Indian J. Plant Physiol., Vol. 13, No. 2, (N.S.) pp. 198-202 (April-June, 2008)

SHORT COMMUNICATION

MANGANESE DEFICIENCY AFFECTS THE GROWTH, METABOLISM AND YIELD OF CHICKPEA

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Received on 14 June, 2008, Revised on 27 June, 2008

The effects of manganese (Mn) deficiency on growth and yield were examined in chickpea (*Cicer arietinum*) *cv* 13.G-256. The plants were grown in purified sand supplied with nutrient solution without Mn. The plants were given Mn at two levels, viz., low (2µM deficient) and normal (10µM) levels in the form of $MnSO_4.H_2O$. In Mn deficiency chlorosis appeared in the young and middle leaves at 35 DAS. Chlorotic leaves turned brown and necrotic. Severe deficiency caused reduction in the branching and flowering. Pods were underdeveloped and seed formation was reduced. In addition to decreased biomass, Mn deficiency also reduced the chlorophyll concentration and photosynthesis as indicated by reduced Hill reaction. Furthermore, amount of Mn, starch, total nitrogen and protein were markedly reduced in various plant parts. The concentration of sugars in leaves and phenols in leaves and seeds were increased. There was a significant reduction in the activities of peroxidase, acid phosphatase and RNase in seeds, although these were enhanced in the leaves. Peroxidase and RNase were higher than acid phosphatase in pod walls. The deficiency of Mn resulted in reduction of seed yield.

***Key words*: *Cicer arietinum*, Mn deficiency, reproductive yield, seed reserves**

The importance of Mn as an essential micronutrient was recognized as early as 90 years ago. Lack of Mn available to plants is a major factor limiting crop production since it plays an important role in maintaining yield, quantity and quality (Brennan and Bolland 2004, Kochain 2000). The availability of Mn is reduced by soil conditions like high pH which oxidizes Mn^{2+} to MnO_2 (Martens and Westermann 1991). Visible symptoms of Mn deficiency are interveinal chlorosis and necrosis of the leaves under advanced stage (Campbell and Nable 1988). Manganese has role in photosynthesis (Mengel and Krikby 2001), photo-oxidation, N metabolism (Marschner 1997), and in the function of enzymes namely, MnSOD (Mn- superoxide dismutase), enzymes of the shikimic acid pathway, biosynthesis of aromatic acids, and many secondary products, like lignin, flavonoids and IAA (Burnell 1988). The deficiency of Mn reduces the grain yield, mainly due to short supply of carbohydrates to the grains (Longnecker *et al*. 1991). Low Mn produces 'split' seeds, decreases seed protein, oil and fatty acids (Wilson *et al*. 1982).

Chickpea is an important crop in India and the seeds are rich in proteins. The low and control levels of Mn on the yield of seeds and metabolism of the leaf and pod-walls of chickpea grown in sand culture were examined.

Chickpea (*Cicer arietinum* L.) cv.13.G-256, plants were grown in refined sand culture (Hewitt 1966) at two levels of Mn, 2 µM (deficient) and 10µM (adequate), supplied as manganese sulphate ($MnSO_4.H_2O$) in polyethylene containers having central drainage hole,

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covered with inverted watch glass lined with glass wool. The container was a shallow dish of 50-55 cm diameter and 30 cm height, containing 10 Kg sand. Plants were maintained under glass house conditions at ambient temperature (25-30 ^{0}C) with 3 replicates in each treatment. Four plants were maintained in each pot and were reduced to one at harvest sampling for final yield. The composition of nutrient solution excluding Mn was 4mM KNO_3, 4mM Ca $(NO_3)_2$; 2mM $MgSO_4$; 1.5mM NaH_2PO_4; 0.1mM NaCl; 100 µM Fe- EDTA; 30 µM H_3BO_3; 1 µM $CuSO_4$; 1 µM $ZnSO_4$; 0.2 µM Na_2MoO_4; 0.1 µM $NiSO_4$ and 0.1 µM $CoSO_4$.

Solution of macronutrients and micronutrients were prepared from analytical grade crystallized salts, purified against traces of Mn (Hewitt 1966) .The contribution of Mn from purified sand, water and nutrient solution was less than 0.02 µM. After the germination of seedlings, nutrient solution including Mn at desired levels were supplied daily except on weekends when pots were flushed with glass distilled water to remove accumulated salts and deleterious substances from the root surface. Plants were maintained in culture till maturity (120 days after sowing).

Plants were examined periodically for changes in growth parameters and visible symptoms of Mn deficiency were recorded. At 120 days after sowing (DAS), plants were sampled for the determination of dry matter, yield and Mn concentration. Mn concentration was measured by atomic absorption spectrophotometer (AAS 4141, Element-AS, ECL, Hyderabad) in oven-dried leaves and air-dried seeds, after wet digestion in nitric and perchloric acids.

In the homogenates of fresh leaves of chickpea (40 DAS) the concentration of chlorophyll, Hill reaction and activity of peroxidase, acid phosphatase and ribonuclease was assayed. Enzyme activity is expressed on protein basis. The above parameters were also estimated in pod wall and seeds of pods (78 DAS). Finely chopped fresh leaf tissue (75 DAS) and mature air dried seeds (120 DAS) were fixed separately in 80 % (v/v) boiling ethanol (1: 10) and ground at room temperature for determination of carbohydrate, nitrogen fractions and total phenols (Khurana *et al*. 2006). At 120 DAS the total seed protein was estimated in mature seeds of harvest sampling (Bradford 1976). The data have been analyzed statistically and standard error (±SE) values are given along with the mean (Sukhatme and Amble 1985).

At 35 DAS, the leaves and apical buds of chickpea plants grown at deficient Mn (2 µM), turned pale yellow. Then entire leaves turned chlorotic severe deficiency resulted in reduced number of branches and leaves. These symptoms were somewhat similar to those found in lentil (Khurana *et al*. 1991) and other legumes (Moraghan 1985). Flowering was delayed and the number of flowers were reduced, many of them aborted and could not produced pods. The pods had reduced number of seeds (Table 1). Similar observations were recorded in sunflower (Heenan and Campbell 1980). This is due to reduce assimilate supply and low pollen viability (Campbell and Nable 1988). Large number of seeds were shriveled and deformed with necrotic cotyledons as a result of poor lignifications of seed coat (Campbell and Nable 1988) and also due to low sink activity.

In Mn deficient chickpea the reduction in biomass (Table 1) might be due to disturbed carbohydrate and protein metabolism. This may have resulted in growth inhibitions and low chlorophyll content (Shenker *et al*. 2004). In Mn deficient chickpea not only anther development is delayed but the germination rate is also very low, which might have caused poor seed yield (Table 1). Mn deficiency resulted in a decreased concentration of chlorophyll (a and b) and Hill reaction activity in chickpea leaves (Table 1), as Mn has a significant role in photosynthetic oxygen evolution (Burnell 1988, Mengel and Krikby 2001) and photosystem II activity. These results are in agreement with the reports on potato (Gopal *et al*. 2006).

In chickpea seeds the quality deteriorated as phenols accumulated and proteins, sugars and starch were reduced (Fig. 1) indicating decline in nutritional value of seeds. The decrease in proteins under low Mn might suggest the role of Mn in ribonucleic acid synthesis. Total nitrogen content decreased in seeds and leaves of Mn deficient chickpea (Table 1; Fig. 1), (Mortensen 1985). The decrease in starch content in leaves and seeds of Mn deficient plants might be due to lowered activity of starch phosphorylase (Khurana *et al*. 1999, Gopal *et al*. 2006). The concentration of sugars

Table 1. Effect of Mn deficiency on yield components, Mn concentration, chlorophyll, Hill reaction activity, concentration of sugars, starch, phenols and nitrogen fractions in chickpea leaves.

Days after sowing	Parameters	Adequate Mn (10µM)	Deficient Mn (2 µM)
120	Dry weight: g plant^{-1}	10.50±0.28	5.50±0.16
	No of pods plant^{-1}	19±1.50	9±0.5 0
	Pods weight: g plant^{-1}	5.14±0.08	2.17±0.03
	No. of seeds plant^{-1}	19±2.00	8±0.00
	Seed weight: g plant^{-1}	4.45±0.21	1.70±0.020
	100 Seed weight: g	23.42±0.40	21.25±0.250
	Manganese: µg g^{-1} dm		
	Leaves	17.71±0.95	11.98±0.86
	Seed	15.29±0.41	8.40±0.20
40	Chlorophyll (mg g^{-1} fw)		
	a	1.27±0.03	0.44±0.01
	b	0.46±0.08	0.14±0.05
	Hill reaction: Change in O.D. 100 mg^{-1} fw	1.40±0.10	0.23±0.05
75	Sugars: % fw		
	Reducing	0.40±0.04	0.41±0.03
	Non-reducing	0.23±0.01	0.29±0.01
	Starch: % fw	0.75±0.04	0.67±0.02
	Phenols: µg g^{-1} fw	13±0.5	19±0.50
	Nitrogen: % fw		
	Protein	0.661±0.024	0.375±0.018
	Non-protein	0.061±0.001	0.047±0.003

(reducing and non-reducing) increased, in Mn deficient leaves (Table 1; Fig. 1), this might be due to inhibited translocation of photosynthates. These results are similar to those reported for wheat (Pearson and Rengel 1997).Owing to Mn deficiency phenols accumulated in leaves (Table 1) and seeds (Fig. 1) and is likely due to increased activity of peroxidase (Khurana *et al*. 1999).

In Mn deficiency the increase in the specific activity of peroxidase in leaves and pod wall (Fig. 1) is similar to that of some other crop plants (Sinha *et al.* 2006). The peroxidase activity declined in seeds (Fig. 1). The increased activity of the enzyme can be used as a biological tool for diagnosing Mn deficiency in crop plants. The stimulation in peroxidase activity might be the results of increased oxidative stress in which there is greater formation of H_2O_2 or higher accumulation of phenols in plants. The increased activity of peroxidase may rescue the system scavenging oxygen free radicals. Mn deficiency resulted in an increase in the activity of acid phosphatase in leaves (Fig. 1) might be due to accumulation of phosphorous and its decreased availability for different metabolic pathways. Increased activity of acid phosphatase in low Mn is a common phenomenon for most of the plant species (Sinha *et al* 2006). The observation on reduced activity of the enzymes in seeds and pod wall (Fig. 1) is in consonance with the observations of Bar- Akiva and Lavon (1967) and in contrast with the results in wheat grains (Kaur *et al*. 1991). In Mn deficient leaves and pod wall of chickpea, the activity of ribonuclease increased (Fig. 1), whereas, its activity decreased in Mn deficient seeds (Fig. 1) probably as a consequence of low protein content due to reduced availability of nitrogen from pod wall to seeds. The concentration of Mn in leaves and seeds of chickpea decreased in Mn deficiency and almost similar to the observations of Brennan and Bolland (2003). It was concluded that the Mn stress not only

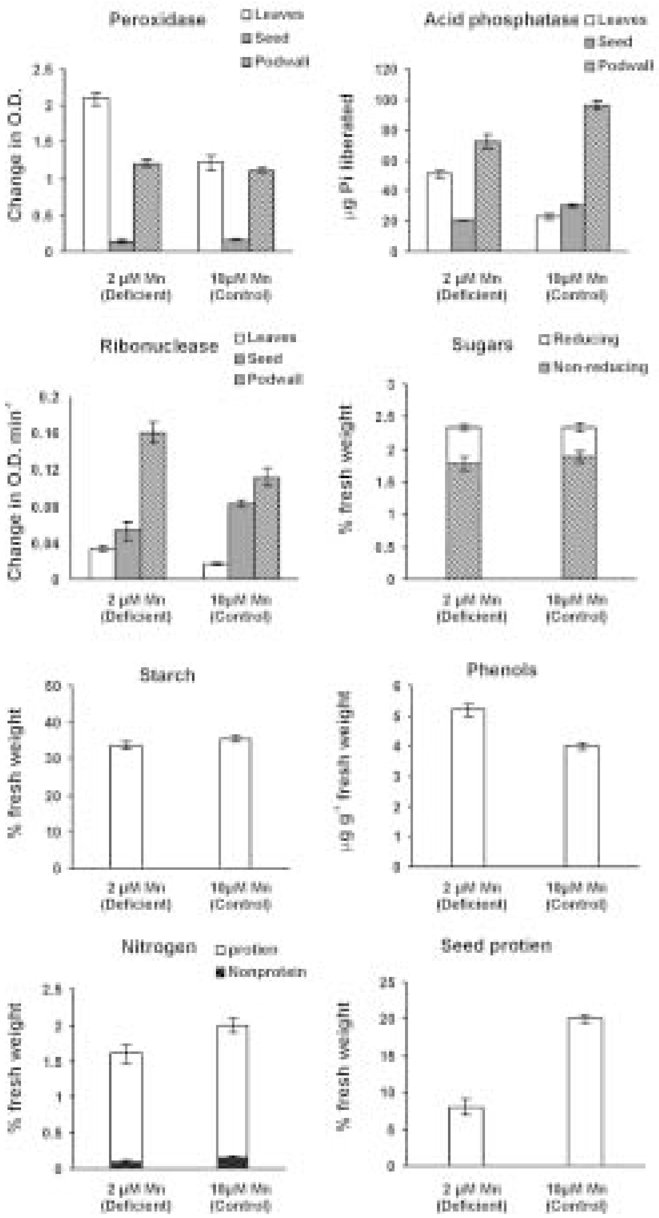

Fig. 1. Effect of Mn deficiency on specific activity of peroxidase, acid phosphatase and ribonuclease enzymes (Leaves, seed and pod wall) and concentration of sugars, starch, phenols, nitrogen fractions and protein (mature seeds) in chickpea. Vertical lines on each bar represent ± SE. Leaves = 40 d, seed and pod wall = 78 d, mature seeds =120 d.

alters the plant metabolism and reproductive yield but also limits nutritive quality of the produce (seeds) suggesting possible role of Mn in seed development.

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