Pak. j. sci. ind. res. vol.38, nos. 3-4, March-April 1995

RESPONSE OF LEGUMES TO SALT STRESS: EFFECT ON GROWTH AND NITROGEN FIXATION OF CHICKPEA *(CICER ARIETINUM* VAR. CM-72)

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(Received October 11, 1988 ; revised February 2, 1995)

In a green house experiment, chickpea (CM-72) was subjected to different salinity levels under un-inoculated and rhizobial inoculation to study effectiveness (nodule formation) and growth of the plant. In a set of inoculated plants, the plants died at 6.0 dS m^{-1} and beyond at flowering stage showing their sensitivity toward salinity. Plant height, dry matter yield (DMY), N-content (mg/plant) decreased with increasing salinity levels in both inoculated and un-inoculated plants at all growth stages. Nodulation was adversely affected due to presence of salinity in the growth medium. Percent crude protein increased with increasing salinity. Percent crude protein comparatively increased in inoculated plants than un-inoculated ones. Chickpea is sensitive to salinity. Seed treatment with rhizobial inoculum may improve the protein content of plant under saline conditions.

Key words. Salt stress, Nitrogen fixation, Chickpea.

Introduction

Salt sensitivity of Legume Rhizobia Symbiosis is an established factor in some cases. This sensivity of the host limits the symbiotic performance in glycine, because nodules are salt resistant [1]. An inhibition of growth of rhizobia is recorded due to the presence of toxic levels of NaCl. Increased soil salinity reduced the biomass and yield of many plants [2]. The same effect is more established in legumes which is due to a direct effect of salinity on nitrogen fixing ability of plants [3]. The rhizobium infects the plant root through the root hair. In the presence of high salt (1.2%), deformation of root hair is recorded [4]. The decrease is further related to suppressed cell division and cell enlargement [5]. Both the above factors result in a decline in nodulation followed by a decline in growth of the plants.

Chickpea is an important cash crop of Pakistan. It is cultivated in arid areas due to its low water adoptability [6]. It is drought tolerant, however it is sensitive to salt stress. Dinitrogen fixation by suitable rhizobia is reported to provide N for increased yield in different crops [7], but under salt stress chickpea has been reported to fail. This failure could be due to symbiotic failure of host [8]. A further investigation is carried out to explore the behavior of chickpea (CM-72) towards nitrogen fixation under salt stress.

Material and Methods

A green house experiments was designed in modified Leonard jar assembly [9] using sterilized sand. Height of the pots was 15cm with 12cm diameter. Chickpea variety CM-72 was used as the test crop. It was sown in three replicates under five different salinity levels e.g. control (1.5) half strength Hoagland solution, 3.0, 6.0, 9.0 and 12.0 dS m^{-1}. The EC of the solution was maintained by using EC meter (Sybron, USA). The experiment was divided into two sets, one of the sets was sown with peat based mixed strain inoculated seeds, according to Burton [10]. Salinity levels were maintained in nitrogen free half strength Hoaglands solution [11] with 1:1 NaCl and $CaCl_2$ $2H_2O$. Solutions were changed on alternate days. Plants were harvested at three different growth stages i.e. seedling (36 days), flowering (110 days) and 50% maturity (138 days) for record of agronomic data and analytical purpose. The plant material was oven dried at 60°C and various plant parts analyzed for N-content using Kjeltech. The data obtained was subjected to statistical analysis according to Sokal and Rohlf [12].

Results and Discussion

Plant height. Increasing salinity reduced the plant height significantly. The reduction in plant height was highly significant at flowering and maturity stages. There were no significant differences recorded in plant height due to inoculation at seedling stage. An increase of 11.5 and 5.70% in plant height was observed under control and 3.0 dS m^{-1} salt concentration at flowering stage (Fig.1). The significant decrease in plant height may be related to the effect of salinity which may have suppressed cell enlargement and cell division [5]. The non-significant effect of salinity at the seedling stage confirmed the findings of Lunin and Gallatin [13] and Attaullah *et al.* [14], who also observed different behavior of plants towards salinity at different stages.

Dry matter yield. Dry matter yield (DMY) of the plants significantly decreased due to salinity inspite of inoculation at seedling stage. A linear decrease in % DM was recorded upto EC 6.0 dS m^{-1}. Above this, an increase of 4.54 and 5.25% DM was recorded at 9.0 and 12.0 dS m^{-1} salt levels, respectively. The growth was enhanced in control (29.8%) due to the presence of inoculum at the flowering stage. Growth was reduced to 20.3% in the presence of EC 3.0 dS m^{-1}. The salt may be responsible for this reduction as described by Abel and Mckenzie [2]. At maturity, DM yield in control exceeded over non-inoculated. Salinity had more pronounced decreasing effect and a significant decrease of 20.48% was recorded at EC 3.0 dS m^{-1}. Results showed that the decrease in DM production was not due to the slow rhizobial growth but due to the presence of salt in the growth medium [15] which reduces the nodulation as well as nitrogen fixing ability of plants [16].

Nodulation. Maximum number of nodules per chickpea plant was observed in control at 50% maturity in the absence of salts while maximum dry matter of nodules was produced at 3.0 dS m^{-1} at flowering stage (Table 1). The plants could not survive beyond 3.0 dS m^{-1} salt level. Similar trend in nodule number and nodule weight under salt stress was reported by Balasubramanian and Sinha [17] in chickpea. Dry matter yield of nodules under saline conditions was reduced at seedling stage wherever number of nodules was increased as compared to control in un-inoculated plants. At flowering stage the number and DMY was significantly high showing the maximum symbiotic activity of rhizobia (Table 2). Lauter *et al.* [15] has reported a delayed and reduced nitrogen fixation in chickpea rhizobium inoculated plants when grown under salt stress. Resistance toward salinity is usually increased with the age of plant and inoculation has also overcome the situation explained by Lauter *et al.* [15]. The increase in DM of nodules per plant observed in the present study agrees with the earlier work.

Dry weight root (DWR). Dry weight root (93.75%) at flowering stage in control was attributed to inoculation of seeds. The increase was reduced to 25% with increasing salt level (3.0 dS m^{-1}) at flowering stage as compared to control.

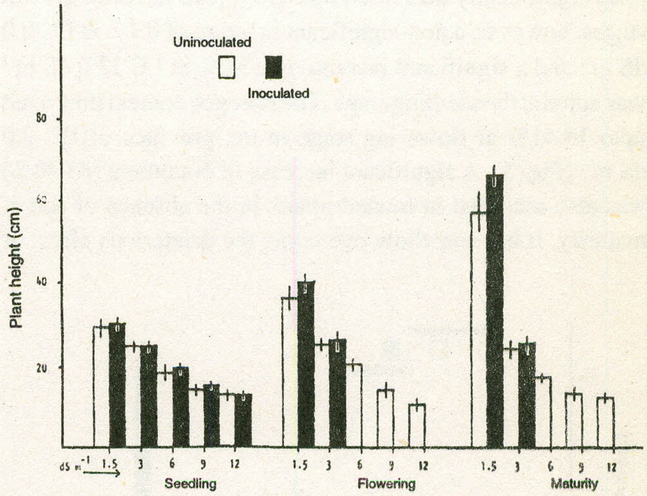

Fig. 1. Effect of NaCl : $CaCl_2 2H_2O$ (1:1) in the absence or presence of rhizobium on the plant height of chickpea (CM-72).

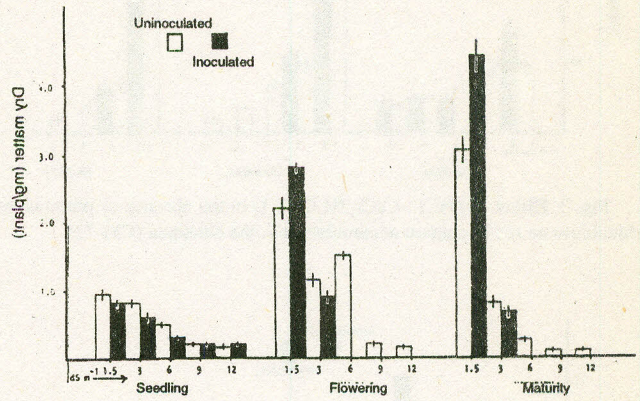

Fig. 2. Effect of NaCl : $CaCl_2 2H_2O$ (1:1) in the absence or presence of rhizobium on the dry matter of chickpea (CM-72).

TABLE 1. ROOT WEIGHT g/PLANT AT DIFFERENT GROWTH STAGES AND SALINITY LEVELS IN CHICKPEA.

Salinity Levels Ds m^{-1}	Root weight g/plant					
	Un-inoculated			Inoculated		
	Seedling	Flowering	Maturity	Seedling	Flowering	Maturity
Control (1.5)	-	0.32±0.02	0.28±0.04	-	0.62±0.16	0.52±0.08
3.0	-	0.12±0.02	-	-	0.15±0.02	-
6.0	-	-	-	-	-	-
9.0	-	-	-	-	-	-
12.0	-	-	-	-	-	-

TABLE 2. NUMBER OF NODULES, THEIR DRY MATTER (MG/PLANT) AND NITROGEN CONTENT (MG/PLANT) IN CHICKPEA.

Salinity levels ds m^{-1}	No. of nodules			Dry matter production			Nitrogen content nodules		
	Seedling	Flowering	Mauurity	Seedling	Flowering	Maturity	Seedling	Flowering	Maturity
Control	23.5±3.3	28.8±8.6	44.6±7.9	21.7±2.4	156.5±12.2	274.4±19.8	1.03±0.1	7.32±0.6	10.78±0.8
3.0	38.3±5.2	20.4±5.8	-	48.9±8.7	351.5±27.5	-	2.06±0.5	0.74±0.1	-
6.0	5.5±3.7	-	-	6.9±0.6	-	-	0.25±0.01	-	-
9.0	-	-	-	-	-	-	0.13±0.00	-	-
12.0	-	-	-	-	-	-	-	-	-

In the absence of salts, the inoculum increased the DW by rhizobium infection of root [4] and a regular supply of nitrogen was maintained to the plants by increasing N-fixation (Table 1). Presence of slat has deformed the root hair [4] and inoculation of rhizobia was restricted hence dual action of both factors (i. e. presence of salt and absence of rhizobia) has retarded the DWR.

Nitrogen content (mg/plant). Nitrogen content of the plant significantly decreased upto EC 6.0 dS m^{-1} at all growth stages, however, a non-significant increase of 0.1% at EC 9.0 dS m^{-1} and a significant increase of 15.0% at EC 12.0 dS m^{-1} was noted at the seedling stage. The nitrogen content improved upto 15.41% at flowering stage in the presence of EC 3.0 dS m^{-1} (Fig. 3). A significant increase of N-content (83.48%) was also recorded in treated plants in the absence of salt at maturity. It has somehow overcome the deleterious effect of salinity due to low salt concentration in the growth medium. There was a significant increase in nitrogen content of nodules in inoculated plants at the seedling stage upto EC 3.0 dS m^{-1}, but it decreased at EC 6.0 dS m^{-1} and higher salinity levels. The flowering stage seems to be adversely affected in this respect. The decrease in N (mg/plant) may be related to a direct effect of salinity on N-fixing ability of the plant [3]. Steinborn and Roughly [16] have also reported the same trend in the pea and mungbean.

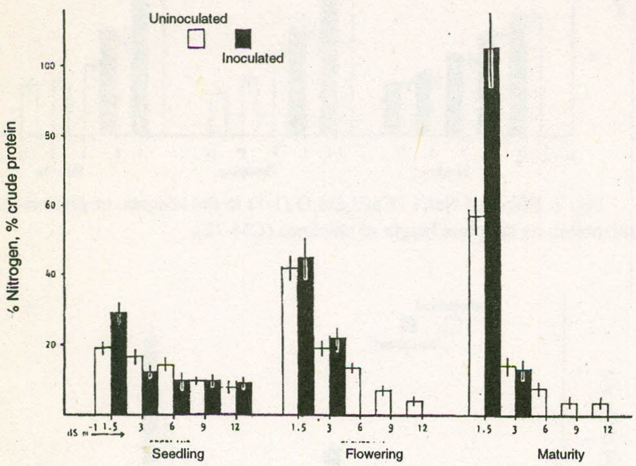

Fig. 3. Effect of NaCl : $CaCl_2 2H_2O$ (1:1) in the absence or presence of rhizobium on the N-content accumulation in the chickpea (CM-72).

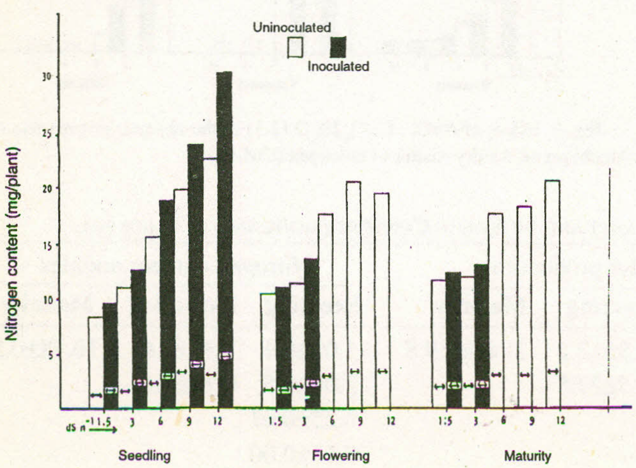

Fig. 4. Effect of NaCl : $CaCl_2 2H_2O$ (1:1) in the absence or presence of rhizobium on nitrogen and crude protein of chickpea (CM-72).

Crude protein content. Crude protein content increased with increasing salinity levels at the seedling stage, but decreased with the growth of plant. Crude protein content of non-inoculated plant significantly decreased in the control at flowering and maturity stages (Fig. 4). Percent nitrogen of the plants has also shown the results as observed in the case of crude protein. The present findings about crude protein content at the seedling stage correlate with those of Prisco and O, Leary [18]. The cumulative effect of salinity on the activity of rhizobia and their N-fixing ability [15] has reduced the growth of plants which resulted in reduced crude protein content.

Conclusion

The chickpea Var CM-72 can tolerate the salinity upto 3.0 dS m^{-1}. Salinity beyond this level adversely affected the process of nodule formation. Seeds treated with rhizobial inoculum accumulated significantly higher percentage of nitrogen and crude protein content than non-inoculated ones.

References

1. J. R. Wilson, Aust. J. Agric. Res., **21,** 571 (1970).
2. G. H. Abel and A. J. Mckenzie, Crop Sci., **4,** 157 (1964).
3. D. N. Mmunns, R. L. Rox and B. L. Koch, Plant and Soil, **46,** 591 (1977).
4. J. C. Tu, Can. J. Pl. Sci., **61,** 231 (1981).
5. R. G. Nieman, Plant Physiol., **40,** 156 (1965).
6. M. Santakumari, C. S. Reddy, A. R. C. Reddy and V. S. R. Das, Natur Wissenschatten. **66,** 54 (1979).
7. K. S. Panwar, Y. P. Singh, U. V. Singh and A. S. Mishra, Ind. J. Agron., **22,** (3), 145 (1977).
8. M. Kheradnam and S. R. Ghoshy, Agron. J., **65,** 329 (1973).
9. J. M. Vincent, *A Manual for the Practical Study of Root Nodule Bacteria.* IBP Hand Book No.15. (Blackwell Sci. Publications, Oxford, 1970).
10. J. C. Burton, *Rhizobium Culture and Use of P. I. in Pellar* (Ed) Microbial Technology (Reinhold Publishing Corporation. New York, 1976).
11. D. R. Hoagland and D. I. Arnon, Calif. Agric. Expt. Stn. Circ., 347 (1950).

12. R. R. Sokal and F. J. Rohlf, Biometry. In: *The Principles and Practice of Statistics in Biological Research,* (W.H. Freeman, San Francisco, 1981), 2nd ed., pp. 1-776.
13. J. Lunin and M. H. Gllatin, Soil Sci. Soc. Am. Proc., **29,** 608 (1965).
14. M. Attaullah, I. Haq, G. R. Sandhu and A. Sattar, Pak. J. Agric. Res., **2** (3), 183, (1981).
15. D. J. Lauter, K. N. Munns and K. L. Clarkin, Agron. J., **73,** 961 (1981).
16. J. Steinborn and R. J. Roughely, J. Appl. Bacteriol., **39,** 133 (1975).
17. V. Balasubramanian and, S. K. Sinha, Physio. Plant., **36,** 197 (1976).
18. J. T. Prisco and J. W. O, Leary, Plant Soil, **39,** 263 (1973).