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Influence of phosphate and zinc on growth, nodulation and mineral composition of chickpea (*Cicer arietinum* L.) under salt stress

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Zn^{2+} at 5 ppm and phosphate at 20 and 40 ppm improved the growth and nodulation of chickpea (*Cicer arietinum* L.) at two levels of salinity (4.34 and 8.3 dS m^{-1}). Augmentation with Zn^{2+} at 5 ppm provided protection to the plant under saline conditions by reducing the Na^+:K^+ ratio in the shoot. The shoot nitrogen content with 5 ppm Zn^{2+} and 20 ppm phosphate was equal to that of a non-saline control. No significant effect on nitrogenase activity was observed.

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Salt stress adversely affects growth, nodulation and nitrogen accumulation in legumes (Singleton & Bohlool 1984). Chickpea, the major pulse crop in India, either fails completely or gives a very poor yield in saline soils (Saraf & Davis 1969). The productivity of soils affected by salt can be maximized by fertilizer application (Lunin & Gallatin 1965). Chickpea responds well to Zn^{2+} application in normal soils (Yadav *et al.* 1987). Ravikovitch & Yoles (1971) reported the beneficial role of phosphate in alleviating the effect of salinity on growth of clover. The present investigation was carried out to study the influence of Zn^{2+} and phosphate on growth, nodulation, nitrogen fixation, nitrogenase activity and mineral composition of chickpea under salt stress.

Materials and Methods

A growth chamber experiment was conducted using Leonard jars (30 × 8 cm) filled with 800 g of washed river sand. Two levels of salinity (4.34 and 8.3 dS m^{-1}; 1 dS m^{-1} = 1 mmho cm^{-1}) were achieved by applying 50 and 100 ml of 1% NaCl, respectively, to the sand before sowing. The treatments consisted of Zn^{2+} at 0, 2.5, 5 and 10 ppm as $ZnSO_4.7H_2O$ and three levels of phosphate (0, 20 and 40 ppm) as single super-phosphate at both levels of salinity. A non-saline treatment was kept as a reference control. Five seeds of chickpea, cultivar 'Pusa 312', inoculated with specific *Rhizobium* strain 'P-114-3' (isolated by first author), were sown in each jar. Each treatment was replicated three times and three plants per treatment were maintained. The experiment was conducted twice under identical conditions. Modified McKnight's solution devoid of Zn^{2+} or phosphate as per the treatment was used as a nutrient solution (McKnight 1949; Gibson 1963). The assemblies were set up in a randomized pattern in a growth chamber at 24 ± 2°C with 12 h light at an intensity of 14,000 lux at bench level. Lighting was provided from cool-beam fluorescent tubes (40 W) supplemented with incandescent bulbs (100 W). The plants were harvested 50 days after seedling emergence. The shoot length and the number of nodules per jar were recorded.

Nitrogenase activity of the root-nodule system was measured by acetylene reduction assay (ARA) by gas chromatography. Nodules were detached from the roots. The samples of different plant parts were oven dried at 80°C and dry matter yield recorded separately. Nitrogen content was measured both in roots and shoots on a Tecator Kjeltec Auto 1030 analyser. A sample (500 mg) of oven-dried shoot was digested with a mixture of conc. HNO_3 and H_2SO_4 and 72% $HClO_4$ (10:1:3, by vol.). The sodium and potassium content of the plant digests were analysed by flame photometry (Richards 1954) and phosphorus by the vanadylmolybdophosphoric acid method (Jackson 1958).

Statistical Analysis

Analysis of variance was done for each parameter and the critical difference (CD) was calculated.

Results and Discussion

Growth and Nodulation

Salinity, induced by NaCl, affected growth and nodulation of chickpea (Table 1). The nodule and shoot dry weights were only 55 and 58%, respectively, of the control at a salinity of 4.34 dS m^{-1}, and at 8.3 dS m^{-1} they were 44 and 45%, respectively. Augmentation with 2.5 ppm Zn^{2+} increased the plant height, nodule and shoot dry weight, though the nodule number increased only with 5 ppm Zn^{2+}. The maximum yield of most of the plant parts, except root dry weight, was with 5 ppm Zn^{2+} at both levels of salinity. At a salinity of 4.34 dS m^{-1}, and with Zn^{2+} between 5 and 10 ppm, the number of nodules were twice the number that occurred in non-saline controls. Similar results were obtained by Yadav *et al.* (1984) for cowpea. Zn^{2+} is required for the production of indoleacetic acid, which is important for nodulation in legumes (Skoog 1940). The yield of all the plant parts except root dry weight, at 4.34 dS m^{-1} decreased with 10 ppm Zn^{2+}. The higher levels of Zn^{2+} evidently interfered with the nutritional balance of the plant (Table 3).

Table 1. Effect of Zn^{2+} and phosphate on growth and nodulation of chickpea under salt stress.

Salinity level (dSm^{-1})		Treatment		Plant height (cm)	Nodule number (jar^{-1})	Dry biomass (g jar^{-1})		
		Level (ppm)	Number			Nodule	Root	Shoot
0.4	Control		T_1	48	35	0.16	0.54	1.85
4.34	Zn^{2+}	0	T_2	32	38	0.09	0.41	1.07
		2.5	T_3	37	32	0.11	0.40	1.23
		5	T_4	36	71	0.18	0.49	1.41
		10	T_5	29	70	0.12	0.51	1.07
8.3		0	T_6	27	29	0.07	0.33	0.84
		2.5	T_7	20	22	0.06	0.28	0.70
		5	T_8	29	64	0.12	0.48	1.19
		10	T_9	24	56	0.06	0.41	0.68
4.34	Phosphate (as P)	0	T_{10}	30	32	0.04	0.28	0.87
		20	T_{11}	33	50	0.06	0.30	0.97
		40	T_{12}	34	53	0.08	0.45	1.17
8.3		0	T_{13}	23	55	0.04	0.24	0.78
		20	T_{14}	29	65	0.05	0.26	0.87
		40	T_{15}	30	59	0.07	0.39	1.08
CD (5%)*				4.32†	NS‡	0.05	0.12	0.37

* Critical difference at 5% probability level.
† Significant at 1% probability level.
‡ NS—not significant.

Table 3. Influence of Zn^{2+} and phosphate on mineral composition of chickpea under salt stress.

Treatment number*	Mineral content (mg/g shoot)		
	Phosphate (as P)	Na^+	K^+
T_1	2.6	1.1	17.6
T_2	2.4	5.0	10.8
T_3	2.6	3.6	18.0
T_4	1.8	1.5	11.9
T_5	2.5	3.6	17.3
T_6	1.8	5.7	12.8
T_7	1.9	11.1	15.3
T_8	2.7	6.6	12.6
T_9	1.7	7.0	14.8
T_{10}	1.6	4.5	18.0
T_{11}	3.1	4.1	11.6
T_{12}	2.9	7.1	15.6
T_{13}	2.4	11.0	14.6
T_{14}	2.9	10.7	15.1
T_{15}	2.9	11.1	8.9
CD (5%)†	0.9	0.94‡	1.98‡

* Treatment number correspond to treatments in Table 1.
† Critical difference at 5% probability level.
‡ Significant at 1% probability level.

Phosphate application, even at 20 ppm, improved all the growth parameters at both the levels of salinity. Increasing the phosphate to 40 ppm did not significantly increase the growth parameters except for the root dry weight. Such an enhanced effect of phosphate on growth was also observed in clover and chickpea (Ravikovitch & Yoles 1971; Dravid *et al.* 1985).

Nitrogen Metabolism

Nitrogen in the shoots increased significantly at 5 ppm Zn^{2+} and 20 ppm phosphate at both the levels of salinity and was equivalent to the non-saline control (Table 2). However, the application of zinc and phosphate could not restore the nitrogenase activity, which was less than 50% of the non-saline control. Nitrogen fixation is limited both by rhizobial requirement for Zn^{2+} as well as indirectly by Zn^{2+} nutrition of the host plant (Yie 1969).

Mineral Composition

The presence of Zn^{2+} in the root medium provided a protection to the plant under saline conditions which was demonstrated by decreased Na^+ and increased K^+ uptake in shoots (Table 3). The Na^+/K^+ ratio in the plant at 4.34 dS m^{-1} due to 5 ppm Zn^{2+} application was equivalent to the non-saline control. Such protective action has been observed in soybean (Gupta & Gupta 1984). Application of phosphate led to a marginal increase in phosphate uptake and in the Na^+/K^+ ratio in shoots. However, the phosphorus metabolism was little affected by salinity and by zinc/phosphate application. The results are in agreement with the work of Nimbalker & Joshi (1975).

The application of phosphate at a salinity of 4.34 dS m^{-1}, but not at 8.3 dS m^{-1}, induced Na^+ uptake. The increased uptake of Na^+ led to a decreased uptake of K^+, which probably maintained an ionic balance in the cytoplasm. At 8.3 dS m^{-1}, although the K^+ uptake was not increased, the phosphate uptake was increased by 20%. Based on these studies, it is difficult to infer that the demand for increased

Table 2. Influence of Zn^{2+} and phosphate on nitrogen nutrition and nitrogen fixation by acetylene reduction activity (ARA) of chickpea under salt stress.

Treatment number*	Nitrogen % (w/w)		ARA activity (μmol ethylene h^{-1} per jar)†
	Root	Shoot	
T_1	1.2	2.5	8.2
T_2	1.2	2.4	2.3
T_3	1.1	2.2	3.3
T_4	1.1	2.5	3.9
T_5	1.1	2.4	2.2
T_6	1.2	2.3	2.3
T_7	1.3	2.4	1.2
T_8	1.2	2.5	3.6
T_9	1.1	2.2	0.8
T_{10}	1.1	2.3	0.8
T_{11}	1.2	2.7	2.1
T_{12}	1.2	2.3	3.3
T_{13}	1.3	2.0	0.8
T_{14}	1.5	2.3	1.4
T_{15}	1.5	2.0	2.2
CD (5%)‡	0.16	0.32	0.60§

* Treatment number corresponds to treatments in Table 1.
† Each jar contained three plants.
‡ Critical difference at 5% probability level.
§ Significant at 1% probability level.

phosphorus is a nutritional one or that it is necessary to maintain the ionic balance in the cytoplasm.

References

DRAVID, M.S., GOSWAMI, N.N. & KAMATH, M.B. 1985 Zinc and phosphate nutrition to some grain legumes in relation to their tolerance under varied soil conditions. *Journal of Nuclear Agriculture and Biology* **14**, 52–56.

Gibson, A.H. 1963 Physical environment and symbiotic nitrogen fixation—I. The effect of root temperature on recently nodulated *Trifolium subterraneum* L. plants. *Australian Journal of Biological Sciences* 16, 328–355.

GUPTA, S.P. & GUPTA, V.K. 1984 Influence of zinc on calcium, magnesium, sodium, potassium and phosphorus nutrition of soybean (*Glycine max* L.) in sodic soils. *Indian Journal of Ecology* **11**, 236–242.

JACKSON, M.L. (ed.) 1958 Soil Chemical Analysis, p. 204. Englewood Cliffs, NJ: Prentice Hall.

LUNIN, J. & GALLATIN, M.H. 1965 Salinity–fertility interactions in relation to the growth and composition of beans—I. Effect of N, P and K. *Agronomy Journal* **57**, 339–342.

MCKNIGHT, T. 1949 Efficiency of isolates of *Rhizobium* in the cowpea group with proposed additions to this group. *Queensland Journal of Agricultural Sciences* **6**, 61–76.

NIMBALKER, J.D. & JOSHI, G.V. 1975 Effect of increasing salinity on germination, growth and mineral metabolism of sugarcane var Co 740. *Journal of Biological Sciences* **18**, 55–63.

RAVIKOVITCH, S. and YOLES, D. 1971 The influence of phosphorus and nitrogen on millet and clover growing in soils affected by salinity—I. Plant development. *Plant and Soil* **35**, 569–588.

RICHARDS, L.A. (ed.) 1954 *Diagnosis and Improvement of Saline and Alkali Soils*, USDA Agriculture Handbook No. 60. Washington, DC: USDA.

SARAF, C.S. & DAVIS, R.J. 1969 Water management for pulse crops. Presented at the Symposium on Water Management, HAU, Hisar.

SINGLETON, P.W. & BOHLOOL, B.S. 1984 Effect of salinity on nodule formation by soybean. *Plant Physiology* **74**, 72–76.

SKOOG, F. 1940 Relationships between zinc and auxin in growth of higher plants. *American Journal of Botany* **27**, 939–951.

YADAV, D.S., ANTIL, R.S., SINGH, M. & KUMAR, V. 1984 Effect of P and Zn on nitrogen fixation in cowpea. *Indian Journal of Agricultural Sciences* **54**, 217–219.

YADAV, O.P., SHUKLA, U.C. & YADAV, D.S. 1987 Critical limits of soil-Zn, shoot-Zn and P/Zn ratios in soil and plant shoot for normal growth of chickpea (*Cicer arietinum* L.). *Journal of Indian Society of Soil Science* **35**, 677–684.

YIE, S.T. 1969 A study of the relationship of zinc concentration to nitrogen fixation in soybean. *Journal of Science and Engineering (Taiwan)* **6**, 1–8.

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