Title
Exploring the Physiological Basis for High Reproduction Sensitivity to Boron Deficiency in Plants

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Introduction

Boron deficiency causes acute failure of reproductive development and fertilization in flowers, leading to decreased seed/grain set and yield (Marschner, 1995). In B-deficient plants, B concentrations were reported to be much higher in flowers, anthers, carpels or stamens than those of leaves, for example, in wheat (Rerkasem, 1996), maize (Lordkaew et al., 2005), canola (Asad et al., 2000) and green gram (Bell et al., 1990). In wheat, male fertility (stamen and pollen) is more sensitive to B deficiency than carpel and embryo fertility (Huang et al., 2000; Rerkasem, 1996), but female fertility is more sensitive to B deficiency in maize (Dell and Huang, 1997; Lordkaew et al., 2005). However, in dicots such as canola, B deficiency was reported to affect both pollen and pistil/embryo sac development and function, when plants were extremely B-deficient (Xu et al., 1993 cited in Dell and Huang 1997).

Several reviews have discussed the B roles in plant reproduction, including the development and functions of male and female flowers, B distribution in pollen and carpel/pistil, and grain/seed set (e.g. Dell and Huang, 1997; Dell et al., 2002; Huang et al., 2001b; Rerkasem, 1996). The reproductive events most sensitive to B deficiency are hypothesized to be (1) anther development and pollen formation, (2) biochemical signaling in the stigma for pollen recognition, (3) pollen germination, and (4) pollen tube growth (Dell and Huang, 1997). In wheat, B deficiency at pre-meiotic interphase impaired the development of the anther and the meiosis event of pollen mother cells was identified as the most sensitive stage to B deficiency ((Huang et al., 2000). However, there has not been evident explanation about the differential sensitivity of male and female fertility to B deficiency, such as those reported in wheat (male sterility) and maize (female sterility) (Huang et al., 2000; Lordkaew et al., 2005; Rerkasem and Lordkaew, 1996).

Notwithstanding the insights gained from the above research, conclusive evidence linking the sensitivity of reproduction to B deficiency and the requirement for higher B levels in the pollen and/or pistil is lacking. This paper presents new results on B fractions in reproductive tissues of maize and then discusses possible reasons behind the higher sensitivity of plant reproduction to B deficiency, from two perspectives: (1) B requirements in structure and functions of the pollen and pistil, and (2) competition between flowers and leaves for B during critical phases of reproductive growth.

Boron requirements in flower

The assertion that plant reproduction has higher B requirements has been largely drawn from two categories of evidence: (1) the much higher B concentrations in pollen (e.g. wheat and canola) and pistil or silk (e.g. canola and maize) of B-deficient plants than vegetative parts (Table 1) and (2) pollination failure and reduced seedset without significant effects on vegetative growth. For examples, plants at low B supply did not exhibit significant B deficiency symptoms in vegetative parts (e.g. leaves of wheat and maize), but had poor pollen viability, failure of pollen germination and fertilization, and low seed/grain set (Table 1). In wheat, male fertility is more sensitive to B deficiency than female fertility, based on successful cross-pollination of B-adequate pollen onto pistils of B-deficient plants (Rerkasem and Lordkaew, 1996). However, in maize, female flowers are more sensitive to B deficiency than the tassel (Dell and Huang, 1997 and unpublished data) (Table 1). However, in dicot species, less information is available to differentiate male from female sensitivity to B deficiency. Nevertheless, in extremely B-deficient canola plants, both anthers and pistils were abnormal but had nearly twice as much B as the youngest open leaves (Table 1). In addition, the number of flowers was decreased by B

deficiency in canola (Asad et al., 2000).

Even though more B is present in pollen and/or pistil than vegetative tissues the physiological and biochemical requirement for the extra B has not been explained.

Structural and functional basis for B reproductive requirement

Boron's structural role as forming the B-RG-II diester in pectins of primary cell walls and configuring/activating glycoproteins in the plasma membrane has been well established (Bolaños et al., 2004; Matoh, 1997). Furthermore, species differences in B requirements for leaf expansion are positively correlated with the pectin contents of leaf tissues (Bell, 1997). On the basis of these structural roles of B in plant cells, it may be postulated that reproductive tissues have a higher content of pectin-like substrates for B-binding than leaf tissues of the same plant.

In a recent study with maize grown in B-resin buffered solution culture containing less than 0.5 □M B (buffered with B-specific resin loaded with 0.2 mg B/g resin, Huang et al., 1999) from pre-meiotic interphase of the tassel onwards for 15 days, we carried out fractionation of cell walls in the leaf, ear (husk, cob, and silk) and pollen, and analysed B distribution in the cell wall fraction (Table 2). In deficient maize plants, the proportion of total B into cell walls was the highest (84.1%) in the youngest open leaf, followed by silk (61.5%), and the pollen (26.7%) (Table 2). In B-adequate plants, B in the cell wall fraction declined to 11% in the youngest open leaf (YOL), 23.8% in the silk and 8% in the pollen.

The tassel of B deficient plants appeared normal and pollen viability was not affected by the 15 days of 0.2 μM B treatment based on a pollen FCR viability test. In contrast, the elongation of silk was severely inhibited. The B-deficient silks appeared to be thin, flattened, and lacked hairy surface and were unable to protrude out of the husk enclosure for receiving pollen. The lack of epidermal extensions on the surface of the silk may also decrease the capture of pollen fall from the tassel, leading to poor fertilization and seed set.

This experiment showed that temporary B deficiency severely impaired the growth and development of the female, but not the male flowers. This contrasts with our previous findings in wheat (Huang et al., 2000; Huang et al., 2001b). However, the comparison of B concentrations and proportion of B in cell wall of the youngest open leaves and silk does not necessarily support earlier suggestions that silk may have a much higher B requirement than vegetative growth. Although the B-deficient silk had a much lower B concentration than the pollen (Table 2), the total amount of B required in the silk would be much higher than the pollen due to its relatively larger biomass at critical stages of flowering. In addition, our data showed that the silk had a higher pectin content (61 mg uronic acid equivalent/kg dwt cell wall) than pollen (28 uronic acid eq/kg dwt cell wall) in cell walls (Huang et al., unpublished data). The higher distribution of B in cell wall of silk than pollen may be one of the reasons for higher B requirements in the female flower than the male (Table 2).

Boron requirements in pollen function (germination and tube growth leading to fertilization) may account for higher B requirements than those of vegetative growth. Pollen grains may have to accumulate a high level of B stored within the cytoplasm in forms that can be readily re-mobilized such as weak chelates with carbohydrates and phenolics. This B reserve could be readily deployed for the synthesis of cell walls of pollen tubes in order to complete the fertilization of the ovary within a short window of time. Even when maize plants were affected by B deficiency, most B in pollen grains was found to be present in the extractable form, rather than in the bound form in the cell walls (Table 2). By contrast, in female flowers, a greater proportion of tissue B than pollen was distributed in the cell wall fraction due to the rich content

of pectin in the silk. In comparison with B distribution in silk and pollen, an even greater proportion of tissue B in the young leaf was cell wall bound. The supply of free B from the style/silk wall cells may also be important to pollen tube growth. Further research is needed on the significance of other B-binding substrates (e.g. callose) in pollen and anthers which may help to explain the high B requirement in plant reproduction.

These initial results on B distribution in cell walls of pollen and silk provide a preliminary working explanation for high B reproductive requirements in flower development and function. More detailed research is required to examine B fractionation of pollen, pollen tubes and the pistil prior to and during pollination and fertilization, in order to understand why plant reproduction has a higher B requirement than vegetative growth.

B transport into flower

Relatively large B requirements for the development of pollen and pistil within a narrow timeframe pose a challenge for timely and adequate B transport into the floral organs. Flowers of cereals at the sensitive stage of meiosis are often small and enclosed within leaf sheath (e.g. wheat florets) and ear of maize. Dicot species such as oilseed rape, lupin and soybean have more exposed flowers, but petals cover the young pistil and stamen. These structural characteristics of flowers produce lower transpiration rates than leaves, thus rendering flowers less competitive for xylem B transport than the leaves. Boron transport into the young ear enclosed within the sheath of flag and penultimate leaves is dependent on the concurrent long-distance transport of xylem-B, which is closely suppressed by low transpiration (Huang et al., 2001a). However, significant phloem transport of B into the flowers was observed in white lupin, regardless of B supply levels (Huang et al., 2008). Efficient B remobilization into the young flowers at critical developmental stages would be an effective way to overcome the weak competition of the flowers for xylem B transport.

Mechanisms of B transport into individual organs of flowers (e.g. stamen, carpel/pistil, petal, etc) remain unclear, after B reaches the end of the connecting rachis or pedicel of the flowers. Information about how B in a flower is allocated into the stamen and pistil remain unclear. In a fertile floret of wheat, anthers contain >8 mg B kg^{-1} dwt (6-7% of the total B in ear), and 5-6 mg B kg^{-1} dwt in carpels (4-5% of the total B in ear), while the dry matter of anther and carpels only account for about 2% of the ear total dry weight (Rerkasem and Lordkaew, 1996). Transfer cells are common cells specialized in efficient short-distance transport of solutes in flowers and fruits (Offler et al., 2003; Pate and Gunnings, 1972), which may play an important role in distributing B into floral organs.

Conclusion

The high B requirements for plant reproduction are reflected in the relatively high B concentrations in reproductive organs, such as pollen, carpel, and silk. However, no physiological and biochemical mechanisms have been demonstrated to explain why the higher B requirements are necessary, compared to those in vegetative parts such as leaves. Apart from the B requirement in the primary cell walls, a readily available B pool may be required for pollen function – such as rapid pollen tube growth. This hypothesis requires more detailed evidence about B distribution in cell walls across diverse range of plant species and biochemical analysis of other B-binding substrate. Female flower sensitivity to B deficiency in maize may be more likely related to the total amount of B required in the formation and elongation of silk through the husk enclosure for pollination and fertilization, due to the much larger biomass and pectin

content than pollen. This high B requirement for plant reproduction is further challenged by the relatively much weaker sink strength for B transport and distribution due to the weaker transpiration activity in young reproductive organs. Detailed mechanisms remain to be elucidated, governing B transport across the short distance of several layers of specialized tracheal cells immediately at the base of the flower. Floral structure characteristics may also contribute to the differential B transport efficiency into the flower organs.

Table 1: Summary of B concentrations in leaf and flower tissues of crop plants.

		Tissues B concentration (mg /kg dwt)				
Crop	B treatment	Youngest open leaf	Pistil or silk	Pollen/ anther/stamen/ flower bud	Responses of flowers	Data source
Wheat	Nil during meiosis stage	0.48 - 0.82	na	na	Decreased anther length, pollen fertility and grain set	(Huang et al., 2000)
	Adequate	4.0 - 5.0	na	na	Normal	
	<0.5 mg B/kg sandy loam	4.0 - 5.0	5.1 - 5.5	7.0 - 7.5 (anther)	Decreased Male fertility and grain set	(Rerkasem and Lordkaew, 1996)
	adequate	3	8	16 (anther + filament)	Normal	(Rerkasem, 1996)
Maize	0 (no added B)	na	4.4	4.4 (pollen)	44.7% sterile pollen & and abnormal silk	(Lordkaew et al., 2005)
	20 □M	na	11.3	9.0 (pollen)	Normal	
	< 0.5 □M	1.8 - 2.9	0.2 - 1.1	1.0 - 2.5 (pollen)	Abnormal & shortened silk	unpublished data, Huang et al 2009
	21.5 □M	37.0 – 47.0	5.6 - 7.7	11.6 - 16.6 (pollen)	Normal	
Oilseed rape	0.35 - 0.56 □M (from germination)	2.6 - 12.9	NA	4.0 – 33.2 (flower bud)	Decreased number of flowers; smaller pollen size	(Asad et al., 2000)
	0.86 □M & above	18 - 20	NA	44 - 49 (flower bud)	Normal flowers & pollen	
	Low	10	19	19 (anther + filament)	na	Zhang et al (1994) in (Dell and Huang, 1997)
	Adequate	17	40	38	Normal	
Green gram	Low	11	19	na		(Bell et al., 1990)
	Adequate	34	50	na	Normal	

Table 2. Boron distribution in cell walls (CW) of silk and pollen of maize plants exposed to deficient B supply in B-buffered solution culture under glasshouse condition. The values are averages of 4 replicates per treatment with standard errors in the parentheses. Plants (1 plant per pot (5 L)) were transferred into the B treatments at the stage of meiosis/tetrad in the anthers for 21 days. Boron concentrations in the treatments were buffered with B-specific resin loaded with specified amount of B. The silk of the 1st ears emerged were sampled for B analysis and fractionation. The pollen was collected cumulatively during the treatment period.

Boron Treatment (□M)	**Organ**	**Total B mg B/kg dwt**	**CW_B% Total B**
< 0.5	Silk	0.51 (0.28)	61.5 (20.5)
21.5		6.62 (0.43)	23.8 (1.74)
< 0.5	Pollen	1.86 (0.37)	26.7 (3.86)
21.5		14.0 (1.30)	8.04 (0.75)
< 0.5	YOL	2.13 (0.26)	84.1 (12.2)
21.5		42.1 (2.23)	11.0 (0.78)

Table 3. The response of silk and cob length of maize ear to B deficiency in B-buffered solution culture under glasshouse conditions. The values are means of 4 replicates per treatment, with standard error (SE).

B Treatment (□M)	Ear position from base	Extruded silk length (mm)		Net cob length (mm)	
		Average	SE	Average	SE
< 0.5	1	22.5	22.5	59.2	2.69
	2	32.5	23.6	70.0	3.54
21.5	1	NA		NA	
	2	154	23.1	93.7	6.57
10.0	1	116	14.9	75.0	5.40
	2	156	14.3	92.2	7.96

Table 4. Published B concentrations in silk and pollen of maize in sand culture irrigated with nutrient solutions with 20 □M or no added B from germination onwards (Lordkaew et al., 2005). The values are means.

B treatment (□M)	Silk	Pollen	Pollen sterility
	mg B/kg dwt		%
0 (no added B)	4.4	4.4	44.7 - 100
20	11.3	9.0	1.0 - 2.9

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