Chapter 2

LITERATURE REVIEW

2.1 The role of boron in plant growth

Boron is one of the seven recognized essential micronutrients required for the normal growth of most plants. This was first demonstrated by Warington *et al.*, (1923) in England for broad beans (Vicia faba), and has been confirmed for a number of other crops.

The role of B in plant metabolism has been a subject of hypotheses. Recent interpretations of the role of B are based on the formation of stable cis-diol borate complexes (Thellier *et al.*,1979). The primary effects of B are reflected by lignin biosynthesis and xylem differentiation (Lewis, 1980), membrane stabilization (Pilbeam and Kirkby, 1983), and altered enzyme reactions (Dugger, 1983). Boron is also required for cell elongation (Birnbaum *et al.*, 1974; Lovatt *et al.*, 1981) and for cell division (Cohen and Lepper, 1977; Kouchi, 1977). A role is reflected under conditions of B deficiency by a decrease in the number of grains per head in rice (Garg *et al.*, 1979), or even a total lack of fertilization in barley (Simojoki, 1972), or a yield depression in wheat (Li *et al.*, 1978; Rerkasem, 1989).

Boron deficiency reduces yield through reproductive growth failure. As the level of B supply increases in maize, grain set was improved. On the other hand, the vegetative growth, including the growth of silks and cob sheaths, either is not responded, or is even somewhat depressed (Figure 1) (Vaughan, 1977). The supply of B required for seed and grain production is usually higher than that needed for vegetative growth (Marschner, 1986). Thus, it was observed in the Heilongjiang case (China) that sterile plants had a normal vegetative growth (Li *et al.*, 1978).

Wheat yield may be limited by B deficiency through the failure of one or more of these reproductive processes: 1) development of reproductive organs, 2) pollen germination and fertilization, and 3) filling of seeds. The failure of each process may thus limit yield.

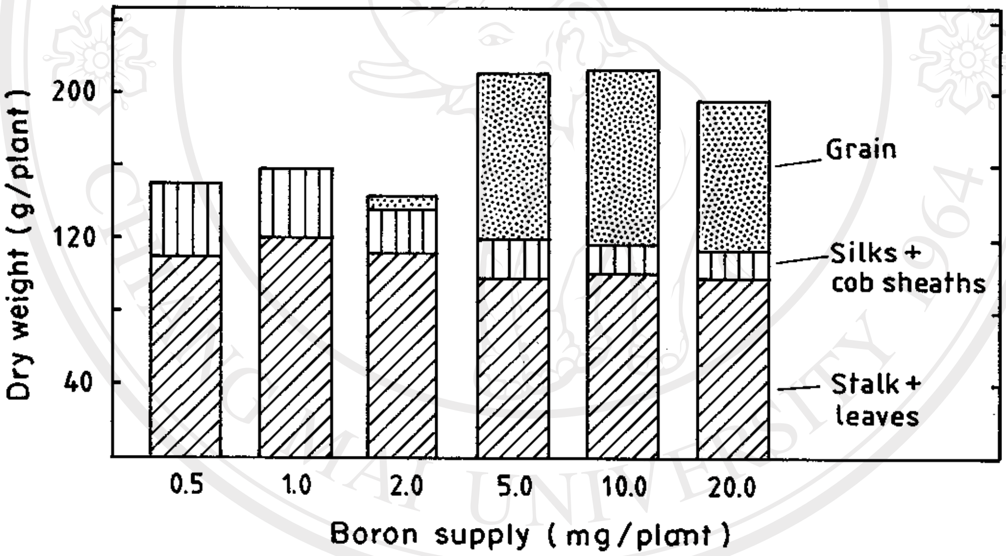

Figure 1. Effects of B supply on the production and distribution of dry matter in maize plants. (Based on Vaughan, 1977)

2.1.1 Development of reproductive organs

Boron may have both indirect and direct effects on reproductive growth. Indirect effects have been suggested to relate to the amount and/or sugar composition of nectar, whereby the flowers of species that rely on pollinating insects become more attractive to insects (Smith and Johnson, 1969; Erikson, 1979). Direct effects of B are reflected by the close relationship between B supply and pollen producing capacity of anthers, as well as viability of pollen grains (Agarwala *et al.*, 1981).

Boron deficient wheat may fail to set grains due to sterility of the male reproductive organs, especially poorly developed anther and abortion of pollen (Li *et al.*, 1978 and Rerkasem, 1989). The pollen grains were irregularly shaped or dented like deflated footballs and had very reduced cytoplasm; the pollen did not accumulate starch and the nuclei, and showed abnormal (Figure 2) (Rerkasem *et al.*, 1989). It has been suggested that B deficiency affected pollen development during the pollen mother cell stage (Li *et al.*, 1978).

Bergmann (1983) has suggested that B concentration in the range of 5-10 mg Bkg^{-1} dry weight (wt) was adequate for growth of wheat plants. He also reported that male sterility and depressed grain set were associated with B levels in flag leaf at booting of 5-6 mg Bkg^{-1} dry wt. Rerkasem *et al.* (1989) suggested that increasing B in flag leaf to 12-13 mg Bkg^{-1} dry wt or more at booting prevented male sterility and increased seed set.

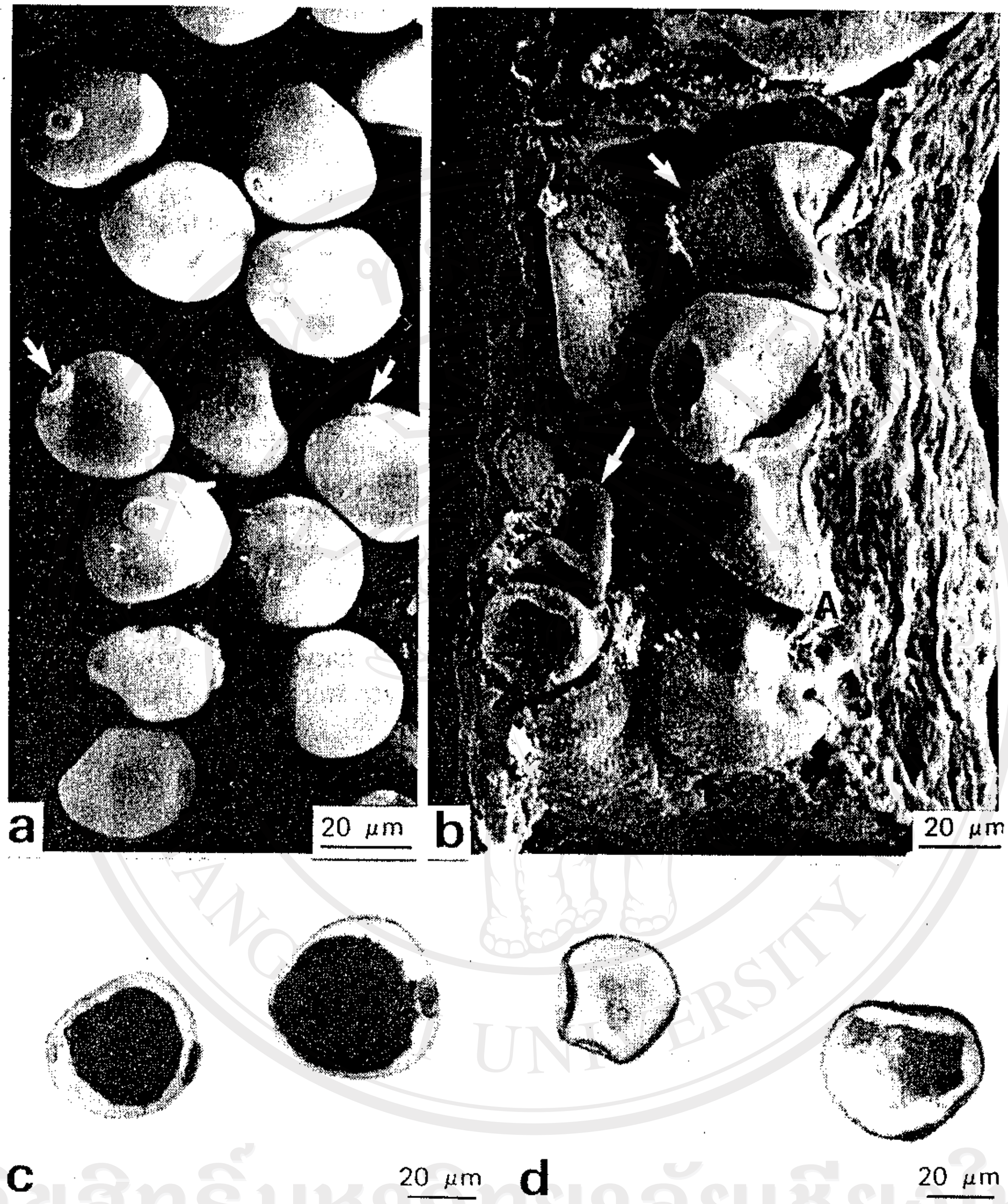

Figure 2. Scanning electron micrographs (a,b) and light micrographs (c,d) of wheat pollen towards anthesis. a) Normal turgid grains with prominent germ pores (arrows) from B+ plant. b) Shrivelled grains (arrows) inside anther tissue (A) from B0 plant. c) Pollen grains from B+ plant mounted in iodine solution. The cytoplasm contains numerous small starch grains. d) Pollen grains from B0 plant showing distorted shape and reduced cytoplasm. (Based on Rerkasem, 1989)

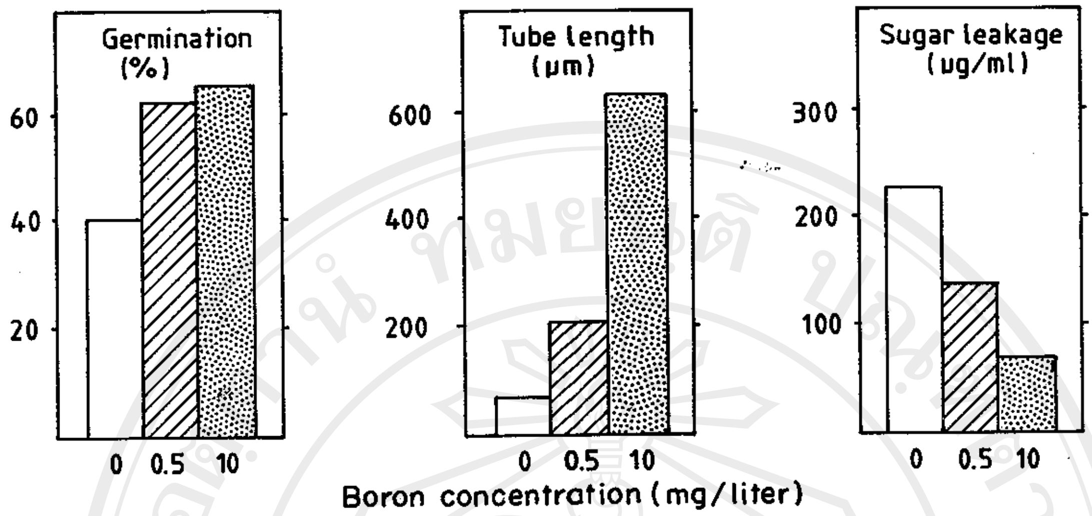

Figure 3. Effect of boric acid concentrations on lily (*lilium longiflorum* L.) pollen germination, tube growth, and leakage of sugar to the medium. (Based on Dickinson, 1978)

2.1.2 Pollen germination and fertilization

Dickinson (1978) demonstrated that B stimulates germination, particularly pollen tube growth in *Lilium longiflorum* (Figure 3). He also showed that leakage of sugars from the pollen decreases while external B concentration increases. Gauch and Duggar (1953) proposed that B combines with sugar to form a sugar-borate complex (ionizable) which is translocated with greater facility than non-borated and non-ionized sugar molecules. Linskens (1955) has shown that, in pollinated styles of *Petunia*, such sugar-borate complexes are formed. Lewis (1980), however, has pointed out that sucrose, the major form of sugar translocated within higher plants, does not possess any cis-hydroxyl pair for complexation with B so that any effect of B in stimulating sugar movement within plants is probably indirect. O'Kelley (1957) showed that during pollen germination and pollen tube growth in *Tecoma radicans* B stimulated the absorption of some sugars like sucrose by the cells and caused a corresponding increase in the rate of oxygen consumption. It is possible that the stimulatory effect of B on pollen tube growth is connected with carbohydrate metabolism (Tupy, 1960). *In vitro* experiments with olive pollen have shown that B stimulates germination and hydrogen ion extrusion; it is believed that B is very important in maintaining an acid pH during germination (Amberger *et al.*, 1990).

According to Lewis (1980), high B levels in the stigma and style are required for physiological inactivation of callose from the pollen tube walls by the formation of borate-callose complexes. When B levels are low, callose levels increase. This induces the synthesis of phytoalexins (including phenols), a defense mechanism similar to that in response to microbial infections (Lewis, 1980; Amberger *et al.*, 1990). The stimulation of tube growth may involve the synthesis of pectic materials required for wall formation of the rapidly elongating pollen tube (Vasil, 1964; Acerbo *et al.*, 1973; and Augsten and Eichhorn, 1976).

Experiments on *petunia hybrida* suggested a role for B in the control of protein secretory activity in pollen tubes, through the redistribution and capture of materials including (and especially) protein from membrane fusion events involving vesicles (Picton and Steer, 1983), which lead to pollen tube extension (Johnson, 1989).

The failure of seed formation in maize suffering from B deficiency has been suggested to be caused by the non-receptivity of the silks to the pollen (Vaughan, 1977). As seen in Figure 1, B supply at less than

2 mg per plant in maize was not sufficient for fertilization and grain set, although vegetative growth was not affected (Vaughan, 1977). Figure 1 also provides the effect of B deficiency on grain yield by strict sink limitation of mineral nutrient deficiency, which is different from typical yield curve in which sink and source are affected to the same extent.

In maize a minimum B content of 3 μgg^{-1} dry wt in the silk is required for pollen germination and fertilization (Vaughan, 1977). The critical B deficient levels in the stigma may, however, vary considerably among cultivars and species. In grapevine (Vitis vinifera) which is known for its high B requirement, fertilization is impaired with 8-20 $\mu g\ Bg^{-1}$ dry wt of the stigma, and 50-60 $\mu g\ Bg^{-1}$ dry wt of the stigma is required to prevent fertilization failure (Gartel, 1974).

In order that effects of B on germination of wheat pollen may be studied under controlled conditions, *in vitro* experiment on wheat pollen may be carried out.

It has been known for many years that B is an essential ingredient in media for pollen germination of most species *in vitro* (Vasil, 1964). Experiment on wheat pollen showed that wheat pollen germination occurs in 0.7% agar media containing 25-125 mgl^{-1} H_3BO_3, 300 mgl^{-1} $CaCl_2.2H_2O$ and 0.75 M raffinose. The optimum level of B in media has been suggested to be 100 mgl^{-1} H_3BO_3 (Cheng and McComb, 1992).

The response of plant to B deficiency may be influenced by temperature. With *Petunia* pollen, Jonhson (1989) showed that B is essential for pollen germination which exhibited peak pollen tube length at 25°C. The wheat experiment in Thailand showed that the degree of B deficiency varied with time of sowing; which suggested that the greater sensitivity to B deficiency of November sowing than October sowing may be related to this sudden decrease in minimum daily temperature (Rerkasem, 1989).

2.1.3 Filling of seeds

General interest in the role of B in carbohydrate metabolism is focused on two aspects: the synthesis of cell wall material and the transport of sugars. The views, however, are conflicting on how the transport of sugars is affected by B deficiency: directly (Gauch and Dugger, 1954) or indirectly (Lewis, 1980).

Under severe B deficiency, the protein content of young leaves decreases and soluble nitrogen compounds, particularly nitrate, accumulate (Hundt *et al.*, 1970). Leaf metabolism and composition might be affected by B deficiency indirectly via its effect on cytokinin synthesis in the root tips: When the supply of B is withheld, both the production and export of cytokinins into the shoots decrease (Wagner and Micheal, 1971). In tobacco, the same phenomenon is true to nicotine alkaloids (Scholz, 1958). While B may facilitate sugar uptake by leaves, the export of photosynthates from the leaves either is unaffected (Weiser *et al.*, 1964), or is impaired by callose formation in the sieve tubes (Venter and Currier, 1977) or by the lack of sink activity of roots and shoot apices in plants suffering from severe B deficiency (Marschner, 1986).

In addition to boron´s affects on sink capacity, these evidences indicate that B deficiency may also result in the process of filling of seeds, fruits and storage organs. However, three experiments on wheat (Rerkasem *et al.*,1989) showed that the weight of individual grains was actually increased with B deficiency. This is more likely to be caused by the normal source size (no effect of B on vegetative growth) in relation to the small sink size (grain set reduction by B deficiency).

2.2 Genotypic variation

2.2.1 Boron uptake and requirement

Plant species differ characteristically in their capacity for B uptake when grown in the same soil (Table 1), which generally reflects typical species differences in the B requirement for growth. The boron requirement of dicotyledons is greater than that of monocotyledons (Marschner, 1986). It has been shown by Tanaka (1967) that the content of strongly complexed B in the root cell walls is 3-5 $\mu g g^{-1}$ dry wt in monocotyledons (e.g. wheat) and up to 30 $\mu g g^{-1}$ dry wt in certain dicotyledons (e.g. sunflower). These differences in B requirement may be related mainly to differences in cell wall composition.

Table 1. Boron content of the leaf tissue of plant species from the same location (mg Bkg^{-1} dry wt)[1]

Plant Species	Boron content
Wheat	6.0
Maize	8.7
Timothy	14.8
Tobacco	29.4
Red clover	32.2
Alfalfa	37.0
Brussels sprouts	50.2
Carrots	75.4
Sugar beet	102.3

[1] Based on Gupta (1979)

Monocotyledons such as wheat, are generally considered to be less sensitive to B deficiency than dicotyledons (Marschner, 1986) because the B requirement of monocotyledons is low. However, the doubling of number of grains per spikelet and the increases in grain yield, in response to B application, found in Thailand are similar to the level of responses found in more sensitive crops, such as black gram, green gram and sunflower grown in the same soil series (Predisritpat, 1988 and Rerkasem *et al.*, 1986). The potential for B deficiency in wheat may also be related to its characteristically poorer capacity to take up B when compared with other crops (Marschner, 1986).

The differences among plant genotypes in their response to low B have been documented. Boron uptake appears to be controlled by the plant roots (Clark, 1983). Brown and Jones (1971) found that there was lower B in the leaves of tomato genotypes grown on a low B susceptible rootstock, but there was more B when grown on a B tolerant rootstock even though the genotypes showed differences in tolerance of susceptibility to low B. Differential accumulations of B have also been reported for various maize genotypes (Clark, 1983). Wheat genotypes have been observed to vary a great deal in the responses to B deficiency (Rerkasem *et al.*, 1989). For example, SW 41 wheat is the most sensitive to B deficiency. SW 23 wheat is moderate in its response; its seed and yield were reduced only with an application of lime to accentuate B deficiency. Genotypes of tolerance to B deficiency are KUHR 12, and Sonora 64; they did not appear to be affected even when lime was applied (Rerkasem *et al.*, 1989)

2.2.2 Distribution and redistribution of B

It has been proposed that: 1) the net uptake of boric acid by higher plants is influenced by the transpiration rate; 2) transport in the xylem is probably directly proportional to the rate of transpiration; and 3) the redistribution of B in the phloem is very limited (Raven, 1980). Gerath *et al.*, (1975) reported that the B distribution in shoots of rape is in response to an increasing B supply; the typical gradient in the transpiration rates among the shoot organs (leaves > pods >> seeds) corresponds to the gradient in B content. In *Pyrus communis* pollen, more B was found in the vesicle and wall fraction and also in the tips of pollen tubes (Dugger, 1983).

The processes that control B movement from tomato roots to other organs were reported to be located in the roots and genetically influenced (Brown and Ambler, 1972). The limiting factor in the long-distance transport of B is most likely to be the high permeability of the sieve tube plasma membrane to B and the correspondingly high leakage of B out of sieve tubes (Oertli and Richardson, 1970).

One early observed symptom of B deficiency distorts apical growth, which has led to the general conclusion that there is little or no reutilization of B from older to younger plant parts (Dugger, 1983). It has been proposed that lower B content in the apices of sunflowers is explained by low reutilization capacity (Maevskaya *et al.*, 1974). However, broccoli plants, when removed to a boron-deficient growth medium at about midpoint in their growth cycle, redistributed internal B from the younger and mature leaves to the youngest tissue (Acerbo *et al.*, 1973).