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Effect of salinity on seed germination, seedling growth, nodulation and leghaemoglobin content in three cultivators of *Macrotyloma uniflorum* (Lam.)VERDC

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ABSTRACT

The objective of this study was to investigate the effect of sodium chloride on seed germination, seedling growth, nodulation and leghaemoglobin content in three cultivars of *M. uniflorum*. Salinity decreased the percentage of seed germination, root and shoot length, number of nodules, size of nodules, fresh weight of the nodules and also leghaemoglobin content. The highest percentage of germination was obtained at control and at 10mM salt concentration. Germination percentage was of a higher magnitude in cultivar-1 at all the salt concentrations tested. Number and size of the nodules, fresh and dry weight of the nodules was high in non-saline control. Leghaemoglobin content was also high in control plants inoculated with all the five salt tolerant rhizobia.

Key words : Salinity, Salt tolerant rhizobia, Nodulation, Leghaemoglobin

INTRODUCTION

Saline soils are major problems contributing to the low productivity in arid and semi-arid regions. It is estimated that 10% of the world's crop lands are affected by salinity. Like mature plant, germinating seeds and seedlings can also be subjected to environmental stresses. Salinity adversely affects germination of seeds and plant growth (Kingsbury and Epstein, 1986). Nitrogen fixation by the symbiotic association of legume-*Rhizobium* is greatly affected by environmental conditions and drought and salt stress are among the most deleterious factors. These stresses limit the nitrogen fixation under natural conditions. The number, size, weight of single nodule and leghaemoglobin content in the nodules has been reported to be inhibited under salt stress conditions (Ibrahim *et al.*, 1970; Wilson, 1970; Kumar and Pramila, 1983).

Horse gram [*Macrotyloma uniflorum* (Lam.) Verdc.] is an important tropical pulse crop and it is extensively cultivated on light red gravel soils of peninsular India. It is cultivated in 1.1 million hectares during both *Kharif* and *Rabi* seasons. It derives its importance from its adaptability to poor and adverse climatic conditions which are unsuitable for other pulse crops. Presently, attempts were made to assess the effect of salinity on seed germination, root length, shoot length, number of nodules, weight of the nodules and leghaemoglobin content in the three cultivars of *M. uniflorum.*

MATERIALS AND METHODS

The seeds of three cultivars of *M. uniflorum viz.*, cultivar-1, cultivar-2 and cultivar-3 were obtained from local fields in Andhra Pradesh. The seeds were surface sterilized with 6% H_2O_2 solution for 30 minuites and rinsed thoroughly with double distilled water. Ten healthy seeds of each cultivar of uniform size were placed on filter paper (Whatman no.1) in a Petri dish.Each Petri dish contained 10ml of the salt solutions *viz.*, control (without salt), 25mM, 50mM, 75mM, 100mM, 125mM, 150mM, 175mM and 200mM (three replicates were maintained for each concentration). The salt solutions were prepared by dissolving sodium chloride in distilled water. The Petri plates were incubated at 34 ± 1^0C. The percentage of seed germination, root length and shoot length were measured at 24 hours interval up to 5 days.

Out of the thirty two rhizobia five salt tolerant isolates *viz.*, HGR1, HGR4, HGR16, HGR17 and HGR24 were selected to study the effect of salinity on nodulation and leghaemoglobin content. Salt tolerant isolates of *M. uniflorum* rhizobia cultured on YEM broth by the method described by Vincent (1970). The experiment was conducted during *Rabi* season. Surface sterilized seeds of *M. uniflorum* were inoculated with 72h old cultures of different isolates. The inoculated seeds were sown in earthenware pots containing sterilized sand and treated with saline water containing 0-50 mM sodium chloride. The non treated pots served as control. The treatments were given 40 days after sowing. The five plants were pulled out randomly from each treatment at all the three growth stages. The number of nodules per plant, size of nodules, fresh and dry weight of the nodules was measured after detaching them from the roots. The leghaemoglobin content of nodules was estimated by the method described by Tu *et al.* (1970).

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RESULTS AND DISCUSSION

Salinity decreased the percentage of seed germination and early seedling growth in three cultivars of *M. uniflorum*. Moreover, the germination behavior showed variation with increasing salinity. This effect of salinity on germination may be osmotic or ionic resulting in reduction in final percentage of germination. The present investigation indicated that seed germination and early seedling growth of *M. uniflorum* under saline conditions depends on the level of salinity and the type of cultivar.

The effect of salinity on seed germination and early seedling growth (Fig.1) revealed that the germination of seeds responded favourably to salinity up to 200mM. These three cultivars showed variation in their response to salt stress. Cultivar-1 showed germination up to 200mM salt concentration, but the percentage of seed germination decreased with increasing salt concentration. Cultivar-2 showed germination up to 150mM, where as cultivar-3 showed germination up to 100mM only. It indicates that cultivar-1 showed more tolerance to salt stress followed by cultivar-2 and cultivar-3. Salinity is more harmful during early stages of germination and seedling growth. These three cultivars showed reduced elongation of the root with increasing salinity. All the three cultivars showed more root elongation at control. In these three, cultivar-2 showed some good response in root length when compared to the remaining two cultivars. All the three cultivars showed reduction in shoot length with increasing salinity over control.

The process of nodulation has been reported to be highly sensitive to saline conditions. Different isolates of *Rhizobium* isolated from *M. uniflorum* growing in different soil samples exhibited well developed nodules on their respective homologous hosts. The nodule number was maximum after 40 days under stress and in control (without salt). Different legume species differ in their relative resistance to salt stress. The number of nodules, fresh and dry weight of nodules per plant increased gradually up to 10mM after that, the number of nodules decreased with salinity in the plants inoculated with HGR1, HGR4, HGR16, HGR17 and HGR24. The number of nodules and fresh weight of nodules were more in the plants inoculated with HGR17. The fresh weight of the nodules was also decreased with increasing salinity in the plants inoculated with HGR1, HGR4, HGR16, HGR17 and HGR24, respectively. Gupta (2002) reported that the increasing levels of salinity adversely affected nodulation and leghaemoglobin contents in various crop plants.

Among the five salt tolerant isolates, the isolate HGR17 showed more nodulation in all the three cultivars followed by HGR1, HGR4, HGR16 and HGR24 (Table 1). These five salt tolerant isolates showed more leghaemoglobin (485µg/g) at control, though the number of nodules and the size of nodules were high at 10mM.

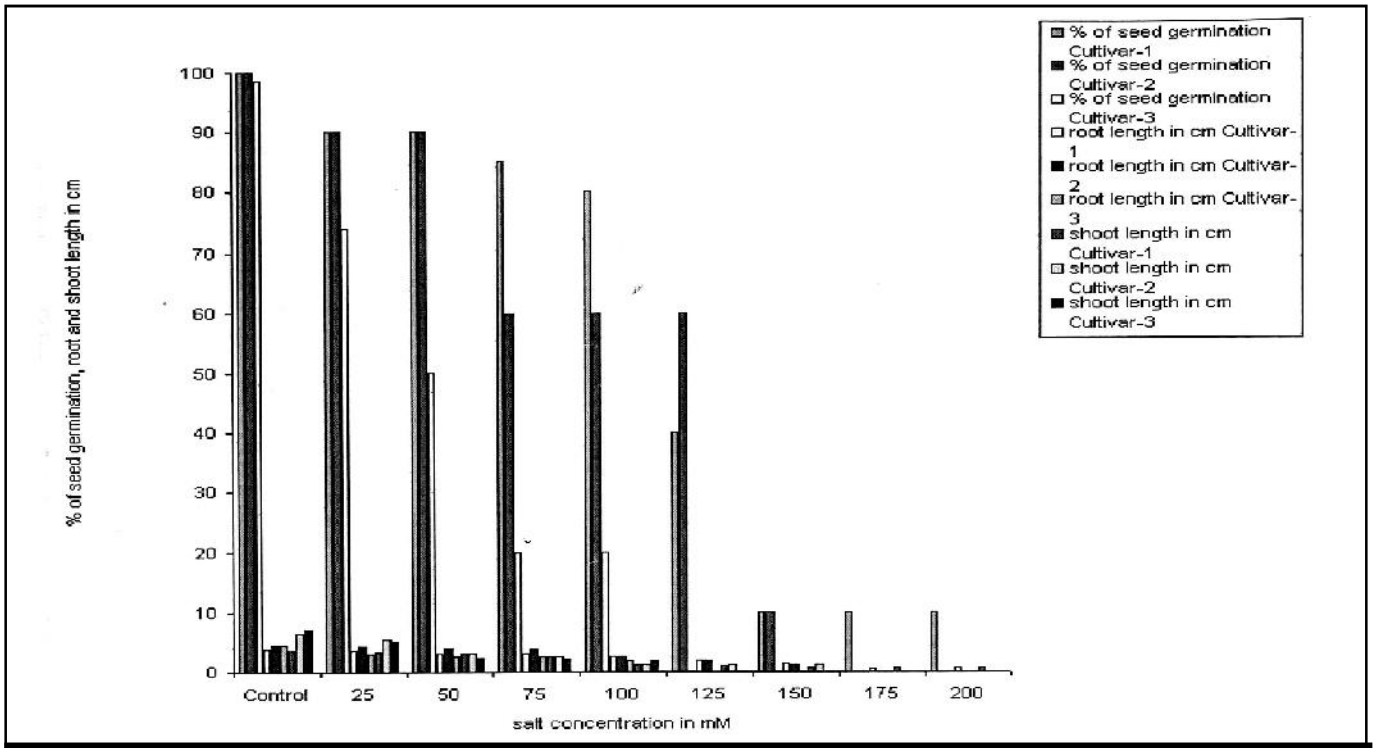

Fig. 1 : Effect of salinity on seed germination, root and shoot length in three cultivars of *M. uniflorum*

Table 1 : Effect of salinity on nodulation and leghaemoglobin content in three cultivars of *M. uniforum* plants inoculated with salt tolerant rhizobia

Parameters	Salinity in mM	Cultivar-1					Cultivar-2					Cultivar-3				
		HGR 1	HGR 4	HGR 16	HGR 17	HGR 24	HGR 1	HGR 4	HGR 16	HGR 17	HGR 24	HGR 1	HGR 4	HGR 16	HGR 17	HGR 24
Size of	Control	5	5	5	6	4	5	5	5	6	5	5	5	5	6	5
nodule	10	6	6	5	6	4	5	5	4	5	6	5	5	4	6	5
(mm)	20	5	5	4	4	3	4	4	3	4	3	4	5	4	6	4
	40	1	2	2	2	2	0	1	1	1	1	1	1	1	1	1
Number of	Control	16	14	15	17	16	15	14	15	17	16	15	14	15	17	16
nodules	10	17	14	15	17	13	16	14	15	17	13	15	14	15	17	16
	20	12	12	13	12	10	10	12	13	12	10	10	12	13	12	10
	30	8	8	6	10	6	7	8	6	10	6	7	8	6	10	6
	40	5	4	4	6	3	4	4	4	6	3	4	4	4	6	3
Fresh	Control	180	175	185	190	162	150	170	182	196	148	142	130	135	142	126
weight of	10	165	164	160	182	154	132	160	154	186	150	137	110	132	128	120
nodules	20	120	138	125	160	120	108	134	136	162	132	100	092	120	010	112
(mg)	30	100	100	102	124	100	094	098	090	130	120	090	086	092	086	092
	40	082	074	068	082	054	054	070	072	092	084	050	062	054	050	060
Dry weight	Control	021	020	022	028	020	020	020	020	026	018	018	020	020	022	018
of nodules	10	018	018	020	024	016	016	014	015	016	016	014	018	018	018	016
(mg)	20	016	016	014	020	014	014	012	010	014	010	011	016	016	016	012
	30	014	014	010	018	014	014	010	008	002	008	010	014	012	012	008
	40	---	---	---	---	---	---	---	---	---	---	006	---	---	---	---
Leghaemog	Control	460	430	460	400	485	380	400	420	400	440	320	380	400	382	370
lobin	10	456	445	306	434	300	330	380	400	380	400	310	372	380	360	330
(µg/ml)	20	428	386	375	315	296	318	330	380	364	320	292	312	290	316	300
content	30	368	355	346	338	325	296	300	322	322	280	270	274	260	290	262
	40	175	162	155	140	134	270	272	220	232	240	220	230	260	280	200

After that the amount of leghaemoglobin decreased with increase in salt concentration. The amount of leghaemoglobin was high in the plants inoculated with the isolate HGR24 followed by HGR1, HGR16, HGR4 and HGR17. At 40mM salt concentration the amount of leghaemoglobin was very low (140-280 µg/g) in the three cultivars inoculated with the five rhizobial isolates. At 50mM salt concentration the plants showed some abnormal growth (bushy appearance, stunted growth). The amount of leghaemoglobin is positively correlated with nodule nitrogen fixation. Salt tolerant isolates of *Rhizobium* were able to grow and fix nitrogen in saline soils. Lower concentrations of sodium have been found to absolutely essential for N_2 fixation in certain diazotrophs (Apte *et al.,* 1987). Statistical analysis using ANOVA revealed that the number and size of the nodules, fresh weight and leghaemoglobin content at all the salt concentrations inoculated with five *Rhizobium* isolates were significant.

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REFERENCES

Apte, S.K, Reddy, B.R. and Thomas, J. (1987). *Appl. Environ. Microbiol.*, **53**:1934.

Gupta Neeraj (2002). Chemical composition and nutritive value of field bean (*Vicia faba* L.) as affected by soil salinity, Ph.D. Theses, M.J.P. Rohilkhand University Bareilly, India.

Ibrahim, A.N., Kamal, M. and Khadar, M.P. (1970). Nodule information and growth of legumes as influenced by certain sodium salts. *Agrolem Talajatan,* **19**:164–172.

Kingsbury, R.W. and Epstein, E. (1986). Salt sensitivity in wheat: A case for specific ion toxicity. *Plant Physiol.,* **80**:651-654.

Kumar, S. and Pramila (1983). Effect of chloride and sulphate type of salinization and desalinization on nodulation and nitrogen fixation in Chickpea. *Indian J. Plant Physiol.,* **26**:396-401

Tu, J.C., Ford, R.E and Garu, C.R. (1970). Some factors affecting nodulation and nodule efficiency in soybeans infected by Soybean mosaic virus. *Phytopathol.*, **60**:1653-1656.

Vincent, J.M. (1970). *A Manual For The Practical Study Of The Root Bodule Bacteria*. IBP hand book, No.15. Black Well Scientific Publications. Oxford pp. 79.

Wilson, J.R. (1970). Response at salinity in Glycine VI. Some effect of a range short term salt stress on the growth, nodulation and nitrogen fixation of Glycine weightii. *Australian J. agric. Res.*, **21**:511-582

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