiency in crops. This has been illustrated by the knowledge about the relationship between mobility and sugar alcohols that has led to genetic engineering to make tobacco more B efficient by introduction of the sorbitol synthesis gene (Bellaloui *et al.*, 1999; Brown *et al.*, 1999).

1.4 Boron mobility in plants

1.4.1 Boron absorption

Boron absorption in plants can be both active and passive uptake. Bowen (1972) reported a fraction of B uptake was regulated metabolically, correlating with the concentration of the $B(OH)_4^-$ in sugarcane leaf tissue, meristematic tissue and excised root. However, Hu and Brown (1997) concluded that uptake of B was most likely a passive, non-metabolic absorption of boric acid. They suggested that the passive absorption rate of B differs between species due to the following: (i) B uptake is under partial metabolic control, and may include an active exclusion mechanism; (ii) root exudation of B complexes 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.

1.4.2 Xylem and phloem transport

Boron is transported from the root to the shoot of plants in the same way as the other nutrient elements. It is moved in the xylem along with the movement of water due to transpiration (Raven, 1980). Data on the direct analysis of nutrient concentration in phloem sap, movement of isotopes, development of deficiency symptoms, measurement of the rate of influx of an element during fruit development, comparison of measured contents in different plant parts, and determination of concentration gradients in plants from older to younger leaves (Van Goor and Van Lune, 1980; Marschner, 1995; Smith and Loneragan, 1997), have been used to explore the retranslocation or phloem mobility of nutrient elements.

1.4.3 Retranslocation of boron

From references over many years, it has generally been reported that B cannot be retranslocated via the phloem. For example, in tomato (*Lycopersicon esculentum*) the first symptoms of B toxicity appeared in the form of chlorosis at the margins and tips of leaves with high B concentration in contrast to the other parts (Oertli, 1993). Boron concentration was found to be much lower in phloem exudate of broccoli (*Brassica oleracea* var. *italica*) than in leaves (Shelp, 1987). However, evidence suggesting the possibility of B mobility in the phloem began to appear in the 1990's. After foliar B spray, Hanson (1991) observed a decrease in the B concentration of treated leaves to similar levels in untreated leaves of apple (*Malus domestica*), pear (*Pyrus communis*), plum (*Prunus domestica*) and cherry (*Prunus ceasus*). Delgado *et al.* (1994) showed that the B concentration of new tissues increased after applying B

to leaves of olive *(Olea europaea*). Moreover, it was found that the amount and proportion of ^{10}B increased in the root system of Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*) seedlings after applying ^{10}B to their shoots (Lehto *et al.*, 2000). Subsequently, they applied ^{10}B to mature leaves of deciduous forest seedlings and found that ^{10}B could be transported to new leaves in *Sorbus aucuparia*, *Alnus incana*, *Ulmus glaba*, *Fraxinus excelsior*, *Betula pubescens* and *Larix sibirica* (Lehto *et al.*, 2004). In an early study, Campbell *et al.* (1975) tested a hypothesis on phloem mobility in peanut by measuring B in the fruit that developed while buried in B-free medium. Finding this the non-transpiring fruit was able to develop in the B-free sand, it was concluded that B was phloem mobile in peanut. However, a more direct evidence of this is still lacking.

1.4.4 Mechanism of boron mobility in plants

The formation of *cis*-diols, such as B-polyol complexes, was proposed as the reason for B being transported in the phloem of some plants (Raven, 1980) and this was later established to be the mechanism of B mobility within plants (Brown and Hu, 1996). Brown and Hu (1996) showed that B was translocated from treated leaves in sorbitol-rich species including, almond (*Prunus amygdalus* syn. *P. dulcis*), apple (*Malus domestica*) and nectarine (*Prunus persica* var. *nectarine*) by the formation of B-sorbitol complexes; while in sorbitol-poor species, including fig (*Ficus carica*), pistachio (*Pistacia vera*) and walnut (*Juglans regia*), there was no evidence of B mobility. Hu *et* al. (1997) analysed the phloem sap of celery (*Apium graveolens*) and the floral nectar of peach (*Prunus persica*). Their results suggested that B existed in the phloem sap of celery as mannitol-B-mannitol complexes and a mixture of sorbitol-

B-sorbitol, fructose-B-fructose or sorbitol-B-fructose was found in peach nectar. Boron mobility had previously been noted in these species (Shelp *et al.*, 1995) but the mechanisms had not been identified. Brown *et al.* (1999) proved that sorbitol directly influences the mobility of B in tobacco (*Nicotiana tabacum*). When they inserted the sorbitol synthesis gene into tobacco, B mobility was enhanced in transgenic plants and these plants showed increased growth and yield in contrast to wild-type tobacco grown with interrupted soil B supply and B supplied as foliar application to mature leaves. The other evidence from this group supported these findings. Bellaloui *et al.* (1999) concluded that sorbitol production of transgenic tobacco significantly affected both B uptake and B transport. Recently, Lehto *et al.* (2004) reported that the occurrence of polyols was related to B mobility in some deciduous forest species such as sorbitol in *Sorbus* and *Prunus*, mannitol in *Fraxinus* and pinitol in *Larix*.

Sugars or sugar alcohols are important transport substances in the phloem and several methods have been used to determine their concentrations. The most direct method is to analyse the sieve tube sap which can be collected after making an incision in the bark but it does not work in all plants. Alternatively, aphid stylets could be used on a large number of plant species but it can be difficult to find suitable aphids (Ziegler, 1975). In some plants, phloem sap can be easily collected, e.g. from the droplets that formed at the basipetal cut end of celery stems which had clearly identifiable vascular bundles, or the droplets of extrafloral nectar of peach (Hu *et al.*, 1997). However, care must be taken to avoid cross-contamination with xylem sap. Pate *et al.* (1974) tried to bleed phloem sap from 150 species of legume by cutting the distal tips of attached fruits as close as possible to the remaining style and found that bleeding only occurred in *Spartium*, *Genista*, *Lupinus* and *Jacksonia*. The exudates

obtained by this method have been confirmed to be uncontaminated with xylem sap due to high sugar content, high levels of amino compounds, very high levels of potassium and low level of calcium (Pate *et al.*, 1974). In addition, this paper also showed that phloem sap could be collected by cutting into other above-ground parts, e.g. young shoot tips of *Spartium and Jacksonia* and the vascular strands of the top three or four internodes of *Lupinus*. However, phloem sap from these parts probably is diluted by water from xylem and other tissues. The analysis of polyols in extracted plant tissues has been used by some workers to test the relationship between B mobility and the existence of these sugars. Letho *et al.* (2004b) found polyols in extracted leaves and stems of some deciduous trees which was related to B mobility, i.e., sorbitol in *Sorbus* and *Prunnus*, mannitol in *Fraxinus*. Similarly, Bellaloui *et al.* (1999) found the presence of sorbitol in extracted tissues of tobacco which was genetically engineered to produce this polyol. The determination of soluble carbohydrates from various plant materials can be made using HPLC (Hu *et al.*, 1997; Stangoulis, 1998) or GC-MS (Hu *et al.*, 1997; Stangoulis, 1998; Bellaloui *et al.*, 1999; Marsilio *et al.*, 2001; Lehto *et al.*, 2004b).

From all of the above studies it is now clear that the mobility of B in the phloem is varied among plant species.

1.5 Methods for studying nutrient mobility

A range of methods have been used to determine the phloem mobility of an element. The development of deficiency or toxicity symptoms is a simple method that does not require laboratory equipment. Symptoms of nutrient deficiency which regularly appear in growing tissue, such as in terminal buds or young leaves, and

toxicity symptoms that typically occur on mature leaves suggest the presence of phloem immobile elements (Marschner, 1995). For example, iron has been proposed to be phloem immobile because deficiency symptoms developed rapidly in young leaves and root tips (Hocking, 1980). During the development of their deficiencies, concentrations of nutrients also remain high in older leaves, e.g. in tomato (*Lycopersicon esculentum*) plants grown in excessive B supply (10 mg B $liter^{-1}$), the first symptoms of B toxicity appeared in the form of chlorosis at the margins and tips of leaves with high B concentration in contrast with the other parts (Oertli, 1993). Analysis of nutrient concentrations in phloem sap provides the direct evidence for determining phloem mobility (Van Goor and Van Lune, 1980; Shelp, 1987). The low concentration of calcium (Ca) in phloem sap is attributed to its failure to move from old leaves (Pate *et al.*, 1975). The much lower B concentration in phloem exudate than in leaves also showed the character of phloem immobility in broccoli (*Brassica oleracea* var. *italica*) (Shelp, 1987). However, it is very difficult to collect phloem exudate and the possibility of contaminating the phloem sap by cutting parenchyma cells and by substances from the apoplasm (Hayashi and Chino, 1990) is always a concern. Experiments using foliar nutrient application and measuring nutrient concentration in treated compared to non-treated tissues have also been used to study nutrient mobility. Hanson (1991) applied foliar B spray (500 mg.$liter^{-1}$) to leaves of apple (*Malus domestica*), pear (*Pyrus communis*), plum (*Prunus domestica*) and cherry (*Prunus ceasus*) and found that the B concentrations in treated leaves decreased to similar levels in nontreated leaves and the highest B concentrations were found in buds. Delgado *et al.* (1994) reported that application B to leaves of olive (*Olea europaea*) at anthesis increased the B concentration in leaf blades, petioles,

bark of bearing shoots, flowers and fruits. This suggests the possibility of B mobility in the phloem. The rate of influx of an element during fruit development was used as an indicator of relative mobility in redistribution in apple by Van Goor and Van Lune (1980) who found highly mobility of potassium (K) and B to magnesium (Mg) and Ca. Another precise approach to study phloem mobility is the use of labeled elements (radioactive or stable isotopes) to follow long-distance transport after application, e.g. the study of labeled phosphorus (^{32}P) and sodium (^{22}Na) in bean (Marschner, 1995). Due to the unavailability of unstable isotopes to trace the movement of B, this element is the least understood of all plant essential elements. It is now possible to utilize the stable isotope ^{10}B as a tracer of B since the introduction of inductively coupled plasma mass spectrometry (ICP-MS) in the mid 1980s (Brown *et al.*, 1992). This led to many reports investigating B mobility in plants. By using ^{10}B as a tracer to shoots of Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*) seedlings (Lehto *et al.*, 2000), it was found that the amount and proportion of ^{10}B increased in the root system of both species. They also found that ^{10}B was transported to new leaves in *Sorbus aucuparia*, *Alnus incana*, *Ulmus glaba*, *Fraxinus excelsior*, *Betula pubescens* and *Larix sibirica* after application of ^{10}B to mature leaves (Lehto *et al.*, 2004).

1.6 Concluding remarks

The lack of information for B retranslocation in tropical crop species led to the investigation in this thesis. As B mobility is species dependent, the variation among tropical species in its mobility in the phloem examined using a range of criteria in plants of different B status. The impact of B mobility in response to B deficiency and withdrawal of B may provide useful information for management of B fertilization in crop production in the future.