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SHORT COMMUNICATION

ZINC STRESS DISTURBED THE PHYSIOLOGY OF FRENCH BEAN

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Zinc stress was induced in french bean cv. Anupama in refined sand at variable levels of zinc from deficient (0.00065 mg/l) to excess (13.0 mg/l) supply. After 35 days, depression in growth followed by initiation of chlorosis at margins of middle leaves was observed under zinc deficiency. At later stage with continuous low supply, zinc deficient plants were very retarded and stunted in growth with dense small leaves. At excess Zn (13 mg/l) level, interveinal chlorosis appeared on young leaves started from the base of the lamina and spread towards the centre. Zinc stress (both deficiency and excess) caused reduction in biomass and economic yield. The concentrations of reducing sugar, starch, protein nitrogen, soluble protein and specific activity of carbonic anhydrase were decreased and stimulation in specific activities of acid phosphatase and ribonuclease in leaves and pods of french bean was observed under zinc stress. The concentration of Zn in dry matter increased with an increase in zinc supply. It appears that the zinc concentration values at deficiency, threshold of deficiency, threshold of toxicity and toxicity in leaves were 18, 32, 117 and 138 µg Zn/g dry matter respectively.

***Key words*: Economic yield, enzymes, french bean, zinc**

In India, the deficiency of zinc is a major cause of widespread crop failure and poor yield (Takkar and Randhawa 1978, Rashid *et al.* 1994). Zinc is an essential micronutrient and involved in many ways in the metabolism of higher plants. The metabolic functions of zinc are based on its strong affinity to form tetrahydral complexes with N-, O- and S- ligands and thereby plays both catalytic and structural role in enzyme system. Deficiency of zinc is reported to reduce the activity of carbonic anhydrase, alcohol dehydrogenase, fructose 1-6 diphosphatase and aldolase, in which zinc is an intergral component (Marschner 1995). Zinc is also a structural component of the cytoplasmic ribosomes and essential for their structural integrity. Disintegration of ribosomes in zinc deficient plants is responsible for decrease in protein synthesis in such condition hence protein levels are markedly reduced under Zn deficiency (Mengel and Kirkby 2001). Besides deficiency, zinc at excess concentration is strongly toxic and impair numerous physiological processes, induce abnormalities in plant morphology and reduce plant growth (Monnet *et al.* 2001, Sinhal *et al.* 2007). The present study describes the involvement and requirement of zinc in various aspects of physiology and analyses the threshold limits of deficiency and toxicity of Zn in french bean.

French bean cv. Anupama was grown in refined sand in rabi season at deficient (0.00065 mg/l), subnormal (0.0065 mg/l), normal (0.065 mg/l) and excess (13 mg/l) zinc, supplied as zinc sulphate to induce zinc deficiency and toxicity in french bean. The whole experiment was carried out in polythene containers in a glass house at an ambient temperature (18^{o}-20^{o} C). The plants were grown till maturity (d 125) with three

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replicates in each treatment. After emergence the seedlings were supplied daily with nutrient solution purified against Zn by dithizone extraction. The nutrient solution omitting zinc was same as described earlier (Agarwala and Chatterjee 1996).

In addition to periodically recording of the visible effects of zinc stress, plants were sampled, different parts (leaves, stem and root) were seperated at d 55 and 75 and washed and then dried in an hot air oven at 70° C to determine biomass and tissue concentration of zinc. At harvest (d 125) only economic yield of french bean was measured. Zinc content in different plant parts was measured after tissue digestion with nitric and perchloric acid (Piper 1942), using atomic absorption spectrophotometer (Elements AS AAS 4141). The data expressed as µg Zn/g dry matter. The deficiency and toxicity limits of zinc (leaves) were worked out at d 75 on the basis of relationship between zinc concentration in leaves and relative yield of plants (Agarwala and Sharma 1979).

The activity acid phosphatase, ribonuclease, carbonic anhydrase and soluble protein content were determined in leaves on d 62 following the methods described elsewhere (Chatterjee and Khurana 2007). Colorimetric methods were adopted for determination of sugars and starch (d 72) in fresh pods of french bean (Chatterjee and Khurana 2007) after fixing the material in boiling 80% alcohol. Using Conway and O'Malley indicator (Conway and O'Malley 1942), the protein-N and non protein-N (d 72) were estimated in alcohol insoluble and alcohol soluble fractions respectively (Chibnall *et al.* 1943). The experiment was set in a randomize block design. All determinations were made in triplicate and the data were analyzed statistically (Panse and Sukhatme 1985).

Both deficient and excess Zn supply suppressed plant growth. Growth depression in Zn deficient plants were perceptible at d 35. At d 45, zinc deficient (0.00065 mg/ l) plants developed foliar symptoms of zinc deficiency as chlorosis at margins of middle leaves. Margins of these leaves showed a border. Finally Zn deficient plants were small in size, stunted with condensed internodes and dense small leaves. Such symptoms were related to decrease in indoleacetic acid level in zinc deficient tomato (Cakmak *et al.* 1989, Marschner 1995). Excess zinc (13 mg Zn/l) induced toxicity symptoms of zinc leading to reduced growth and chlorosis of young leaves after 50 days of metal supply in french bean.

Biomass, pod yield and seed yield were depressed in low and excess zinc. Poor anther development with reduced pollen fertility inducing male sterility under low zinc condition was reported earlier in maize (Sharma *et al.* 1990) might be the cause of the economic yield failure in french bean (Table 1). The depression in biomass of french bean in low zinc is due to impeded protein formation, low photosynthetic rate and non-utilization of carbohydrates as a result of low sink activity (Marschner 1995). Low rate of stem elongation or shortening of internodes due to low auxin activity and tryptophan content in zinc deficient plants might be the reason of reduced growth and low biomass in zinc deficient french bean plants. The increase in zinc level from deficiency to excess (0.00065 to 13 mg/l) increased the concentration of zinc in all parts but the maximum amount of Zn was accumulated in leaves (Table 1). The zinc concentration values at zinc deficiency, threshold of deficiency, threshold of toxicity and toxicity in french bean leaves were observed to be 18, 32, 117, 138 µg Zn/g dry matter respectively. The value of deficiency and toxicity observed here are in agreement with Marschner (1995).

Zinc deficiency stimulated the activity of acid phosphatase (Table 2) in french bean leaves and this was probably due to accumulation of inorganic phosphorus in such conditions (Marschner 1995). The activity of carbonic anhydrase decreased under zinc deficiency and increased with an increase in zinc supply (Table 2) as zinc is a constituent of this metalloenzyme. Zinc deficiency has been shown to decrease intercellular CO_2 concentration that results from the decreased activity of carbonic anhydrase under zinc deficiency (Marschner 1995). In french bean, the concentration of soluble protein decreased in zinc deficient leaves whereas stimulated activity of ribonuclease was observed (Table 2) under these conditions (Chatterjee and Khurana 2007). Low protein content and higher amino acid content in zinc deficient plants are the results of reduced

Table 1. Influence of variable zinc on biomass, economic yield and tissue concentration of zinc in french bean.

		Zinc level (mg/l)			
		0.00065	**0.0065**	**0.065**	**13.0**
		Biomass and yield			
Biomass (g/plant)	d 55	3.613±0.194	4.610±0.202	5.664±0.560	3.808±0.047
	d 75	5.155±0.104	8.912±0.265	12.345±0.716	7.900±0.440
Pod wt. (g/plant)	d 125	5.99±0.127	7.74±0.236	15.13±0.355	8.97±0.237
Seed wt. (g/plant)	d 125	4.50±0.236	5.84±0.138	11.42±0.387	6.88±0.271
		Zinc concentration (μg/g dry matter)			
Leaves	d 55	13±0.947	24±2.067	68±4.120	88±5.35
Stem	d 55	15±0.440	35±2.684	54±2.900	73±1.73
Root	d 55	25±1.250	34±0.570	56±3.464	102±6.93
Pods	d 75	15±0.520	35±1.154	46±2.310	53±5.19
Young leaves	d 75	18±0.69	44±1.576	72±4.04	171±9.24
Old leaves	d 75	22±0.69	33±2.90	57±2.42	90±2.60
Stem	d 75	20±0.98	41±1.87	55±1.38	93±8.08
Root	d 75	36±2.31	54±2.88	74±6.35	187±10.39

Table 2. Influence of variable zinc on specific activities of acid phosphate (mg Pi liberated), ribonuclease (change in OD/min), carbonic anhydrase (EU) in leaves and carbohydrate and nitrogen fractions (% fw) in fresh pods of french bean.

	Zinc level (mg/l)			
	0.00065	**0.0065**	**0.065**	**13.0**
Acid phosphatase	116±2.886	72±0.577	55±0.866	93±2.309
Ribonuclease	0.071±0.003	0.044±0.002	0.027±0.00	0.046±0.007
Carbonic anhydrase	34.5±1.732	62.2±1.556	94.2±0.693	123.5±7.217
Reducing sugar	0.402±0.013	0.418±0.010	0.455±0.009	0.461±0.012
Non reducing sugar	0.762±0.013	0.712±0.007	0.635±0.003	0.753±0.006
Starch	0.77±0.017	0.86±0.006	2.45±0.086	0.95±0.023
Protein nitrogen	0.112±0.046	0.205±0.016	0.408±0.015	0.062±0.0001
Non protein nitrogen	0.140±0.011	0.115±0.003	0.082±0.001	0.105±0.008

transcription, translation and enhanced rate of RNA degradation. A clear inverse relationship exists between zinc supply and ribonuclease activity and also between ribonuclease activity and protein content (Marschner 1995). Involvement of zinc in RNA metabolism, as indicated by marked increase in ribonuclease activity is well documented (Sharma *et al.* 1990). At excess zinc supply (13 mg Zn/l), low protein content in french bean leaves was observed. There was a decreased in concentration of reducing sugars, starch and protein nitrogen and increase in concentration of non-protein nitrogen in pods of french bean in deficiency and excess of zinc (Table 2). During Zn stress (0.065 mg Zn/l) decrease in concentration of reducing sugar might be an expression of decreased synthesis of assimilates due to hampered photosynthetic activity and carbohydrate

metabolism. The decrease in starch concentration under zinc deficiency could be a result of reduced starch synthetase activity (Jyung *et al*. 1975). Low concentration of protein nitrogen and soluble protein in zinc deficient french bean is well documented (Marschner 1995). Since, nitrogen is one of the primary essential nutrients involved as a constituent of biomolecules, such as nucleic acids, nitrogen bases, coenzymes and protein, any deviation in these constituents would severely inhibit the growth and economic yield of plants.

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