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Full Length Research Paper

Assessment of disturbances caused by interaction of multinutrient deficiencies of sulphur, zinc and boron in mustard

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In higher plants, due to indiscriminate use of fertilizers, sometimes multiple deficiencies or toxicity is created in the growing medium, which disturbed the yield and metabolism of plants. To observe the effect of sulphur (S) deficiency on zinc (Zn) and boron (B) nutrition of mustard (*Brassica compestris* L.) var. T9, plants were grown in refined sand in complete nutrition (control - 2 mM S, 1 µM Zn and 3 µM B), low S (0.02 mM), low Zn (0.01 µM), low B (0.3 µM), low S-low Zn, low S-low B, low Zn-low B, and low S-low Zn-low B. The deficiency of S decreased the biomass, seed yield, concentration of chlorophyll, S and B, Hill reaction activity and increased the concentration of Zn, accumulation of carbohydrates and activity of starch phosphorylase, peroxidase, acid phosphatase and carbonic anhydrase. The magnitude of Zn deficiency effects were accentuated by low S and were reversed to large extent by low B. The parameter that were affected are, intensity of visible symptoms of low Zn, biomass, seed yield, concentration of chlorophyll and Zn, activity of peroxidase, acid phosphatase, carbonic anhyrdrase and accumulation of sugars and starch. Similarly the effect of low B, increase in symptoms of B deficiency, reduction in biomass, seed yield, concentration of B, chlorophyll and increase in the activity of peroxidase, acid phosphatase and starch phosphorylase were aggravated by low S and mitigated to the same extent by low Zinc. These results revealed a synergism between S and B as well S and Zn and antagonism between Zn and B.

Key words: Mustard, interaction, yield, tissue concentration, metabolism.

INTRODUCTION

Due to indiscriminate use of fertilizers in higher plants, the occurrence of single deficiency of nutrients is a common feature but sometimes multiple deficiencies or toxicity is created in the growing medium. These anomalies are many times difficult to recognize by visible symptoms only. Although, according to Robson and Pitman (1983), interaction between nutrients in higher plants occur when the supply of one nutrient affects the absorption, distribution or formation of another nutrient. Thus depending on nutrient supply, interactions between nutrients can either induce deficiencies or toxicities and can modify growth response. At several occasions the nutrient may not be either deficient or toxic then also the assessment of interactions can be made by considering nutrient concentration and contents within the plants.

In recent years, the deficiency of S is coming up as a matter of concern from many pockets of Indian soils. Apart from this, most of these soils are low in Zn and B and hence, plants growing on them may show these deficiencies (Sarkar et al., 2002). In such conditions it becomes difficult to recognize and distinguish these deficiencies altogether. This paper describes the occurrence of multiple deficiencies of S, Zn and B in mustard and how they affect the plant metabolism when

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Abbreviations: CA, Carbonic anhydrase; **S,** sulphur; **Zn,** zinc; **B,** boron; **LSD,** lease significant difference; **EDTA,** ethylenediaminetetraacetic acid; **ANOVA,** analysis of variance; **ROS,** reactive oxygen species.

grown in refined sand at low concentrations of these nutrients either singly or in combination.

MATERIALS AND METHODS

Plants of mustard (*Brassica compestris* L.) var. T9 were raised in 5 kg polyethylene containers at various combinations of S, Zn and B and in a glass house at an ambient temperature (15 to 32°C). Plants were maintained in culture up to 115 days growth with six replicates and two plants in each treatment. The treatments were as follows: (i) Control (2 mM S, 1 µM Zn and 3 µM B), (ii) low S (0.02 mM), (iii) low Zn (0.01 µM), (iv) low B (0.3 µM), (v) low S + low Zn + low B, (vi) Low S + low Zn, (vii) low S + low B, (viii) low Zn + low B. The composition of complete nutrient solution for control plants was: 4mM KNO_3; 4mM $Ca(NO_3)_2$; 2mM $MgCl_2$; 1.5mM NaH_2PO_4; 10µM $MnSO_4$; 1µM $CuSO_4$; 0.2µM Na_2MoO_4; 0.1µM $CoSO_4$; 0.1µM $NiSO_4$ and 1mM NaCl.

The stock solutions of macro and micronutrients were prepared from Aanalar reagent grade salts. Solutions of macronutrients were freed from Zn contamination by phosphate adsorption method of Hewitt (1966). Fe- ethylenediaminetetraacetic acid (EDTA) was prepared according to the method of Jacobson (1951) by complexing the equimolar solutions of ferrous sulphate and sodium EDTA.

In deficient (low) treatments of S, Zn and B alone or in various combinations, each element in particular treatment was omitted from the complete nutrient solution and was supplied directly to the nutrient solution at required deficient levels either singly or in combinations. The experiment was arranged in factorial randomized block design. Care was taken to keep the contribution of Zn and B from purified sand, water and purified nutrients to below 0.001 µM. The concentration of S that was added through micronutrient was taken in to consideration. The solution was supplied daily except on weekends when the sand was flushed with distilled water.

Plant material was thoroughly washed and rinse with distilled water at day 47 (not given in this paper) and 115 was dried in an electric oven at 70°C for 48 h to determine dry matter and concentration of S, Zn and B. The oven dried leaves after wet digestion with nitric and perchloric acid (10:1) were estimated for Zn by atomic absorption spectrophotometer. Sulphur was estimated by the method of Chesnin and Yein (1951) and boron by Wolf (1971). The concentration of chlorophylls (a and b), Hill reaction activity, and activity of peroxidase (E.C.1.11.1.7), acid phosphatase (E.C.3.1.3.2), starch phosphorylase (E.C. 2.4.1.1) and Carbonic anhydrase (E.C.4.2.1.1), concentration of sugars, starch were also estimated in mustard leaves.

Measurements of biochemical parameters were made in young leaves after 43 days when symptoms of deficiency (all elements) appeared. The concentration of chlorophylls (a and b), Hill reaction activity, and activity of peroxidase, acid phosphatase, carbonic anhydrase were estimated according to Chatterjee et al. (2005). Sugars, starch and activity of starch phosphorylase was estimated by the method described by Sinha et al. (2001).

All determinations were carried out in triplicate and the data were analysed statistically for variance and lease significant difference (LSD) by one way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Mustard when grown in refined sand at single deficiency of each S, Zn and B and in combination with low (0.02 mM) and normal (2 mM) S at normal (1 µM) and deficient (0.01 µM) Zn and normal (3 µM) and deficient (0.3 µM) B, and at low S-low Zn-low B and at normal S (low Zn-low B) together, developed deficiency symptoms of each nutrient either singly or in combination.

The growth depression was most marked in plants at deficient S > low S-low B > low S-low Zn > combined deficiency of S-Zn-B > low B >low B-low Zn > low Zn. Visual symptoms of S deficiency appeared earlier in plants grown at low S-low Zn, but was most marked in plants at low S alone, followed by low S-low B and low S-low Zn. This shows that S deficiency was accentuated more by low B than low Zn. Zinc deficiency appeared earlier in plants at low S-low Zn than in combined deficiency of S-Zn-B. The intensity of symptoms was more in plants at low S-low Zn > low Zn-low B > low S > low Zn-low B > low Zn.

Mustard when grown in refined sand at single deficiency of each S, Zn and B and in combination with low (0.02 mM) and normal (2 mM) S at normal (1 µM) and deficient (0.01 µM) Zn and normal (3 µM) and deficient (0.3 µM) B, and at low S-low Zn-low B and at normal S (low Zn-low B) together, developed deficiency symptoms of each nutrient either singly or in combination. In mustard, the symptoms of S deficiency initiated first at low S only. At d 26, the plants were highly depressed in growth and at d 37 chlorosis and purple pigmentation of young leaves occurred, from apex and margins of leaf lamina. Leaf size was very much reduced, lamina appeared thick and brittle. At severity the entire leaf turned purple in colour. At this stage growth of plants was very much reduced and flowering was delayed. These symptoms of low S are similar to those described by Chaubey et al. (2000) and Mengel and Kirkby (2001).

Zinc deficiency symptoms were next to appear on plants grown at low S-low Zn. At single deficiency of Zn, the symptoms were delayed by 10 days. These effects initiated as chlorotic patches on the interveinal areas of old leaves of plants, later the affected areas became dry and necrotic. At severity (day 40) young leaves were bluish green in colour; thick, brittle and scorching appeared on some of the affected old leaves. Area of leaves reduced and growth of plants was depressed. Flowering initiated earlier in low Zn than in other treatments.

At day 40, visual symptoms of B deficiency appeared as chlorosis of old leaves of plants at low B only. In another 2 to 3 days, the margins of the chlorotic leaves appeared necrotic. At day 66, these necrotic margins developed purplish tinge, leaves were thick, brittle, reduced in size and bent downward. Plants showed shortening of internodes, number and size of leaves reduced and shed prematurely (Malewar et al., 2001).

The growth depression was most marked in plants at deficient S > low S-low B > low S-low Zn > combined deficiency of S-Zn-B > low B > low B-low Zn > low Zn. Visual symptoms of S deficiency appeared earlier in plants grown at low S-low Zn, but was most marked in plants at low S alone, followed by low S-low B and low S-low Zn. This shows that S deficiency was accentuated

Table 1. Interactive effect of sulphur, zinc and boron on dry weight, economic yield and tissue concentration (S, Zn and B) in mustard.

mM Sulphur	Control	Deficient Zn (0.01 µM)	Deficient B (0.3 µM)	Deficient Zn – B (0.01 to 0.3 µM)
		d 115: Dry weight: g plant^{-1}		
2.0	35.05	30.10***	10.72***	21.08 ^{NS}
0.02	1.26*	2.36 ^{NS}	1.99***	2.67***
		Pod weight: g plant^{-1}		
2.0	11.50	11.09**	-	9.45***
0.02	0.160**	0.20***	0.17***	0.29***
		Seed weight: g plant^{-1}		
2.0	5.03	3.55**	-	3.30***
0.02	0.01***	0.01***	0.03***	0.02***
		d 115: Sulphur concentration in leaves: % dry matter		
2.0	0.67	1.04**	1.58***	1.14***
0.02	0.04***	0.29***	0.12^{NS}	0.06***
		Sulphur concentration in seeds: % dry matter		
2.0	0.66	0.81*	-	0.40**
0.02	0.32***	-	-	0.20***
		d 115: Zinc concentration in leaves: µg g^{-1} dry matter		
2.0	49	11***	34***	8***
0.02	64***	13***	49^{NS}	10***
		Zinc concentration in seeds: µg g^{-1} dry matter		
2.0	44	16***	-	12***
0.02	77**	-	-	19***
		d 115: Boron concentration in leaves: µg g^{-1} dry matter		
2.0	63	175***	9***	12***
0.02	83***	108***	4***	9***
		Boron concentration in seed: µg g^{-1} dry matter		
2.0	15	19**	-	6***
0.02	34***	-	-	11***

d= days of growth; *, **, ***, at P = 0.05, P = 0.01 and P = 0.001 respectively; NS = not significant at P= 0.05.

more by low B than low Zn. Zinc deficiency appeared earlier in plants at low S-low Zn than in combined deficiency of S-Zn-B. The intensity of symptoms was more in plants at low S-low Zn > low Zn-low B > low S > low Zn-low B > low Zn. These zinc deficiency symptoms in mustard are in consonance with those reported for other plant species (Marchner, 1995). Boron deficiency symptoms (Sharma, 2006) were more marked in combined deficiency of S-B > low B > low S-low Zn > low B > low Zn-low B. Low S accentuated boron deficiency symptoms more. This synergism between B and S was also reflected when the reduction in biomass and economic yield was aggravated by combined deficiency of both nutrients (Sarkar et al., 2002; Khurana and Chatterjee, 2002).

At maturity (day 115), the biomass of mustard was maximum (Table 1) in control treatment (normal S-normal Zn-normal B) and compared to this it decreased most in low S followed by low S-low B >low S-low Zn > low S-low Zn-low B > low B > low Zn-low B > low Zn. The effects of S deficiency were most pronounced, even with low B and low Zn, more with low B. The reduction in biomass was more in low B, than low zinc, might be due to excess accumulation of auxins and phenols in boron deficiency (Sharma, 2006) resulting in early necrosis of growing tips which in turn retards the growth of plants and reduces the

biomass in low B either singly or in combination with low Zinc. This may suggest a synergistic role of S and B as well as S and Zn, but not of Zn and B the latter is antagonistic. The decrease in biomass in the deficiency of each S, Zn and B nutrient is well documented as in all these cases, the carbohydrate and protein metabolisms are disturbed (Marschner, 1995).

In all these treatments compared to that of control, the formation of inflorescence was also affected (Table 1). In sulphur deficiency and low S-low B, almost negligible flowers and pods were produced, whereas in B or S deficiency no inflorescence appeared. The decline in seed production under B deficiency supports the views of Sarkar et al. (2002) in soybean. This might be attributed to the production of aborted and mal formed embryo sacs or increased incidence of male sterility as observed in boron deficient maize (Agarwala et al., 1981). In low S also, the seed formation was drastically reduced (Table 1) even with adequate boron. Almost no seed production in combined deficiency of both S and B might reflect the essential role of elements in the reproductive phase of the plant. The effect of S deficiency was apparent in all combinations of low Zn and low B. In zinc deficiency, low production of seeds might be due to retarded development of anthers and pollen grains, which is the consequence of suppression of male sexuality in low zinc conditions. It might also be due to delayed microspore development resulting in pollen sterility (Sharma et al., 1990). It is further suggested that requirement of zinc for developing anthers is crucial at the time of micro-sporogenesis. This suggests synergistic role of S and other two nutrients. The effect of low Zn was less pronounced as that of low B-hence again determines the antagonistic role of both nutrients.

The concentration of S decreased (Table 1) variably in leaves at all the levels of low S with or without other two nutrients. But its concentration was increased more by low Zn than low B. The concentration of S was maximum in normal S with low Zn and low B. Here also a synergism between S and Zn is reflected as it has been reported earlier in mustard (Chatterjee et al. 2005). In mustard leaves, the concentration of zinc decreased (Table 1) in low Zn with all combinations. The concentration of zinc was increased by low S from the values of low Zn alone (Chatterjee et al., 2005), whereas B deficiency lowered the values of zinc at low levels of B supply. Zinc along with S and as well as with B is showing antagonism as far as their concentrations are concerned. On the other hand, the concentration of B increased (Table 1) in leaves in low Zn (Singh et al.1990). In S deficiency with low B its values were decreased from that of normal S at low B and low Zn-low B. But in low B irrespective of S supply its concentration decreased markedly. This shows an additive effect of S and B.

Compared to that of control, the concentration of chlorophyll in mustard leaves reduced variably (Table 2) in all other treatments. But the effects of low S is more pronounced (Marschner, 1995) than that of Zn and B. In low zinc, the depression in chlorophyll concentration might be due to known role of zinc in maintaining chlorophyll synthesis through –SH group protection of the oxidation prone δ aminolevulinic acid dehydrogenase and protochlorophyllide reductase (Myśliwa-Kurdzdiel and Strzalka, 2002; Myśliwa-Kurdziel et al., 2004). The decrease in chlorophyll concentration was accentuated by S deficiency. This is probably due to a high proportion of the protein is located in the chloroplast where the chlorophyll molecules comprise prosthetic groups of the chromoproteid complex. Thus under S deficiency storage of S containing aminoacids cysteine and methionine not only inhibits protein synthesis but also decreases the chlorophyll content in leaves or decrease in chlorophyll concentration in mustard leaves might be due low availability of iron for the synthesis of chlorophyll (Marschner, 1995) in low S conditions.

The Hill reaction activity in leaves increased (Table 2) manifold in Zn deficiency alone as well as at low Zn-low B. In the rest of the treatments and as compared to control, its activity decreased, the decrease was most pronounced in low S and low S-low Zn-low B. Zinc and Mn are known to behave antagonistically which is also discernible in stimulated Hill reaction activity in low Zn, might be due to increased availability of Mn for this reaction. Whereas, S and Mn are known to show synergism. This is obvious from lowered activity of Hill reaction in S deficiency. S deficiency accumulated total sugars (Table 2) most pronouncedly whether singly or in combination in low Zn. Total sugars accumulated also in low S, low S-low Zn, low S-low B, low S- low Zn -low B, low B and low Zn-low B. The accumulation of sugars in combined deficiency of S and B, S and Zn and S-B-Zn suggest the involvement of these nutrients in the translocation and utilization of sugars and in the production of lower molecular carbohydrates (Mengel and Kirkby 2001). The reducing sugars accumulated more than non-reducing sugars. S deficiency in mustard accentuated the concentration of starch variably in all combinations; it is most accumulated in S deficiency alone. The accumulation of starch in S deficiency in mustard (Table 2) is in contrast to the reports on green gram, where low starch formation has been reported in deficiency of S. This might also support that B has a direct role in translocation and utilization of carbohydrates (Sharma, 2006) where as Zn has a specific role in photosynthesis as well as in synthesis and hydrolysis of carbohydrates (Mengel and Kirkby, 2001). Its concentration also increased by low B but not to the extent of low S and decreased by low Zn. These effects of low B and low Zn were also reflected in combined deficiency of Zn and B, suggesting synergism.

The activity of starch phosphorylase in mustard leaves was stimulated (Table 2) by low S singly or in combination with low Zn and low B. In zinc deficiency with and without S, the enzyme activity increased but in

Table 2. Interactive effect of sulphur, zinc and boron on concentration of chlorophylls, Hill reaction activity, sugars, starch and specific activity of some enzymes in mustard.

mM Sulphur	Control	Deficient Zn (0.01 µM)	Deficient B (0.3 µM)	Deficient Zn – B (0.01 to 0.3 µM)
		Hill reaction activity: Change in O.D. 100 mg^{-1} fresh weight		
2.0	0.75	4.0***	0.25^{NS}	0.3***
0.02	1.0***	0.4***	3.1***	0.88^{NS}
		Total chlorophyll: mg g^{-1} fresh weight		
2.0	0.54	0.46**	0.38***	0.41**
0.02	0.22***	0.38**	0.4***	0.41*
		Peroxidase: Change in O.D.		
2.0	0.84	1.22***	1.06**	01.1***
0.02	1.13***	1.34***	1.68***	1.46*
		Acid phosphatase: µg Pi liberated		
2.0	266.70	353.60***	41.40***	276.80^{NS}
0.02	92.75***	632.60**	622.4**	71.0***
		Starch phosphorylase: µg Pi liberated		
2.0	7.1	12.9***	6.25***	6.1^{NS}
0.02	8.0*	16.9**	11.9***	15.2***
		Carbonic anhydrase: EU		
2.0	401	163***	185*	257**
0.02	52.4**	176***	167***	131**
		Sugars: % in fresh weight		
		Reducing:		
2.0	0.16	0.14*	0.24***	0.26*
0.02	0.34***	0.26***	0.21*	0.16^{NS}
		Non reducing:		
2.0	0.13	0.09**	0.14***	0.18***
0.02	0.15*	0.20***	0.12^{NS}	0.13**
		Starch: % in fresh weight		
2.0	0.37***	0.34**	0.66*	0.42^{NS}
0.02	2.33***	1.23*	1.35**	0.87***

d= days of growth; *, **, ***, at P = 0.05, P = 0.01 and P = 0.001 respectively; NS = not significant at P= 0.05.

low B its activity decreased. But when B was low with and without Zn (with normal S), the activity of starch phosohorylase was low. The increase in its activity might be due to higher starch formation in these conditions.

In mustard leaves, the activity of peroxidase (Table 2) was stimulated in all the deficiencies either singly or in combination. But the effect of B deficiency was more marked than that of Zn deficiency (Cakmak, 2000). Zinc has a protective role against oxidative stress and therefore it has been suggested that zinc is one of the key element regulating protein synthesis and protects cellular protein form reactive oxygen species (ROS)-mediated oxidative damage (Arvind and Prasad, 2005). S deficiency has further increased the affects of low B and low Zn either singly or in combination. The stimulation in peroxidase activity might be the result of increased oxidative stress in which there is greater formation of H_2O_2 or higher accumulation of phenols in plants.

In all three deficiencies (singly or in combination) the activity of acid phosphatase increased (Table 2) variably but the increase was most marked when Zn and B were deficient along with low S either separately or together. The enzyme activity also increased when Zn and B were deficient alone or together (with normal sulphur). In all these conditions, there is higher accumulation of inorganic phosphorus making low availability of

phosphorus for incorporation in different biomolecules. These observations are in partial consonance with the observations of mustard and maize (Sinha et al., 2000; Sinha et al., 2001).

The activity of carbonic anhydrase (Table 2) was maximum in control treatment and compared to test treatment, it decreased variably in all other treatments, but the effects of Zn deficiency was most marked. Effect of Zn on photosynthesis may individually involve inhibition of carbonic anhydrase (CA) activity (Badger and Price, 1994). S deficiency with low Zn increased its values from that of zinc deficiency alone. This shows an antagonistic relationship between both the nutrients otherwise by looking at other parameters, S and Zn both behaved synergistically.

Conclusions

In mustard synergistic interaction between S and Zn as well as between S and B was discernible when these nutrients were low in supply. The parameters affected were biomass, economic yield, leaf contents of S, Zn and B, carbohydrate fractions, activity of starch phosphorylase, peroxidase, acid phosphotase and carbonic anhydase in leaves. An antagonism to some extent was reflected between B and Zn when the magnitude of some parameters affected by either deficiency, foliar symptoms of either deficiency, biomass, economic yield, concentration of chlorophyll, B, Zn, activity of carbonic anhydrase, peroxidase and acid phosphotase were reversed by each nutrient.

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