Role of Micronutrients in Reproductive Physiology of Plants

Nalini Pandey[*]

Plant Nutrition and Stress Physiology Laboratory, Botany Department, University of Lucknow, Lucknow, 226007, India

Correspondence: * nalini_pandey@rediffmail.com

ABSTRACT

Plant reproductive biology is a key developmental process and has a great impact on plant productivity. Reproduction in plant involves the interaction between the male and female gametophyte which depend on the sporophyte for their nutrient requirement. Till recently, it was thought that adequate supply of micronutrients (Fe, Mn, Cu, Zn, Mo and B) was required during the period of active vegetative growth. It has lately been shown that good vegetative growth of plants does not necessarily go hand in hand with a high seed yield. In a large number of crops even under conditions of moderate micronutrient deficiencies when biomass production is marginally reduced, the reproductive yield is severely decreased. This suggests a requirement of micronutrients for floral induction and reproductive development independent of the requirement for production of necessary assimilates. Sufficient evidence has emerged to show that the micronutrients, in particular Cu, Zn and B, are critically required for reproductive development and that their requirement is possibly higher than what can be met by retranslocation from the vegetative parts of the mother plants. Application of these micronutrients during an early stage of reproductive phase make substantial improvement in pollen fertility, pollen-stigma interaction, seed setting and seed quality. While there are numerous reports of response to micronutrient fertilization benefiting harvest yield of plants, information on involvement of the micronutrients in plant reproductive development is limited. In recent years the identification of a number of genes for the floral organs and specific transcription factors like the zinc-fingers has given new impetus to the role of micronutrients in transcriptional regulation of reproductive development. In this review the current status on the systematic studies on the role of micronutrients in reproductive biology of plants is discussed.

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INTRODUCTION

Plant reproductive biology is the key process in plant development which has a direct impact on agriculture. Reproduction in angiosperm initiates with flowering and leads on to the gametophytic phase, followed by fertilization and seed set. The gametophytic phase in angiosperms is highly reduced and is restricted to the pollen grains (male gametophyte) and ovules (female gametophyte). Pollen grains are formed on maturity from the pollen mother cells after meiosis in the anther and bear the generative and sperm cells which are contained within the pollen wall made up of lipids, proteins and pigments. At flower maturity pollen grains are dispersed from the anther for pollination and at this stage the pistil is fully developed into stigma, style and ovary. The stigma is the first to receive the pollen grain, bind them, and enable their hydration and germination to form pollen tubes and their passage into the style (Lord 2003; Sanchez *et al.* 2004). Water and nutrients (proteins, polysaccharides, lipids) are immediately transported into the grains from the stigma exudates in case of wet stigmas or from stigma papillae as in dry stigmas (Heslop-

Harrison and Shivanna 1977). The pollen coat protein rich matrix (tryphines), waxes and long chain lipids also help to initiate signals required for adhesion and hydration (Wolters-Arts *et al.* 1998; Zinkl *et al.* 1999). Enzymes secreted by pollen grains as well as the stigma and style such as acid phosphatase, peroxidase, ribonuclease, esterase and protease actively participate in these processes and facilitate the pollen tube invasion of stigma (Hiscock *et al.* 2002; Edlund *et al.* 2004; Radlowski 2005). The pollen tube carries the sperm cells which fertilize the ovule and ultimately form seeds.

Although the processes discussed above are conserved across angiosperms, there is a vast diversity in the structures of pollen and stigma and their interactions. The conditions under which plants grow also greatly influence the development of the gametophytes. The formation of viable pollen grain involves the exchange of nutritional, structural and regulatory compounds between the sporophyte and the male gametophyte. Environmentally determined differences in pollen germination are potentially important because differences in pollen deposition and viability may affect fertilization and seed set. Pollen sterility and ovule abortion has

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Invited Review

been shown to result from a variety of environmental factors such as high temperature (Frova *et al.* 1986; Shivanna *et al.* 1991; Young *et al.* 2004), water stress (Saini 1997), UV-B radiation (Demchik and Day 1996; Koti *et al.* 2004), soil salinity (Sun *et al.* 2004) and nutrient stress (Young and Stanton 1990; Sharma *et al.* 1991; Lau and Stephenson 1993; Furuyama and Dzelzkalns 1999; Rehman and Yun 2005).

Reproductive development of plants is known to be adversely affected by micronutrient deficiencies (Agarwala *et al.* 1979; Graham 1975; Dell 1981; Sharma *et al.* 1987; Sharma 1992; Pandey *et al.* 1995; Pandey and Sharma 1999, 2001; Pandey *et al.* 2002; Asad *et al.* 2003 Pandey *et al.* 2009). The importance of micronutrients for reproductive development was realized when it was observed that the effect of their deficiency was more profound on the reproductive yield of plants than on the vegetative yield. Studies carried out subsequently on the gametophytic development of plants deficient in micronutrients, point to a more direct involvement of micronutrients in development of pollen grains and pollen-stigma interactions (Jewell *et al.* 1988; Sharma *et al.* 1990; Pandey *et al.* 1995, 2006). Considerable progress has been made in the recent years on the involvement of micronutrients on reproductive development. With the identification of a number of genes for the floral organ and specific transcription factors like the Zn-finger (Kubo *et al.* 1998; Grossniklaus *et al.* 1998; Takatsuji 1999; Brive *et al.* 2001), the role of micronutrients in transcriptional regulation of reproductive development has gained new appreciation (Kapoor *et al.* 2002; Takahashi *et al.* 2003; Payne *et al.* 2004; Suárez-López 2005). The present review discusses the involvement of micronutrients Fe (iron), Mn (manganese), Cu (copper), Zn (zinc), Mo (molybdenum) and B (boron) at the structural, physiological, biochemical and transcriptional level of reproductive development.

Iron

Iron exists in two oxidation states – ferric (Fe^{3+}) and ferrous (Fe^{2+}) and easy inter-convertibility of the two oxidation states ($Fe^{3+} \leftrightarrow Fe^{2+}$) accounts for its role in a wide range of oxidation-reduction reactions. Iron acquires high biological activity on binding to proteins. A large number of Fe enzymes, with a wide range of substrates, catalyze an array of reactions that are essential to primary metabolism and biosynthesis of secondary metabolites. Iron enzymes and electron carrier proteins form integral components of mitochondrial and photosynthetic electron transport systems. The effect of Fe on reproduction may be related to its role in general metabolism, in particular to its being a constituent of enzymes. Many Fe enzymes, recently cloned using molecular biology techniques, are known to play a role in metabolism of fatty acids, biosynthesis of gibberellins, ethylene and jasmonic acid, which play important roles as signalling molecules in regulation of developmental processes. Katyal and Agarwala (1982) observed that Fe concentration varies in plant types but plants with less than 50 µg Fe g^{-1} dry weight showed significant decrease in reproductive yield whereas optimum yield was obtained in plants containing 100 to 250 µg Fe g^{-1} dry weight.

Iron was reported to be essential for photoperiodic induction of flowering (Hilman 1967). Iron content was found to be crucial for floral initiation and was found to promote flowering in *Lemna* (Gupta and Maheshwari 1970; Khurana and Maheshwari 1983; Nishioka *et al.* 1986). Pandey and Sharma (2001) reported that Fe deficiency (0.56 mg L^{-1}) in green gram (*Vigna radiata*) not only delayed flowering but also reduced the number and size of pollen grains formed. Structural development of pollen grains in the Fe deficient plants was disrupted, with irregularities in exine depositions and the reticulate pattern of the tectum. Redressal of Fe deficiency by 5.6 mg Fe L^{-1} partially reversed the structural changes and viability of pollen grains in green gram.

Involvement of Fe in pollen-stigma interaction cannot be ruled out since Fe is a constituent of peroxidase and

angiosperm stigmas have long been known to exhibit high levels of peroxidase activity when they are most receptive to pollen (Bredemeijer 1979). Recently McInnis *et al.* (2006) have identified a novel stigma specific class III peroxidase gene, SSP (stigma-specific peroxidase) expressed exclusively in the specialized secretory cells (papillae) of the stigmas of *Senecio squalidus* L. (Asteraceae). Since most plant peroxidase genes are expressed in a variety of tissue types in response to stress (Valério *et al.* 2004), the precise papilla cell-specific expression and localization of SSP suggests that it may play a key role in stigma function such as loosening stigma cell wall components to allow penetration and growth of pollen tubes within the stigma. Acting indirectly through H_2O_2 metabolism, SSP and other stigma peroxidases may carry signals for species-specific pollen recognition by the stigmas. The high constitutive levels of peroxidase activity in stigmas (and style) may also contribute to enhanced protection against pathogen attack (Do *et al.* 2003). Stigma peroxidases, such as SSP, may also be important elements of a ROS/H_2O_2-based system of defense and this is suggestive of a new role of ROS and its related enzymes in angiosperm reproduction, specifically in pollen–stigma interactions and protecting stigmas from microbial attack.

The cloning of ripening related cytochrome P450 gene from *Avocado* fruits (Bozak *et al.* 1990) led to the cloning and characterization of many membrane (ER) localized cytochrome P450 encoding genes (designated CYP for cytochrome P450). Enzymes coded by the CYP genes have since been shown to be involved in fatty acid metabolism and in the biosynthetic pathways of gibberellins, ethylene and jasmonic acid (Chapple 1998). Molecular cloning and functional expression of gibberellin oxidases from *Arabidopsis* has revealed that several steps in the biosynthesis of gibberellins from GA_{12} aldehyde are catalyzed by 2-oxoacid dependent non-heme dioxygenases (Hedden and Phillips 2000). The terminal step of ethylene biosynthesis involving oxidation of 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene is catalyzed by the non-heme Fe enzyme ACC oxidase (Bouzayen *et al.* 1990, 1991; Kende 1993). Martinis and Mariani (1999) suggested that female sterility was caused by suppressed ethylene formation resulting from ACC oxidase inhibition. They reported that abnormalities in the lengths of pistils in *naat* tobacco might be a consequence of an ethylene formation disorder caused by Fe deficiency. Thus role of Fe in gibberellins and ethylene synthesis may have far reaching consequences in plant reproductive boilogy (Sun and Gubler 2004).

In a recent study, Takahashi *et al.* (2003) have shown severe suppression of reproductive development in transgenic tobacco plants lacking in synthesis of nicotianamine (NA). Nicotianamine plays a role in the chelation of the cationic micronutrients and their delivery to the reproductive tissues. Chlorotic leaves and abnormally shaped sterile flowers were observed in transgenic tobacco plants (*naat* tobacco) that constitutively expressed the NAAT (nicotianamine amino transferase) gene. Since endogenous NA was consumed as a result of NAAT overproduction in *naat* tobacco the resulting NA shortage caused these disorders. Grafting of *naat* tobacco onto wild-type tobacco reversed interveinal chlorosis and aberrant flower shape but did not reverse sterility. However grafting of *naat* tobacco onto *nas* (nicotianamine synthase) tobacco not only reversed chlorosis in leaves, abnormal flower shape, pollen maturation defects, late anther dehiscence and sterility but also increased the concentrations of Fe, Cu, and Zn in the flowers. The grafting experiment also showed that NA is essential for formation of normal inflorescence, production of normal pollen grains and for seed maturation. The expression of *NtNAS* in floral organs as well as the stamen filament indicates that NA promotes metal transport into the inflorescence for the maturation of pollen in the anther. That NA-metal complex-specific transporter could transport NA-Fe to each flower organ and seed was reported by Vert *et al.* (2002). They observed that the *IRT1* promoter-GUS fusion

showed GUS staining in the anther filament and Fe which could not be complexed by NA was transported to the flowers by *IRT1* (Iron regulated transporter). In *naat* tobacco lacking in NA there was a delay in anther dehiscence. Since allene oxide synthase, a key enzyme for synthesis of jasmonic acid (required for anther dehiscence), has heme-Fe (Kubigsteltig *et al.* 1999), its synthesis might be affected by NA indicating yet another role of Fe for reproduction. NA also may regulate the Fe in Fe-requiring proteins such as DME (DEMETER) which is expressed primarily in the central cell of the female gametophyte, the progenitor of the endosperm (Choi *et al.* 2002). DME is required for endosperm gene imprinting and seed viability and homozygous *dme-1* mutant plants produced siliques in which almost all seeds were aborted.

Manganese

Manganese exists in several oxidation states of which the most dominant and stable is manganous (Mn^{2+}). By undergoing changes in its oxidation states it functions as a cofactor or activator of several enzymes (Burnell 1988). Activation of enzymes by Mn accounts for its role in photosynthesis, carbohydrate, nitrogen and secondary metabolism and detoxification of oxygen free radicals. Manganese is a component of the oxygen evolving complex associated with the photosystem II (PSII). The complex contains cluster of four Mn ions bound to the amino acid residues of the D I protein of the PS II reaction centre. Manganese effect on photosynthesis and activity of enzymes is a major factor contributing to low yield of Mn-deficient plants. The plant critical range for Mn deficiency is 10 to 25 μg Mn g^{-1} dry weight and values below this lead to severe losses in reproductive yield which is suggestive of its role in reproductive development.

The requirement of Mn for generative development is much higher than that for vegetative growth even under conditions of adequate supply. Thus floral and fruiting organs are extremely sensitive to conditions of deficiency and their production is inhibited (Reuter *et al.* 1988). Longnecker *et al.* (1991) reported inhibition in tillering and in the number of ears formed in Mn deficient barley (*Hordeum vulgare*) plants. In lentil *(Lens culinaris)*, pods and seeds were not formed and flowers were shed at less than 0.55 mg Mn L^{-1} (Khurana *et al.* 1991). In cotton (*Gossypium hirsutum*) Mn deficiency caused inhibition of tertiary branching, which resulted in decrease in flower number and capsule yield (Pandey *et al.* 2002). Application of Mn-EDDHA (ethylene diamine di o-hydroxyphenyl acetic acid) with the nutrient solution was found to increase flower induction and number of flowers in *Lemna minor* (Krajncic and Nemec 2003). Low levels of Mn decreased the number and length of the inflorescence and flowers formed in strawberry (Shahrokhi *et al.* 2008). The number of ripe fruits formed, the vitamin C content and total soluble solid was also increased by application of 1.5 g L^{-1} as manganese sulfate in strawberry (Lieten 2004; Shahrokhi *et al.* 2008).

Manganese deficiency has been reported to decrease pollen producing capacity, size of the pollen grains, their viability, pollen tube growth (Sharma *et al.* 1991; Sharma 1992) and activities of some anther enzymes (Kaur *et al.* 1988). The critical requirement of Mn for reproductive development in wheat can be ascribed to the much higher reduction in reproductive yield than vegetative yield and more so for the pollen grain than the ear formation (Sharma 1992). In wheat, Mn deficiency limited grain yield not only by inhibiting tillering and the number of ears plant^{-1} but also by causing pollen sterility leading to poorer number of seeds ear^{-1}. Cross pollination studies revealed that pollen grains from Mn-deficient plants (0.0055 mg L^{-1}) produced fewer grains per spikelet in normal as well as Mn-deficient plants whereas spikelets in emasculated ears of Mn-deficient plants after pollination with pollen grains from normal plants formed nearly the same number of grains spikelet^{-1} as in self pollinated normal plants. This suggests that although

Mn deficiency produces fewer tillers, it does not affect the ovule fertility as much as the pollen viability. The loss of fertility in Mn-deficient pollen seems to be a result of retarded growth of the sporophyte and its inability to provide adequate Mn to the gametophyte (pollen grains). Poor pollen fertility and shortage of carbohydrates leading to low seed yield has been widely reported in Mn-deficient plants (Marschner 1995; Mengel *et al.* 2001).

Manganese deficiency greatly reduces seed yield and causes seed disorders such as dark discoloration ("marsh spots") on cotyledons of pea (*Pisum sativum* L.) and cracks of the testa ("split seeds") in seeds of *Lupinus* spp. (Walton 1978; Campbell and Nable 1988; Römheld and Marschner 1991; Longnecker *et al.* 1996). Hocking *et al.* (1977) observed that at critically low levels of Mn (7.5 μg Mn g^{-1} dry weight), lupins exhibited abnormal proliferations of the endosperm, partial extrusion of the embryos through seed coat as well as brown coloration and splitting of the seed coat. Foliar applications of Mn as manganese sulphate (up to 10 kg ha^{-1}) has been shown to increase the fruit set and seed yield in Mn deficient plants such as soyabean, lupins, sugar beet and wheat (Mascagni and Cox 1985; Brennan 1996; Last and Bean 1991; Modaihsh 1997). Recently Dordas (2009) observed that foliar application of Mn (100 and 200 g Mn L^{-1}) during anthesis in cotton significantly increased the number of bolls formed indicating that Mn has a direct effect on fertilization and especially on boll formation and retention in cotton.

Besides seed yield Mn deficiency also impairs the seed quality (Ohki *et al.* 1979; Wilson *et al.* 1982; Campbell and Noble 1988; Khurana *et al.* 1999). Seedling establishment and the initial growth of the plant is also affected by Mn deficiency. It was observed that seeds that were developed in plants with adequate Mn supply show high seed vigor and were able to germinate and grow better than seeds produced from Mn deficient plants (Longnecker *et al.* 1996; Romheld and Marschner 1991). In *Arabidopsis* an analysis of manganese levels at different stages of seed development (Otegui *et al.* 2002) revealed that Mn (as Mn-phytate) is stored in the endoplasmic reticulum of the chalazal region of the endosperm, and is mobilized to the embryo at the late bent-cotyledon stage. They observed that disappearance of Mn-phytate from the endosperm coincided with the accumulation of two Mn binding proteins of the embryo – the MnSOD and the 33KD protein of the oxygen evolving Mn-complex of PS II. Thus Mn stored in the endosperm as Mn-phytate functions in the assembly of Mn-proteins during embryo development (Otegui *et al.* 2002).

Recently Mn has been assigned a role in providing protection to the nutrient-rich stigma. The role of Mn in providing protection to the receptive surfaces of stigmas from microbal attack in spite of its nutrient-rich secretions seems to draw analogy from the nectar which are protected from microbial infection by ROS and H_2O_2 due to the presence of a superoxide dimutase–Nectarin 1 (Carter and Thornburg 2004). Nectarin I (NEC1) is a germin-like protein (GLP) that has manganese superoxide dismutase activity. The MnSOD activity generates high level of H_2O_2 in the nectar which functions as an antimicrobial defense to protect the gynoecium and developing ovules from microbial attack and is expressed during nectar production prior to anthesis and ceases after floral pollination (Carter and Thornburg 2000, 2004).

Copper

Copper is a redox-active metal with interconvertible cuprous (Cu^+) and cupric (Cu^{2+}) forms which enable it to function in oxidation-reduction reactions in cellular metabolism. Most Cu in plants is complexed to proteins. As a constituent of Cu protein plastocyanin, it plays a key role in photosynthetic electron transport. Involvement of plastocyanin in electron transport accounts for decrease in photosynthetic rates in Cu deficient plants leading to low yield. As a cofactor of several enzymes (polyphenol oxidase, ascorbate

oxidase and amine oxidase) it affects the ligninification of plant cells and pollen fertility. It provides protection from superoxide ions since it is a cofactor of Cu/Zn superoxide dismutase. The critical concentration of Cu deficiency is 1 to 5 µg Cu g^{-1} dry weight and concentrations of Cu below this result in significant decrease in yield. The Cu concentration in plants showing optimum yield range between 8 to 33 µg Cu g^{-1} dry weight (Robson and Reuter 1981).

Copper plays a major role in reproductive development of plants, which accounts for loss of yield under Cu deficiency. Several aspects of reproductive development are influenced by Cu. Copper deficiency has been reported to delay flowering and cause reduction in the number of flowers in large number of crops (Graves and Sutcliffe 1974; Graham 1976; Davies *et al.* 1978; Graves *et al.* 1979; Reuter *et al.* 1981). Delayed flowering in Cu-deficient plants (Davies *et al.* 1978; Reuter *et al.* 1981) has been attributed to decrease in the activity of polyphenol oxidase and ascorbate oxidase leading to accumulation of IAA which is inhibitory to flowering. Tanaka *et al.* (1982) reported that flowering in *Lemna paucicostata* 6746 and *Lemna gibba* G3 does not take place if the Cu from the medium is chelated by the addition of EDTA. Deficient Cu supply (<0.063 mg L^{-1}) in safflower (*Carthamus tinctorius* L.) delayed and impaired formation of the capitulum and the floret formed were totally sterile and failed to develop seeds (Pandey and Sharma 1999).

Development of anthers and pollen grains is very sensitive to plant Cu status and higher concentration of Cu in anthers and ovaries than in other floral parts suggests involvement of Cu in their development (Knight *et al.* 1973). The role of Cu in microsporogenesis and pollen fertility may account for the more severe reduction in seed yield than dry matter production by Cu deficient plants (Graham 1975). Graham (1975) gave evidence for male sterility in Cu deficient wheat plants and showed that the deficiency interfered with development of pollen grain near the stage of meiosis of pollen mother cell and that decrease in seed setting is a function of male sterility and not ovule sterility. They observed that cross pollination of Cu sufficient plants with pollen grains of Cu-deficient plants drastically reduced the number of grains formed (just 2 head^{-1}), whereas when Cu-deficient plants were fertilized with pollen grains of Cu-sufficient plants an appreciable number of seeds were formed (47 head^{-1}). Graham (1976) reported that application of Cu (0.4 mg Kg^{-1} soil) to Cu-deficient wheat plants produced excellent yield if applied any time between sowing to early boot stage reiterating their contention that microsporogenesis was interrupted by Cu deficiency at or near meiosis.

Inadequate supply of Cu not only limits the size of anthers (Graham 1975; Agarwala *et al.* 1980; Dell 1981) but also prevents their dehiscence. Pollen sterility and impairment in development of endothecium was consistently observed by Dell (1981) in plants (wheat, oat, barley, sweet corn, sunflower, petunia and sub-clover) grown under Cu-deficient conditions. They observed that at low Cu supply (<500 µg Cu pot^{-1}) the inflorescences were developed but flowers formed were male sterile showing either staminodes (50 µg Cu pot^{-1}) or arrow shaped shriveled stamen without tetrads (100 µg Cu pot^{-1}) or stamens with sterile pollen grains and reduced lignification (300 µg Cu pot^{-1}). The sterile pollen grains formed were small deformed and lacked cytoplasm or starch. The endothecium failed to develop in Cu-deficient anthers and there were reduced thickenings surrounding the stoma which is critical for its rupture and thus release of pollen grains. They attributed the failure of anther dehiscence in Cu-deficient plants to poor lignification of anther cell walls resulting from decrease in activity of Cu-containing enzymes involved in biosynthesis of lignins.

Jewell *et al.* (1988) reported the formation of a non-functional tapetum in the anthers of Cu-deficient barley plants which was expansionary and failed to supply nutrients to the developing pollen grains. Abnormal (polyploid) pollen grains were observed in Cu-deficient plants of durum
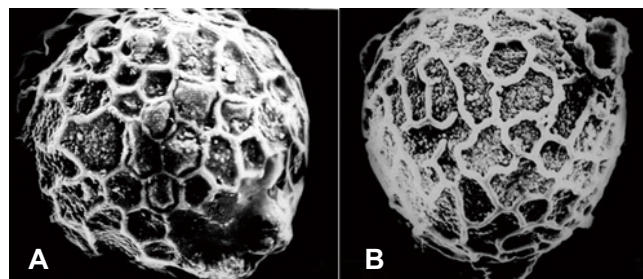

Fig. 1 Scanning electron microscopy (SEM) images of Cu-sufficient (A) and Cu-deficient (B) pollen grains of green gram plants. Reprinted from **Pandey N, Gupta M, Sharma CP** (1996) Ultrastructural changes in pollen grains of green gram subjected to copper deficiency. *Geophytology* **25**, 147-150, ©1996, with kind permission of the Paleobotanical Society, India.

wheat (Azouaou and Souvré 1993). In Cu-deficient wheat, number and size of pollen grains is severely restricted, pollen grains lack dense cytoplasmic contents and the high starch content, characteristic of normal, viable pollen grains (Agarwala *et al.* 1980; Jewell *et al.* 1988). Pandey *et al.* (1996) observed ultrastructural changes in pollen grains of green gram following Cu deprivation (0.0063 mg L^{-1}) and resupply. Copper deficiency decreased the size and pore diameter of pollen grains and increased the thickness of exine. As compared to the reticulate pattern in Cu sufficient pollen grains (**Fig. 1A**), the exine architecture was also altered in Cu deficient pollen grains. The lumen showed pilate projectons of the baculae and muri were thick with irregular undulated walls (**Fig. 1B**). In the pollen grains that were produced following resupply of Cu (0.063 mg L^{-1}), the ultrastructural changes were minimized and germination percentage was increased.

Copper also affects the maturation and quality of seeds and Cu deficient rice plants are reported to show decrease in seed reserves of starch, soluble sugars and protein (Nautiyal *et al.* 1999). Recently Mesejo *et al.* (2006) showed that application of CuSO$_4$ proved beneficial in forming seedless *Citrus* fruits.

Zinc

Zinc plays a structural as well as functional role in plants. Unlike other cationic micronutrients, it has only one oxidation state (Zn^{2+}) and binds by ionic bonds forming a tetrahedral geometry. It forms a structural component of a large number of proteins with catalytic or regulatory functions. Zinc plays a role in transcriptional regulation and as 'Zn-fingers' provide stability to many regulatory proteins. Over the years, the role of 'Zn-fingers' has gained immense importance in reproductive development (**Table 1**).

Zinc is critical for the reproductive development of plants. Inadequate supply of Zn to plants affects different aspects of reproduction ranging from flowering, floral development, anthesis, gametogenesis, fertilization and seed set. The critical deficiency concentration for Zn ranges between 15 to 20 µg g^{-1} dry weight. Deficient supply of Zn (<0.065 mg Zn L^{-1}) has been shown to severely retard reproductive yield even though the dry matter production was only marginally depressed (Sharma *et al.* 1979, 1987, 1990).

Deficiency of Zn induced pollen sterility in wheat (Sharma *et al.* 1979) and repressed male sexuality in maize (Sharma *et al.* 1987). On withholding Zn supply at the onset of tassel emergence, maize plants show retarded tassel formation, reduction in size of anthers and poor development of sporogenous tissue. Sharma *et al.* (1987) showed that anthers failed to develop and instead of forming sporogenous tissue (pollen mother cells), the anthers developed vessels in Zn-deficient maize plants. The anthers from these plants had low Zn concentration (18 µg g^{-1} dry weight) and stimulated activities of catalase, peroxidase, ribonuclease and acid phosphatase. Resupply of Zn (0.13 mg Zn L^{-1}) to

Table 1 Zinc-finger proteins involved in reproductive development in plants.

Proteins	Function	Expression	Plant species	References
ZPT2-1	DNA-binding protein for *EPSPS* gene	petal, seedlings	*Petunia*	Takatsuji *et al.* 1992
ZPT2-2, ZPT2-3	unknown	petal and stamen, stress-inducible	*Petunia*	Takatsuji *et al.* 1994
SUPERMAN	cell division in floral organ	stamen primordial	*Arabidopsis*	Sakai *et al.* 1995
CONSTANS	Promotes flowering	apical meristem	*Arabidopsis*	Putreill *et al.* 1995
ZPT3-1, ZPT3-2, ZPT4-1, ZPT4-2, ZPT4-3, ZPT2-6	Gametogenesis	tapetum, microspores, anthers, pollen and stomium	*Petunia*	Kobayashi *et al.* 1998
ID1	Floral transition	meristem	*Zea mays*	Colasanti *et al.* 1998
FIS1	seed and embryo development	gametophyte	*Arabidopsis*	Chaudhury *et al.* 1999
EMF2	Flowering	meristem	*Arabidopsis*	Yoshida *et al.* 2001
TAZ1	tapetum development	tapetum	*Petunia*	Kapoor *et al.* 2002
RIE1	seed development	embryo	*Arabidopsis*	Xu and Li 2003
Moc3	mating efficiency, asci formation	nucleus	*Schizosaccharomyces pombe*	Goldar *et al.* 2005
MEZ1	pollen meiosis	anther	*Petunia*	Kapoor and Takatsuji 2006
ZOS	reproductive development	panicle, seeds	*Oryza sativa*	Agarwal *et al.* 2007
IMA	flower and ovule development	floral meristem	*Solanum lycopersicum*	Sicard *et al.* 2008

deficient plants after flowering failed to produce normal pollen grains in the anthers suggesting that Zn deficiency during the pollen formation stage caused irreparable damage to the developing pollen grains. The anthers of the Zn-deficient flowers showed repression of male sexuality leading to poor fertilization and seed setting. Sharma *et al.* (1990) observed that if Zn was restored to Zn-deficient plants prior to formation of pollen mother stage then pollen fertility could be restored to a large extent. However if Zn deficiency was induced before microsporogenesis, then even though the vegetative yield and ovule fertility was normal (as evidenced from seed set by cross pollination of ovules in Zn-deficient plants with normal pollen grains), the fertility of pollen grains was reduced. Induction of Zn deficiency post release of microspores failed to effect pollen fertility. Thus plants have a high requirement of Zn which is critical for microsporogenesis and pollen fertility.

Polar (1975) showed that in *Vicia faba* the developing anthers and pollen grains had a much higher concentration of Zn. Ender *et al.* (1983) showed that the pollen tubes of lily accumulated a larger concentration of Zn in the tip of the growing tube and that at least a part of it played a role in fertilization. The pollen of Zn-deficient plants were small in size, failed to germinate and exhibited changes in exine morphology (Pandey *et al.* 1995, 2000, 2006, 2009). The exine of Zn-deficient pollen grains of black gram (*Vigna mungo*) show highly sinuous, lobed muri having a waxy covering and raised baculae as compared to the reticulate and uniform muri present in the exine of the Zn-sufficient pollen grains (**Fig. 2A, 2B**). Changes in exine pattern are reported to be caused by changes in the extracellular matrix of the pollen grains resulting from structural and biochemical changes during microsporogenesis. The later phase of pollen development is known to be associated with changes in mRNA and protein levels in pollen grains (Willing *et al.* 1988; Wetzel and Jensen 1992) and this may possibly lead to alterations in the exine pattern of Zn-deficient pollen grains. It is reported that Zn deficiency decreases the RNA content of pollen grains, possibly because of enhanced levels of ribonuclease activity (Sharma *et al.* 1979, 1987). The thickness of the exine and wide and raised muri with waxy depositions in Zn-deficient pollen grains may also act as a barrier to pollen hydration which is a prerequisite for pollen germination and pollen tube growth (Zinkl *et al.* 1999).

More severe limitation in seed setting than *in vitro* germination of pollen grains under Zn deficiency does not rule out the role of Zn in pistil functioning (Pandey *et al.* 1995, 2006). Impaired pollen-stigma interaction due to Zn deficiency induced changes in stigmatic size, morphology and exudations has also been observed (Pandey *et al.* 1995, 2006, 2009). Scanning electron microscopic (SEM) examination of the stigmatic surface of Zn-deficient field bean
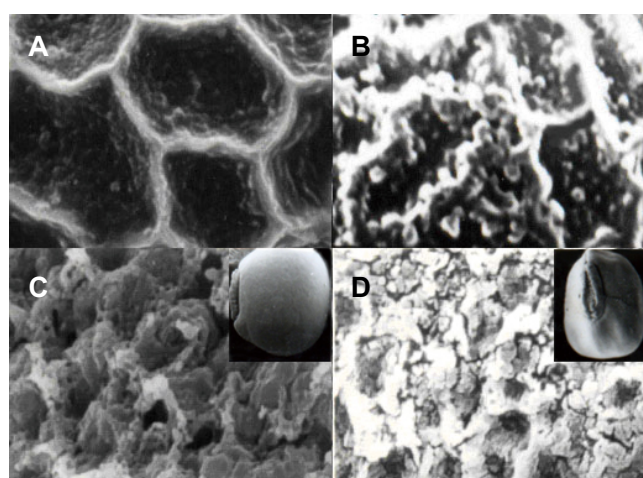

Fig. 2 Scanning electron microscopy (SEM) images of Zn-sufficient (A) and Zn-deficient (B) pollen grains. Pollen grains of A show uniform muri and those of B show sinuous lobed muri, with waxy depositions filling the cavity between baculae. SEM images showing seed coat topography (whole seed inset) of Zn-sufficient (**C**) and ZnD (**D**) seeds. Reprinted from **Pandey N, Pathak GC, Sharma CP** (2009) Impairment in reproductive development is a major factor limiting reproductive yield of black gram under zinc deficiency. *Biologia Plantarum* **4**, 723-727, ©2009, with kind permission of Springer Science and Business Media, Dordrecht, The Netherlands.

and lentil flowers revealed decrease in pollen receptive area of the stigmatic head (Pandey *et al.* 1995, 2006). The Zn-deficient stigmas of these plants have been shown to have a persistent cuticle over stigmatic papillae which prevented secretion of stigmatic exudates leading to poor adhesion of the pollen grains. The rupture of cuticle for pollen germination requires activities of enzymes like cutinases and esterases (Dafni and Maues 1998; Hiscock *et al.* 2002). In stigmatic exudates and pollen grains of Zn deficient black gram (Pandey *et al.* 2009) the activity and isoforms of acid phosphatase which is inhibitory to pollen tube growth was enhanced (**Fig. 3A, 3B**) whereas the activity of esterase which is conducive to pollen germination was poorly expressed (**Fig. 3C, 3D**). Alleviation of Zn deficiency (0.1 μM ZnSO$_4$) by raising Zn supply (1 μM ZnSO$_4$) prior to flowering decreased the severity of Zn deficiency effects on pollen and stigma morphology, pollen fertility and seed yield (Pandey *et al.* 2006, 2009).

As a constituent of Zn-finger proteins Zn plays an important role in floral initiation and gametogenesis. In plants, as many as 30 proteins have been reported that have one, two, three or four TFIIIA-type Zn-fingers in transcription
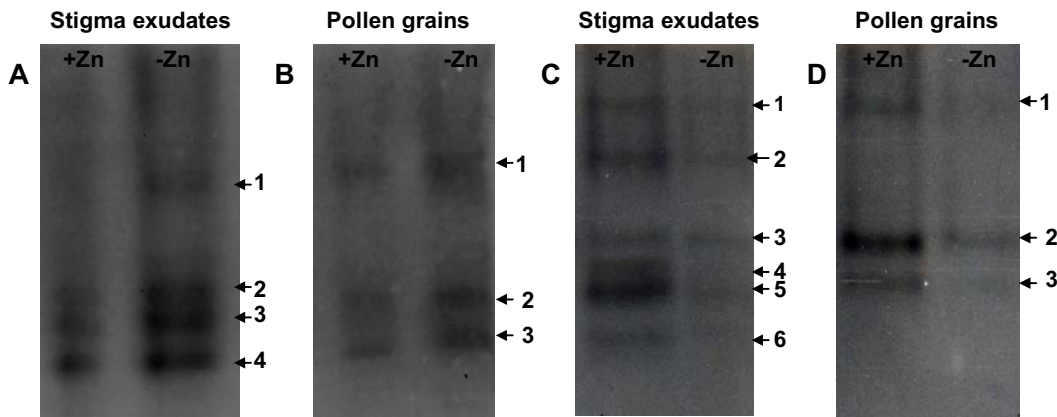
Fig. 3 Activity staining of native gels showing isoforms of acid phosphatase (A, B) and esterase (C, D) in stigma exudates (A,C) and pollen extracts (B, D) of Zn-sufficient and -deficient plants. Reprinted from **Pandey N, Pathak GC, Sharma CP** (2009) Impairment in reproductive development is a major factor limiting reproductive yield of black gram under zinc deficiency. *Biologia Plantarum* **4**, 723-727, ©2009, with kind permission of Springer Science and Business Media, Dordrecht, The Netherlands.

factors as DNA-binding motifs (Takatsuji *et al.* 1992; Sakai *et al.* 1995). In the TFIIIA-type Zn-finger (also referred to as Cys2/His2 or classical Zn-finger) two cysteines and two histidines tetrahedrally coordinate a Zn atom to form a compact finger structure containing a β-hairpin and a α–helix (Choo and Klug 1997). Several proteins that contain the TFIIIA type Zn-finger have been reported from *Petunia*, *Arabidopsis* and other plant species and have been assigned regulatory functions (Kubo *et al.* 1998; Takatsuji 1999). Thus SUPERMAN, AtZFP1, PetSPL3 and BcZFP1 have been implicated in the developmental regulation of various floral and vegetative organs, presumably through the control of cell division and/or expansion in particular cell types (Takatsuji 1999). Tissue- and development-specific Zn-finger proteins containing either two, three, or four Zn-fingers have been shown to be expressed specifically in anther during pollen development and their expression patterns suggest their involvement in the regulation of developmental events during gametogenesis (Kobayashi *et al.* 1998). Thus *ZPT3-2* is expressed specifically in the tapetal tissue and supports the development of male gametophytes; the *ZPT3-2* expression is from the tetrad stage till degeneration of tapetal layer; *ZPT4-3* and *ZPT3-1* are expressed in the microspores and *ZPT2-6* is expressed in mature pollen grains and in stomium cells prior to dehiscence (**Table 1**).

The *Arabidopsis MS1* (*MALE STERILITY1*) gene that is homologous with the PHD-finger motifs (C4HC3 Zn-finger-like motifs) was also reported to be a critical sporophyte-controlling factor for anther and pollen development (Wilson *et al.* 2001). The PHD (plant homeo domain) finger is a modified Zn-finger and is found in plants as a transcriptional regulator of genes involved in defense against pathogens (Korfhage *et al.* 1994) and in genes that are required for reproductive development and fertility. It plays a role in chromatin organization and transcriptional regulation. *Arabidopsis* male-sterile mutant exhibits meiotic defects. Meiocytes from mutant plants appeared normal up to diakinesis, after which they exhibited signs of apoptosis, including defects in chromosome behavior, cytoplasmic shrinkage, and chromatin fragmentation, followed by meiocyte cell death before cytokinesis. The *MMD1*encodes a PHD finger which is expressed preferentially in male meiocytes (Yang *et al.* 2003). The *MMD1* gene was found to be identical to *DUET* a gene reported by Reddy *et al.* (2003) who showed its requirement for male meiotic chromosome organization and suggested that in the absence of *DUET* function, male meiocytes undergo defective progression through the meiotic cell cycle.

Kapoor *et al.* (2002) reported *TAZ1* (*TAPETUM DEVELOPMENT ZN-FINGER PROTEIN1*) which encodes a protein with three Zn-finger motif and shows biphasic expression pattern in *Petunia*. In the premeiotic phase, *TAZ1* transcripts were found to accumulate in all cell types of the anther except the tapetum and gametophytic tissues. However, the postmeiotic phase of anther development was characterized by its expression exclusively in the tapetum indicating its role in microspore maturation. Silencing of *TAZ1* resulted in premature degeneration of tapetum and pollen abortion soon after their release from pollen tetrads in petunia. A few pollen grains that survived showed reduced flavonol accumulation, defects in pollen wall formation, and poor germination rates. No effect was however found on female fertility, because cross-pollination of *TAZ1*-silenced flowers by wild-type pollen yielded normal seed set.

Reproductive development, including gametogenesis in Zn-deficient plants, could also be inhibited because of limited delivery of Zn to the reproductive organs. Besides its role in translocation of heavy metals Fe, Cu, Zn and Mn, the role of NA (nicotianamine) for development of reproductive organs has already been discussed with respect to Fe. Takahashi *et al.* (2003) have suggested that NA may also be required for intracellular regulation for Zn-finger proteins. Zinc deficiency may result in *naat* tobacco due to shortage of NA and thus affect the functions of Zn-finger proteins, leading to sterility. Keeping in view its role as an intercellular metal carrier, the absence of NA may inhibit the transfer or the removal of Zn from the Zn-finger proteins thereby affecting the interaction between the transcription factors within the cells. FIL (*FILAMENTOUS FLOWER*) is one of the Zn-finger proteins (having two Zn ions) involved in morphogenesis and its binding to DNA or interaction with other transcription factors is regulated by the release of one Zn ion by EDTA (Kanaya *et al.* 2001). FIL is thought to interact with LFY (LEAFY) and APETALA1 (Sawa *et al.* 1999) and required for floral organ formation, determining their correct numbers and positions (Chen *et al.* 1999). Zinc is also related to female fertility, because Zn-finger Polycomb group proteins are necessary for proper female gametophyte and seed development (Grossniklaus *et al.* 1998; Brive *et al.* 2001). Payne *et al.* (2004) isolated a recessive, conditionally male-sterile *Arabidopsis* mutant, *knuckles* (*knu*). They identified *KNUCKLES* which encodes a small protein containing a single C2H2 Zn-finger and functions as a transcriptional repressor. KNU expression occurs early during the development of gynoecium and persists near its base until after ovule primordia appear. KNU suppresses overgrowth of basal gynoecial structures such as the nectaries and gynophore to allow for full development of the ovaries, and prevents the placentae therein from acquiring floral meristem identity.

Zinc also plays a role in floral induction which converts the vegetative meristem into a reproductive one. Colasanti *et al.* (1998) showed that the *indeterminate1* gene (*id1*) controls the transition to flowering in maize. The *id1* gene encodes a protein with Zn-finger motifs, suggesting that the

id1 gene product functions as a transcriptional regulator of the floral transition. SUPERMAN (*At*SUP) contributes to the maintenance of floral whorl boundaries (Sakai *et al.* 1995). CONSTANS (CO) has been assigned a role in long-distance signaling in the photoperiodic regulation of flowering in *Arabidopsis*. CO encodes a Zn-finger transcription factor that directly induces other genes involved in floral induction, such as SUPPRESSOR OF OVEREXPRESSION OF CO 1 and FLOWERING LOCUS T (Samach *et al.* 2000; Takada and Goto 2003). Ayre and Turgeon (2004) have implicated CO as a mediator between photoperiod perception and the transition to flowering in *Arabidopsis*. They observed that CO expression in tissues at the inception of the phloem translocation stream in leaves was sufficient to accelerate flowering at the apical meristem under non inductive (short-day) conditions and substitute for photoperiod in generating a mobile flowering signal. Kim *et al.* (2008) have recently shown that regulation of CO by light quality plays a key role in the regulation of flowering time and that plants in which flowering is promoted by Far Red light expressed higher levels of *CO* mRNA.

A number of plant ZFPs (Zn-finger proteins) from *Petunia* and *Arabidopsis* have also been implicated in floral organogenesis, gametogenesis and pollen abortion (Yoshida *et al.* 2001; Kapoor and Takatsuji 2006). Goldar *et al.* (2005) identified *moc1–moc4* genes in *Schizosaccharomyces pombe* that overcome a partially sterile strain. Of these, Moc3 was found to be a novel protein containing a Zn-finger (Zn(2)-Cys(6)) motif while Moc4 was identical with a Zn-finger protein Zfs1. They constructed a deletion mutant of the moc3 gene and found that its disruptant was lower in mating efficiency and formed aberrant asci.

Agarwal *et al.* (2007) observed that the genome of *Oryza sativa* codes for 189 C2H2 Zn-finger transcription factors of which 39 genes were up-regulated more than 2-fold, during reproductive development, as compared to that at vegetative stages. Of these 18 were found to be specific to panicle development and 12 genes were seed specific. They named these rice proteins as ZOS (ZPTs of *Oryza sativa*). Sicard *et al.* (2008) reported the INHIBITOR OF MERISTEM ACTIVITY (IMA) gene encoding a mini Zn-finger (MIF) protein from tomato (*Solanum lycopersicum*) which regulates the processes of flower and ovule development. They observed that IMA controls the number of carpels during floral development and under expression of IMA prevents the formation of gametophytic tissue, which is replaced by an undetermined sporophytic tissue.

Seeds of Zn-deficient plants take a longer time to mature (Boawn *et al.* 1969; Baylock 1995; Morghan and Grafton 1999). Gupta *et al.* (1994) reported alterations in seed coat topography, which could possibly inhibit their germination. Thick waxy depositions in the seed coat of seeds from Zn-deficient black gram plants (**Fig. 2C, 2D**) reduced the germination of seeds (Pandey *et al.* 2009). Supply of Zn (0.065 mg L^{-1}) to Zn-deficient (0.0065 mg L^{-1}) plants prior to initiation of flowering partially mitigated the effects of Zn on seed and their germinability suggesting the role of Zn in seed development (Pandey *et al.* 2006, 2009). The positive role of Zn-finger transcription factor on seed germination has been shown in *Arabidopsis* (Liu *et al.* 2005). Based on the study of Zn levels at different stages of seed development Otegui *et al.* (2002) have reported accumulation of Zn, as Zn-phytate, in the chalazal vacuoles of the endosperm and its disappearance at the late globular stage in *Arabidopsis*. They observed that as per their need during the course of embryo development, Zn-phytate stored in the chalazal vacuoles of the endosperm functions as the Zn source for the assembly of several diverse Zn-proteins which are expressed during embryogenesis.

Chaudhury *et al.* (1999) cloned two genes, *FIS1* and *FIS2* (fertilization-independent seed development), in *Arabidopsis* which confer female gametophytic phenotypes. These two genes control both fertilization independent seed development and post pollination embryo development in *Arabidopsis*. *FIS2* encodes a protein with a C_2H_2 Zn-finger motif and *FIS1* encodes a protein with homology to the *Drosophila Polycomb* group gene. Sagasser *et al.* (2002) reported TT1 (Transparent Testa 1) which represents a novel type of plant Zn-finger protein with similarity to *Zm*ID1 and *St*PCP1 (*Zea mays and Solanum tuberosum*) and is responsible for the yellow seed color, attributed to the lack of condensed tannins in the seed coat of the *tt1* mutant. The yellow appearance of mature *tt1* mutant seeds is due to lack of TT1 function in young endothelium cells which resulted in inhibition of pigment synthesis. They observed that *tt1* is the first Zn-finger factor required for cell-specific flavonoid accumulation mutation and had no effect on flavonoid biosynthesis in vegetative plant parts, but specifically affected the pigmentation of a single cell layer of the seed coat. TT1 acts as a transcriptional regulator rather than an enzyme involved in seed pigment formation. In plants few genes encoding RING (Really Interesting New Gene) Zn-finger proteins have been documented which affect plant growth and development. Xu and Li (2003) identified a novel gene, *RIE1*, encoding a RING-H2 Zn-finger protein in *Arabidopsis thaliana* with an important role in seed development. Kim *et al.* (2007) found a Zn-finger containing glycine-rich RNA binding protein, atRZ-1a, that retarded germination and seedling growth in salt stressed *Arabidopsis* by modulating the expression of several germination responsive genes in an ABA-dependent way.

Molybdenum

Molybdenum is a metal of the second transitional series and exists in four oxidation states of which the hexavalent (Mo(VI)) form is the most stable. Over 30 enzymes catalyzing oxidation-reduction reactions contain a Mo cofactor (MoCo) in the form of molybdopterin (Mendel and Haensch 2002). The role of Mo in assimilation of nitrate and dinitrogen fixation involves oxidation-reduction reactions during electron transport and is critical for the nitrogen metabolism of plants. The critical concentration of Mo is not only lowest amongst all micronutrients, it also varies widely in plant species and with the form of nitrogen they utilize. Thus the nodulating legumes have a higher requirement for Mo than the non-nodulating ones. Likewise Mo requirement for plants grown with reduced (ammonical) form of N is lower than those grown with nitrate-N. The deficiency values for Mo range between 0.01 to 0.5 μg g^{-1} dry weight. (Gupta 1997).

Molybdenum nutrition has a profound effect on plant reproductive development and seed yield (Kaiser *et al.* 2005). Studies on Mo nutrition of maize (Agarwala *et al.* 1978, 1979), sorghum (Agarwala *et al.* 1988) and wheat (Chatterjee and Nautiyal, 2001) have shown that reproductive development, including grain yield, is reduced by Mo deficiency. Molybdenum deficiency in maize (Agarwala *et al.* 1978a) shows reduction in cob size, failure of styles to protrude out of the husk and poor seed set. Agarwala *et al.* (1979) observed an appreciable delay in formation of tassels and poor development of anthers in Mo deficient maize plants (0.0001 mg L^{-1}) showing moderate foliar symptoms. In plants severely deficient in Mo (0.00001 mg L^{-1}) not only was the development of tassels and their emergence delayed but their size was markedly reduced. The lemma and the palea borne on these were also severely chlorotic and papery. In these plants the anther size was severely restricted and they appeared shriveled (because of lack of sporogenous tissue). The anthers produced fewer and smaller pollen grains which were highly vacuolated and had poor viability. The Mo-deficient pollen grains lacked cytoplasmic contents, stained poorly for starch and showed poor *in vitro* germination. The loss of viability of the pollen grains could be a consequence of low activity of invertase in Mo-deficient pollen grains indicating poor sucrose utilizing capacity. A marked increase in the number of melon (*Cucumis melo* L.) fruits produced on acid soil deficient in Mo, in response to Mo application also suggests a role of Mo in pollen fertility (Gubler *et al.* 1982).

Molybdenum deficiency has recently been suggested as the primary cause of a bunch development disorder called *Millerandage* or 'hen and chicken' in grapevines (Williams *et al.* 2004). The poor fertilization of the ovaries results in the formation of a bunch of grapes that develop unevenly with unfertilized and underdeveloped ovaries along with the fully matured berries.

Molybdenum also effects the seed development and vigor. Gurley and Giddens (1969) observed that sowing soybean (*Glycine max*) seeds rich in Mo content in soils deficient in Mo was beneficial for establishment of seedlings and seed yield. Soil application and seed treatment (70 to 200 g Mo ha^{-1}) with Mo increased the seed yield significantly in legumes (Adams 1997; Deo and Kothari 2002). Effect of Mo deficiency on development of wheat seeds, their chemical composition and vigor has been described by Chatterjee and Nautiyal (2001). They reported decrease in endosperm reserves such as the concentration of starch, sugars, protein and non-protein nitrogen in these seeds. Qualitative changes such as decrease in prolamins, glutelin and globulin and increase in albumin were also observed in the seeds of Mo-deficient plants. The increase in the relative proportion of albumins in these seeds could be a factor contributing to their poor viability and loss of vigor. In seeds of chickpea (*Cicer arietinum*) also, Mo deficiency leads to decrease in relative concentration of legumins and vicilins and increase of albumins (Nautiyal *et al.* 2005).

Molybdenum deficiency reduces seed dormancy leading to pre-harvest sprouting of cereals grains. Low Mo seeds show premature sprouting or vivipary in maize (Tanner, 1978; Farwell *et al.* 1991). Foliar application of Mo has been shown to control premature sprouting of grains in wheat (Cairns and Kurtzinger, 1992). The deficiency response is perhaps related to ethylene production involving the role of Mo as a cofactor of aldehyde oxidase, which catalyzes the conversion of abscisic aldehyde to abscisic acid (Seo and Koshiba 2002). Abscisic acid is responsible for suppressing premature germination through the induction of primary dormancy.

Boron

Boron is a metalloid existing in three valency states. It forms complexes with hydroxyl radicals to form *cis*-diol groups which forms the basis of its structural role in plant cell walls (Loomis and Durst 1992). Boron cross-linking of cell wall rhamnogalacturonan II provides the cell wall with a structure capable of turgor driven growth like that of the pollen tube (O'Neill *et al.* 2004). Its deficiency induces changes in membrane potential and H$^+$ extrusion resulting in impairment of membrane function (Cakmak and Romheld 1997) which could also have an impact on pollen tube growth and extension. Crops differ widely in their B requirement. Thus gramminaceous monocots (cereals) have a much lower requirement of B than the dicotyledons (crucifers and legumes). The critical level of B deficiency remains 5 to 10 µg g^{-1} dry weight in most crops below which drastic reductions are observed in reproductive yield.

Boron plays an important role in reproductive development. The effect of B deficiency is more pronounced on the reproductive growth especially flowering, fruiting, seed setting and seed yield than on vegetative growth (Sherrell 1983; Loomis and Durst 1992; Noppakoonwong *et al.* 1997). The reproductive parts of flowers such as anthers, ovary and stigma show aberrations even without prior expression of symptoms of B deficiency during vegetative growth. Under B deficient condition these floral parts possess a much higher concentration of B than the other parts (Gauch and Dugger 1954; Adams *et al.* 1975; Xu *et al.* 1993), reiterating an involvement of B in plant reproductive development. Thus while the B requirement in anthers for successful grain set is 10 mg B kg^{-1} dry matter, leaves of wheat having less than 2 mg B kg^{-1} dry matter fail to show symptoms of B deficiency (Rerkasem *et al.* 1997). Inadequate supply of B is reflected in delayed and restricted

flowering, premature bud abscission, pollen sterility, decreased seed set and poor seed yield. Severe reduction in number and size of flowers was observed in plants subjected to B deficiency (Adams *et al.* 1975; Zhang *et al.* 1994). Decrease in the number of flowers in B-deficient plants may be caused by the breakdown of the apical meristem restricting emergence of flower bearing branches and premature bud abscission. Several factors restricting the supply of B to the reproductive organs may be responsible for limitation in reproductive development and seed yield of B-deficient plants. Slow rate of transpiration due to the foliar coverings of the reproductive parts and the inflorescence may result in passive delivery of B to meet the requirement of the reproductive organs (Hansen and Breen 1985). Various environmental constraints such as temperature, light, humidity and water could also restrict transport of B during the critical stages of reproductive development (Dell and Huang 1997). The immobility of B from leaves and other mature tissues to the floral parts (Brown and Shelp 1997) and poor access of the pollen grains and the embryo sacs to the vascular supply are major factors for poor B delivery to the reproductive organs (Rawson and Nippakoonwonge 1996; Dell and Huang 1997; Brown *et al.* 2002).

Male gametogenesis has been reported to be particularly sensitive to low B supply (Rerkasem and Loneragan 1994). Deficiency of B can result in plants becoming functionally male sterile especially in cereals: barley (Simojoki 1972), wheat (Li *et al.* 1978) and rice (Garg *et al.* 1979). However cases of female sterility have also been reported in maize (Vaughan 1977) and avocado (Coetzer and Robbertse 1987). Several studies have shown an involvement of B in microsporogenesis and male fertility (Zhang *et al.* 1994; Rerkasem *et al.* 1997; Huang *et al.* 2000). Boron involvement in pollen development and fertility was first reported by Lohnis (1937) who observed atrophy of anthers in B-deficient plants of several cereal crops. Small stamens have been observed in B-deficient oilseed rape (*Brassica napus* L) which also shows an abnormally developed tapetum (Zhang *et al.* 1994). It was observed that in B-deficient maize plants emergence of tassels and anthers was delayed, the anthers lacked sporogenous tissue and stamens turned into floral appendages or staminodes (Agarwala *et al.* 1981). In wheat florets each sterile spikelet appear to be normal with fully developed lemma and palea but anthers are absent or poorly developed being small and misshapen with few malformed pollen grains (Rerkasem *et al.* 1991; Cheng and Rerkasem 1993). In wheat under severe conditions of deficiency, anthers develop as small arrow-shaped structures largely devoid of cells in the anther locules and exhibit inhibition of floret fertility (Huang *et al.* 2000).

Boron deficient plants show arrest of microsporogenesis beyond the pollen mother cell stage (Xu *et al.* 1993). Anatomical investigation of pollen development in a B-sensitive (SW41) and B-insensitive (Sonora) wheat failed to detect any abnormality in pollen until after the uninucleate vacuolate stage and pollen grains formed were sterile at anthesis (Rerkasem *et al.* 1997). Visible impairment in pollen development in B-deficient wheat did not appear until after the microspores were released from the tetrads. This means that meiosis proceeded satisfactorily unlike the case in Cu deficiency where microsporogenesis is impaired during early meiosis (Graham 1975). However the effect of B deficiency is expressed structurally later in pollen development and at anthesis. The pollen grains in well-formed anthers appear empty, misshapen, shrivelled, or may be normal in shape but lack reserves of storage materials such as starch. Rawson (1996a) found that the critical period for pollen development extends after emergence of the flag leaf tip to shortly after the flag leaf has become fully expanded. The flag leaf may play an important role in translocation of carbohydrate for pollen development. Huang *et al.* (2000) showed that there may be two phases of pollen development during which B deficiency impairs pollen viability: (a) meiosis to young microspore and (b) mitosis-I to pollen maturation. They showed that the period from premeiotic

interphase through meiosis to late tetrad was the most sensitive stage of microsporogenesis in wheat while the period from mitosis-I to II during which starch accumulation occurred in pollen grains was less sensitive. During the first phase, impaired formation of pollen cell walls and cell expansion may be the primary consequence of B deficiency whereas during the second phase, carbohydrate delivery necessary for starch accumulation may result in pollen grains poor in starch content.

Pollen germination and pollen tube growth are particularly sensitive to B and pollen viability is impaired by B deficiency (Vasil 1964; Duggar 1983). In B-deficient maize, pollen size and germination is severely reduced and was observed even before induction of foliar symptoms of B deficiency (Agarwala *et al.* 1981; De Wet 1989). Rerkasem *et al.* (1993) reported that impaired development of anthers and poor pollen germination results in a failure of fertilization in B-deficient wheat. *In vitro* germination tests also show that B was required for pollen germination and tube growth in wheat (Cheng and Rerkasem 1993), avocado (Smith *et al.* 1997) and *Picea meyeri* (Wang *et al.* 2003).

Boron plays an essential role in the structure and function of cell walls and cellular membranes (Cakmak and Romheld 1997; Matoh *et al.* 1997; Ishii *et al.* 1999) and as such a role for B in pollen cell walls would be inevitable. Boron deficiency may alter the deposition of callose, cellulose and pectate in cell walls of anther and pollen cells. In wheat, low B did not alter the structural appearance of the mature pollen wall under the transmission electron microscope (Rerkasem *et al.* 1997a). By contrast, Smith *et al.* (1997) observed that the exine was less extensively developed in pollen from B-deficient avocado. In a recent study in *Pisum sativum* (Pandey and Gupta 2009, unpublished data) B deficiency failed to produce any significant changes in the exine ultrastructure but markedly reduced the size and viability (% germination) of the pollen grains.

Cytoskeletal changes induced by B deficiency observed in tips of meristematic cells (Baluska *et al.* 2002; Yu *et al.* 2002) suggest possible involvement of B in cytoskeletal changes preceeding pollen germination and pollen tube growth. Matoh *et al.* (1998) detected borate-rhamnogalacturonan-II (RGII) complex in the cell walls of pollen tubes. It has been reported that the walls of growing tips of pollen tubes are composed predominantly of pectic polysaccharides including RG-II and that the normal growth of pollen tubes requires borate- and Ca-dependent cross-linking of pectin (O'Neill *et al,* 2004). Recently, Iwai *et al.* (2006) showed that *NpGUT1* gene encoding a glycosyltransferase involved in the borate cross-linking of pectin RG-II is required for male and female reproductive tissues and fertilization. Any alteration in the composition of the main side chain A in RG-II in the vicinity of the apiosyl residue provokes important developmental and reproductive disorders. Delmas *et al.* (2008) reported that the synthesis of the 3-deoxy-D-manno-2-octulosonic acid (Kdo) component of RG-II is required for pollen tube growth and elongation. Their observations using the Kdo-8-P synthase (*AtkdsA2*-V and *AtkdsA2-S*) gene expression are strikingly similar to the effects induced by *NpGUT1* misexpression and indicative of pollen tube defect related to the RGII within the plant cell wall.

Besides microsporogenesis and pollen germination B also affects pollen receptivity of the stigma, pollen tube growth through the stylar tract and development of ovule (Birnbaum *et al.* 1974). Non-receptiveness of silks in B-deficient maize plants was found to result in poor setting of grains (Vaughan 1977). The B deficient oilseed rape showed morphological aberrations in stigmatic papillae and developed abnormal embryo sacs. Enhanced accumulation of phenolic compounds on the stigmatic surface was found to inhibit the germination of pollen grains on the stigma of B-deficient *Campsis grandiflora* (Dhakre *et al.* 1994). Robbertse *et al.* (1990) reported that a gradient of B concentration along the style facilitated the growth of pollen tubes. Shen *et al.* (1994) observed that the rate of pollen tube elongation was inhibited in B-deficient plants.

The high requirement of B for reproductive development of plants has a direct bearing on seed yield (Mozafar 1993; Rerkasem *et al.* 1993; Cheng and Rerkasem 1993; Rawson 1996b). Under acute B deficiency, the crop fails to set seeds. Low grain yield of wheat is attributed to male sterility caused by reduced transport of B to anthers, where it is critically required for microsporogenesis and pollen fertility (Huang *et al.* 1996; Rerkasem 1996; Rawson 1996a; Subedi *et al.* 1998). Boron is also important for post fertilization development and seed maturation. Seeds of low B black gram plants showed poor germination and lack of vigor (Bell *et al.* 1989). Sinha *et al.* (1999) observed enhanced accumulation of phenolic compounds and decrease in oil content in seeds of low B *Sesamum* plants. In sunflower, Chatterjee and Nautiyal (2000) found that withholding B supply, even as late as the time of anthesis, produces morphological aberrations in seeds and reduces the seed content of non-reducing sugars, starch and oil. Asad *et al.* (2003) observed that foliar application of B (0.3 to 1.3 Kg ha^{-1}) during the reproductive phase enhanced seed yield upto three fold in sunflower. Singh *et al.* (2007) also reported that foliar spray of B improves fruit yield and quality of strawberries. Recently Sharma and Singh (2009) observed that low B (and Ca) resulted in development of sunken pits on fruit peel of mango ('fruit pitting disorder'). The pitted fruits had lower concentration of B (22 mg kg^{-1}) concentrations than normal fruit (38 mg kg^{-1}).

CONCLUSIONS

Studies on reproductive development following deprivation of micronutrient supply to plants, point to a more direct involvement of micronutrients (specifically Zn and B) in reproductive development. The physiological roles for micronutrients in plant reproduction have yet to be fully defined and there is a great need for experimentation in this area. Many of the studies that have been undertaken do not give definitive information because plants were critically deficient at the time of flowering and structural changes were examined long after the primary effect took place. Reproductive development of plants may be severely limited as in case of Cu, Zn and B, even when deficiency is induced nearing onset of reproductive phase, by which time plants have accumulated enough photosynthates. Therefore study of the effects of nutrient deficiency on reproductive development would be more pertinent if deficiency is induced just prior to the reproductive phase and the stage in reproductive growth which is most sensitive to the deficiency is identified. Identification of the reproductive stage most sensitive to micronutrient requirement would not only help in improving yield through fertilization (soil or foliar) but would also help in nutrient biofortification of seeds. The ultra structural changes in pollen grains observed as a consequence of deficiency of micronutrients need to be explored as changes produced in the pollen coat are important for pollen hydration and adhesion to stigma and fertilization thereof. There is also a need to characterize the pollen-stigma interaction and changes in the exudate enzymes and proteins in response to micronutrients. The involvement of transcription factors like the Zn-finger and polycomb group of proteins in reproductive development reiterate the role of micronutrients in reproductive development and their functional roles need to be further examined.

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