



## SHORT COMMUNICATION

# CHANGES IN METABOLISM AND BIOCHEMICAL COMPOSITION IN RESPONSE TO MICRONUTRIENT LIMITATIONS IN COWPEA

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To study the response of manganese (Mn), copper (Cu) and boron (B) deficiency on metabolism and developmental aberration in biochemical composition, cowpea (*Vigna unguiculata* L.) cv. Pusa Komal was grown in refined sand at adequate (complete nutrient solution) and three deficient levels of Mn (2  $\mu$ M), Cu (0.1  $\mu$ M) and B (3  $\mu$ M). Plants subjected to deficient supply developed characteristic foliar symptoms of each deficiency accompanied by marked depression in growth, dry matter, economic yield, total seed protein, concentration of Mn, Cu and B, chlorophyll content and Hill reaction activity. Exposure of cowpea plants to each deficiency induced changes in enzyme activities of peroxidase, acid phosphatase, ribonuclease, polyphenol oxidase and altered the concentration of sugar, starch and phenols in leaves and seeds indicating specific role of these micronutrients in metabolism and deposition of reserves in the seeds during seed setting of cowpea.

**Key words:** *Vigna unguiculata*, enzymes, seed reserves, economic yield, micronutrient

Most of the agricultural lands in India have been widely reported to be deficient in manganese (Mn), copper (Cu) and boron (B) (He *et al.* 2005). Deficiencies of each micronutrient in agronomic and horticultural crops can lead to plant stress (Marschner 2002) and necessitate their prompt management for sustained increases in crop yield. Higher plants require an adequate supply of Mn, Cu and B for their normal physiological and biochemical functions. Their deficiencies account for a wide gap between the production potential of the major food crops and their productivity on low micronutrient soils.

In higher plants Mn and Cu act as cationic in nature and form stable complexes with organic ligands, proteins and function as biological catalysts. They exist in more than one oxidation state and participate in redox reaction

and electron transport whereas B, a metalloid is known to be involved in structural and regulatory roles. Both Mn and Cu deficiency alters chloroplast structure and reduces chloroplastic pigments (Kastori *et al.* 1995 and Yruela 2005). As a cofactor of SOD, Mn and Cu offers protection against damage from the reactive oxygen species (ROS) (Alscher *et al.* 2002) that induces oxidative stress and play important role in signal transduction (Apel and Hirt 2004). Limited availability of the metal co-factor weakens the antioxidant defence mechanism and exposes the plants to greater damage from ROS. B has recently been implicated in oxidative stress causing peroxidative damage to cellular membrane that eventually causes impairment of several cellular functions (El-Shintinaway 1999). Mn deficiency retards both fertilization and seed maturation (Sharma, 2006) and limits grain yield due to shortage in carbohydrate supply

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for grain filling (Longnecker *et al.* 1991), ascribed to critical requirement of Mn for reproductive development whereas the role of Cu in development of both male and female reproductive phases of various plants have also been emphasized (Owuoché *et al.* 1995). Low B during reproductive development can cause devastating yield loss through male sterility (Rerkasem 1996 and Ahmed *et al.* 2007) and floral abnormalities (Sharma 2006).

Cowpea (*Vigna unguiculata* L.) is a major pulse crop with high nutritive value grown across a wide range of environment in India and worldwide. As information regarding the effect of these micronutrient deficiencies on reproductive phase of legumes with particular aspect to seed development and alteration in seed reserves is meagre, the present study will add a resource on the biochemical and physiological roles of these micronutrients in plant and the diverse ways in which they react to limitation in micronutrients supply, contributing to the knowledge on global constraints in crop production by growing the plants in refined sand at deficient and adequate levels of these micronutrients.

Cowpea cv. Pusa Komal was raised from seeds in purified sand culture with following treatments :- (i) Complete nutrient solution (Adequate) (ii) Deficient Mn (2  $\mu$ M) (iii) Deficient Cu (0.1  $\mu$ M) (iv) Deficient B (3  $\mu$ M). Plants were maintained under glass house conditions at ambient temperature (25 – 30°C) with 3 replicates in each treatment. Prior to sowing, seeds were treated with 0.01% HgCl<sub>2</sub> for 5 minutes and then washed several times with distilled water to remove any surface contamination. After emergence, four plants were maintained in each pot and were thinned to one at harvest to sample for economic yield. The composition of complete nutrient solution was: 4 mM KNO<sub>3</sub>; 4 mM Ca (NO<sub>3</sub>)<sub>2</sub>; 2 mM MgSO<sub>4</sub>; 1.5 mM NaH<sub>2</sub>PO<sub>4</sub>; 0.1 mM NaCl; 100  $\mu$ M Fe-EDTA; 30  $\mu$ M H<sub>3</sub>BO<sub>3</sub>; 1  $\mu$ M CuSO<sub>4</sub>; 10  $\mu$ M MnSO<sub>4</sub>; 1  $\mu$ M ZnSO<sub>4</sub>; 0.2  $\mu$ M Na<sub>2</sub>MoO<sub>4</sub>; 0.1  $\mu$ M NiSO<sub>4</sub> and 0.1  $\mu$ M CoSO<sub>4</sub>. In deficient treatments of Mn, Cu and B the particular nutrient(s) were omitted from the complete nutrient solution and were supplied as manganese sulphate (MnSO<sub>4</sub>), copper sulphate (CuSO<sub>4</sub>) and boric acid (H<sub>3</sub>BO<sub>3</sub>) respectively, directly to the nutrient solution at required deficient level(s). The contamination of Mn, Cu and B from purified sand, water

and nutrient solution for Mn, Cu and B were 0.02  $\mu$ M, 0.001  $\mu$ M and 0.1  $\mu$ M respectively and taken into consideration while supplying the nutrient.

After emergence of seedlings, nutrient solution was supplied at desired level daily except on weekends when pots were flushed with glass distilled water in Mn and Cu treatment and with B-free deionized water in B treatment to remove accumulated salts from the root surface. Plants were maintained in culture till maturity (83 days after sowing). After manifestation of growth differences and visible foliar symptoms of Mn, Cu and B, plants were sampled for the determination of dry matter, reproductive yield and tissue concentrations. Mn and Cu concentrations were determined by atomic absorption spectrometry whereas B concentration was estimated by colorimetrically (Wolf 1971) in oven dried leaves and air dried seeds (d 83), after wet digestion in nitric and perchloric acid (10:1). In the homogenate of fresh leaves of cowpea (d 60) the concentration of chlorophyll and Hill reaction activity was measured. The enzyme activities of peroxidase, acid phosphatase, ribonuclease and polyphenol oxidase in leaves at d 59 and in seed at d 60 were assayed (Nautiyal *et al.* 1999). Finely chopped fresh leaf tissue (d 65) and matured air dried seeds (d 83) were fixed separately in 80% (v/v) boiling ethanol (1:10) and ground at room temperature for determination of carbohydrate and total phenols (Gopal *et al.* 2006). At d 83 the total seed protein was estimated in mature seeds of harvest sampling (Bradford 1976). The experiment was laid out in completely randomized block design and data was analysed statistically (Panse and Shukhatme, 1985).

Cowpea plants subjected to Mn, Cu and B deficiencies, exhibited growth depression which was first discernible in Mn deficiency (2  $\mu$ M) at d 15, followed by B deficiency (3  $\mu$ M) at d 17 and later at d 20 in Cu deficiency (0.1  $\mu$ M). At 15 DAS (days after sowing), symptoms of low Mn manifested first on lamina of middle leaves as interveinal chlorosis which gradually intensified as irregular brown spots. In Cu deficient leaf the interveinal areas of young leaves turned chlorotic and developed minute brown spots. The visible symptoms of low boron appeared as necrosis of the margins of young expanding leaves, became severely distorted, puckered

and crumpled. Cowpea grown in refined sand exhibiting symptoms of Mn deficiency which are somewhat similar to those described earlier for soybean (Morghan 1985) while that of Cu deficiency are similar to those reported earlier for sunflower (Nautiyal *et al.* 1999). Flowering was delayed in all the three deficiencies (Mn, Cu and B) and a reduction in the number of flowers (Pandey and Sharma 2002).

Owing to deficient supply of Mn, Cu and B, the biomass and economic yield of cowpea decreased variably and the depression in dry weight was most discernible at low Mn (60%) than that of low B (56 %) and low Cu (35%) as compared to adequately supplied plants (Table 1). The number and weight of seeds were

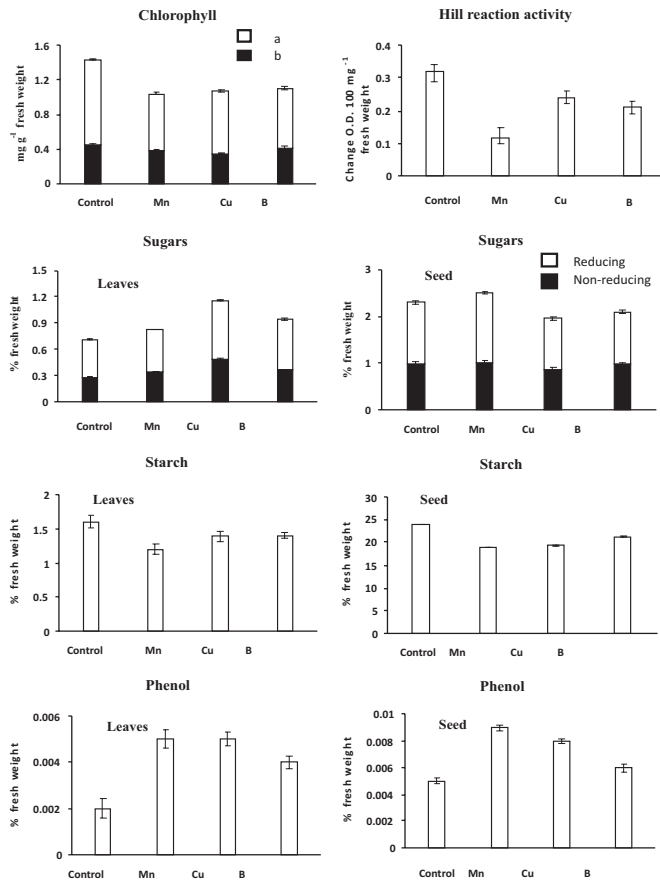
also reduced and seed weight was highly depressed in B deficiency (66%) (Table1). The reduction in dry weight in each of the three deficiencies may be due to disturbed carbohydrate and protein metabolism as has been observed in potato (Gopal *et al.* 2006) and tobacco (Yu *et al.* 1999). The decline in biomass in low Cu might reflect sink limitation, which in turn might be responsible for lowered synthesis of soluble carbohydrates (Nautiyal *et al.* 1999), thus affecting lower economic yield and poor quality of seeds. The decreased biomass as well as economic yield in low B, is due to lower RNA synthesis or low phosphate incorporation in nucleic acids as these processes are most important for development of meristematic tissue. Pods and seeds were not formed properly in Mn, Cu, and B deficient cowpea plants due to low assimilate supply or extremely low viability of pollens (Marschner 2002). However, low seed yield in B deficient cowpea might be attributed to male sterility caused by reduced transport of B to the flowering parts, where it is critically required for microsporogenesis and pollen fertility (Rerkasem 1996).

**Table 1.** Effect of Mn, Cu and B deficiency on dry matter, economic yield and concentration of Mn, Cu and B in different parts of cowpea at d 83 ( $\pm$  SE).

Control	Treatments		
	2 $\mu$ M Mn	0.1 $\mu$ M Cu	3 $\mu$ M B
	Dry weight (g plant <sup>-1</sup> )		
10.89 $\pm$ 0.09	4.35 $\pm$ 0.14	7.12 $\pm$ 0.04	4.75 $\pm$ 0.06
	Seed weight (g plant <sup>-1</sup> )		
2.73 $\pm$ 0.03	0.96 $\pm$ 0.04	1.06 $\pm$ 0.02	0.92 $\pm$ 0.01
	No. of seed plant <sup>-1</sup>		
19	8	10	7
	Mn concentration ( $\mu$ g g <sup>-1</sup> dry matter)		
	Leaves		
36.0 $\pm$ 2.2	20.12 $\pm$ 1.28	—	—
	Seed		
28.9 $\pm$ 2.22	19.0 $\pm$ 1.00	—	—
	Cu concentration ( $\mu$ g g <sup>-1</sup> dry matter)		
	Leaves		
6.56 $\pm$ 0.08	—	2.24 $\pm$ 0.02	—
	Seed		
7.07 $\pm$ 0.10	—	2.34 $\pm$ 0.03	—
	B concentration ( $\mu$ g g <sup>-1</sup> dry matter)		
	Leaves		
24.8 $\pm$ 1.2	—	—	10.1 $\pm$ 0.98
	Seed		
19.2 $\pm$ 0.99	—	—	9.80 $\pm$ 0.76

At d 83 the concentration of Mn, Cu and B in cowpea decreased markedly, under the deficiency of each nutrient in both seeds and leaves. However, the concentration of each nutrient was comparatively higher in leaves than seeds except in Cu (Table1). The reduced concentration of Mn, Cu and B are directly proportional to the supply and are in agreement with the observations of Pandey and Sharma (2002), Nautiyal *et al.* (1999) and Zhao and Oosterhuis (2003). The relatively low concentration of Mn and B in seeds than leaves under limited availability of Mn and B suggests low xylem input of these nutrients to seed as compared to its input through the phloem (Huffman and O' Halloran 2001 and Li *et al.* 2001).

The concentration of chlorophyll and Hill reaction activity in cowpea leaves were drastically reduced in each of the three deficiencies. But the reduction was most marked in followed by Cu and B in chlorophyll Mn, deficiency followed by B and Cu deficiency (Fig. 1). The decrease in chlorophyll concentration and Hill reaction activity in low Mn and Cu leaves suggests the role of Mn and Cu in photosynthetic activity. The role of Mn in oxidation of water in PS II is well documented



**Fig. 1.** Effect of Mn, Cu and B deficiency on chlorophyll concentration, Hill reaction activity, sugars, starch and phenol content in leaves and seeds of cowpea. Vertical lines represent  $\pm$ SE (n=3)

(Marschner 2002). Both Cu and Mn alter the ultra structural changes in chloroplasts (Yachandra *et al.* 1993 and Casimiro *et al.* 1990) and induce decrease in chlorophyll content. The reduced chlorophyll concentration and Hill reaction activity in boron deficient leaves are in agreement to the report on sunflower (Kastori *et al.* 1995).

The specific activity of peroxidase and acid phosphatase was increased variably at low Mn, Cu and B in both leaves and seeds (Table 2). The elevated peroxidase activity at low Mn, Cu and B might be attributed to rapid diffusion of H<sub>2</sub>O<sub>2</sub> produced in the cytosol during protective activities against oxidative damage or might be due to high accumulation of phenols and low protein formation (Roy and Bera 2002). The

**Table 2.** Effect of Mn, Cu and B deficiency on specific activities of some enzymes in leaves and seed and total protein in seeds in cowpea ( $\pm$  SE).

Plant part	Treatments			
	Control	2 $\mu$ M Mn	0.1 $\mu$ M Cu	3 $\mu$ M B
Peroxidase: Change O.D.				
Leaves	5.50 $\pm$ 0.13	9.90 $\pm$ 0.45	7.50 $\pm$ 0.22	9.86 $\pm$ 0.34
Seed	1.15 $\pm$ 0.01	1.34 $\pm$ 0.03	1.23 $\pm$ 0.04	1.76 $\pm$ 0.02
Acid phosphatase: $\mu$ g Pi liberated				
Leaves	106 $\pm$ 23	143 $\pm$ 32	137 $\pm$ 21	120 $\pm$ 14
Seed	13 $\pm$ 2.0	26 $\pm$ 1.9	22 $\pm$ 1.0	14 $\pm$ 0.89
Ribonuclease: Change O.D. min <sup>-1</sup>				
Leaves	0.625 $\pm$ 0.02	0.127 $\pm$ 0.03	0.137 $\pm$ 0.02	0.227 $\pm$ 0.03
Seed	0.056 $\pm$ 0.001	0.091 $\pm$ 0.006	0.041 $\pm$ 0.002	0.021 $\pm$ 0.001
Polyphenol oxidase: Change O.D. min <sup>-1</sup>				
Leaves	0.129 $\pm$ 0.03	0.197 $\pm$ 0.02	0.108 $\pm$ 0.04	0.189 $\pm$ 0.02
Total protein: % fresh weight				
Seed	21.0 $\pm$ 1.83	8.40 $\pm$ 1.98	8.80 $\pm$ 1.23	7.98 $\pm$ 0.99

stimulated activity of acid phosphatase in leaves, and seeds in Mn deficiency might be due to accumulation of inorganic phosphorous in different metabolic pathways (Gopal *et al.* 2006). Low Cu and B decreased the activity of ribonuclease in cowpea leaves and seeds. However, in Mn deficiency the activity decreased in leaves and increased in seeds (Table 2). In Mn deficient leaves, the activity of ribonuclease decreased as a consequence of low protein content or due to reduced availability of nitrogen whereas its increased activity in seeds might be due to decrease RNA content under such condition. The activity of poly phenol oxidase decreased considerably in low Cu and increased variably in low B and Mn in leaves (Table 2). The activity of poly phenol oxidase decreased considerably in low Cu suggesting the role of Cu in its synthesis. The increase in poly phenol oxidase activity in Mn and B deficient cowpea might be not only due to enhanced accumulation of existing phenols but also synthesis of new phenolic compounds (Chamacho Cristobal *et al.* 2002).

The concentration of sugar increased in each of the three deficiencies (Mn, Cu and B) in cowpea leaves. In seeds, the concentration of sugars was decreased in Cu

and B deficiency while increased in Mn deficiency. These results are in partial consonance with the observation on other plant species (Asad *et al.* 2003 and Gopal *et al.* 2006). However the concentration of starch decreased in both, leaves and seeds at low Mn, Cu and B (Fig. 1). The decreased starch content in Mn deficiency might be due to lowered activity of starch phosphorylase. The concentration of phenols in leaves and seeds increased variably in each of the three deficiencies (Fig. 1). The enhanced accumulation of phenols in B deficient plants led to enhanced generation of the superoxide ions (O<sub>2</sub><sup>-</sup>), causing peroxidative damage to cellular membranes. The concentration of protein in seeds decreased variably in Mn, Cu and B deficiency (Table 2). The decreased protein concentration at low Mn suggest the requirement of Mn for catalyzing RNA polymerase in protein synthesis and is in agreement with the results of Roy and Bera (2002) in mungbean. However, in Cu deficiency the decreased concentration of protein might be the consequence of the accumulation of non-protein nitrogen in seeds, substantiate the disturbed protein metabolism in the deficiency (Nautiyal *et al.* 1999). The concentration of protein decreased in low B supply might be due to decreased RNA concentration or its role in facilitation of nitrate uptake (Ruiz *et al.* 1998).

It is concluded from the present study that the exposure of each deficiency of Mn, Cu and B to cowpea plants significantly reduced biomass, seed yield, concentration of Mn, Cu and B, chlorophyll content and Hill reaction activity and induced changes in enzyme activities of peroxidase, acid phosphatase, ribonuclease, poly phenol oxidase as well as altered the concentration of sugar, starch and phenols in leaves and seeds indicating specific role of these micronutrients in metabolism and deposition of seed reserves during seed setting of cowpea.

## REFERENCES

- Ahmed, M., Jahiruddin, M. and Main, M.H. (2007). Screening of wheat genotype for boron deficiency. *J. Plant Nutr.* **30**: 1127-1138.
- Alscher, R.G., Erturk, N. and Heath, L.S. (2002). Role of superoxide dismutases (SODs) in controlling oxidative stress. *J. Exp. Bot.* **53**: 1331-1341.
- Apel, K. and Hirt, H. (2004). Reactive oxygen species: metabolism, oxidative stress and signal transduction. *Ann. Rev. Plant Biol.* **55**: 373-400.
- Asad, A., Blamey, F.P.C. and Edwards, D.G. (2003). Effects of boron foliar applications on vegetative and reproductive growth of sunflower. *Ann. Bot.* **92**: 565-570.
- Bradford, M.M. (1976). A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Ann. Biochem.* **79**: 248-244.
- Camacho-Cristobal, J.J., Anzellotti, D. and Gonzalez-Fontes, A. (2002). Changes in phenolic metabolism of tobacco plants during short-term boron deficiency. *Plant Physiol. Biochem.* **40**: 997-1002.
- Casimiro, A., Barroso, J.P. and Pais, M.S. (1990). Effect of copper deficiency on photosynthetic electron transport in wheat plants. *Plant Physiol.* **79**: 459-464.
- El-Shintinawy, F. (1999). Structural and functional damage caused by boron deficiency in sunflower leaves. *Photosynth.* **36**: 565- 573.
- Gopal, R., Dube, B.K. and Chatterjee, C. (2006). Effect of Mn stress on yield, productivity and metabolism of potato. *Ind. J. Hort.* **63**:174-177.
- He, Z.L., Yang, X.E. and Stoffella, P.J. (2005). Trace elements in agro-ecosystems and impact on the environment. *J. Trace Elements Med. Biol.* **19**: 125-140.
- Huffman, D.L. and O' Halloran, T.V. (2001). Function, structure and mechanism of intracellular trafficking proteins. *Ann. Rev. Biochem.* **70**: 677-701.
- Kastori, R., Plesnicar, M., Pankoic, D. and Sakae, Z. (1995). Photosynthesis, chlorophyll fluorescence and soluble carbohydrates in sunflower leaves as affected by boron deficiency. *J. Plant Nutr.* **18**: 1751-1763.
- Li, C., Dannel, F., Pfeffer, H., Romheld, V. and Bangerth, F. (2001). Effect of boron starvation on boron

- compartmentation, and possibly hormone-mediated elongation growth and apical dominance in pea (*Pisum sativum*) plants. *Physiol. Plant.* **111**: 212-219.
- Longnecker, N.E., Graham, R.D. and Card, G. (1991). Effects of manganese deficiency on the pattern of tillering and development of barley (*Hordeum vulgare*). *Field Crops Res.* **28**: 85-102.
- Marschner, H. (2002). Mineral Nutrition of Higher Plants. London: Academic Press, New York.
- Morghan, J.T. (1985). Manganese deficiency in soybeans as affected by ferric EDTA and low soil temperature. *J. Soil Sci. Soc. Am.* **49**:1584-1586.
- Nautiyal, N., Sharma, C. P. and Chatterjee, C. (1999). Role of copper in improving the seed quality of sunflower (*Helianthus annuus*). *Indian J.Agric.Sci.* **69**: 210-213.
- Owuoche, J.O., Briggs, K.G., Taylor, G.J. and Penny, D.C. (1995). Response of eight Canadian spring wheat (*Triticum aestivum* L.) cultivars to copper: Copper content in the leaves and grains. *Canadian J. Plant Sci.* **75**: 405-411.
- Pandey, N. and Sharma, C.P. (2002). Metabolic changes in cotton plants subjected to copper deficiency and recovery. *Indian J. Plant Physiol.***7**: 31-34.
- Panase, V.J. and Sukhatme, P. V. (1985). Statistical methods for agricultural workers. New Delhi, India: Indian Offset Press.
- Rerkasem, B. (1996). Boron and plant reproductive development. In: H.M. Rowson and K.D. Subedi (eds.), Sterility in Wheat in Sub-tropical Asia:Extent, Causes and Solutions, pp. 32-55. ICIAR, Canberra.
- Roy, S.B. and Bera, A.K. (2002). Effects of mercury and manganese on amylase, protease and peroxidase enzyme activities in germinated seedlings of mungbean. *Adv. Plant Sci.***15**: 201-206.
- Ruiz, J.M., Baghour, M., Bretones, G., Belakbir, A. and Romero, L. (1998). Nitrogen metabolism in tobacco plants (*Nicotiana tabacum* L.): Role of boron as a possible regulatory factor. *Int. J. Plant Sci.* **159**:121-126.
- Sharma, C.P. (2006). Plant Micronutrients. USA: Science Publishers.
- Wolf, B. (1971). The determination of boron in soil extracts, plant materials, compost, water and nutrient solutions. *Commun. Soil Sci. Plant Anal.* **2**:363-374.
- Yachandra, V.K., De Rose, V.J., Latimer, M.J., Mukerji, I., Sauer, K. and Klein, M.P. (1993). Where plants make oxygen: A structural model for the photosynthetic oxygen- evolving manganese complex. *Sci.* **260**: 675-679.
- Yruela, I. (2005). Copper in plants. *Braz. J. Plant Physiol.* **17**:145-146.
- Yu, Q., Osborne, L.D. and Rengel, Z. (1999). Increased tolerance to Mn deficiency in transgenic tobacco overproducing superoxide dismutase. *Ann. Bot.* **84**: 543-547.
- Zhao, D. and Oosterhuis, D.M. (2003). Cotton growth and physiological responses to boron deficiency. *J. Plant Nutr.* **26**: 855-867.