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Effect of salinity and inoculation on growth, nitrogen fixation and nutrient uptake of *Vigna radiata* (L.) Wilczek

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Abstract

This study reports the effect of salinity and inoculation on growth, ion uptake and nitrogen fixation by *Vigna radiata*. A soil EC_e level of 7.5 dS m^{-1} was quite detrimental causing about 60% decline in dry matter and grain yield of mungbean plants whereas a soil EC_e level of 10.0 dS m^{-1} was almost toxic. In contrast most of the studied strains of Rhizobium were salt tolerant. Nevertheless, nodulation, nitrogen fixation and total nitrogen concentration in the plant was drastically affected at high salt concentration. A noticeable decline in acetylene reduction activity occurred when salinity level increased to 7.5 dS m^{-1}.

Introduction

Vigna radiata is an important pulse crop in Pakistan. Being a legume it could produce food rich in protein without addition of nitrogen fertilizers. Therefore, this crop is especially suitable in developing countries like Pakistan where availability of protein is insufficient and fertilizers are expensive.

Nodulation in *Vigna radiata* under field condition is very poor (Idris *et al.*, in press; Ramaswamy and Nair, 1965). It may be due to absence of Rhizobium in such soils. Alternatively other environmental factors such as salinity (Abdel Ghaffar *et al.*, 1982; Lauter *et al.*, 1981), high temperature and drought (Marshall, 1964; Mass and Hoffman, 1977; Vincent *et al.*, 1962) may effect the nodulation and nitrogen fixation of leguminous plants. For example a few studies (Bernstein and Ogata, 1966; Wilson, 1970) show that the effect of salinity on nitrogen fixation ability of soybean and alfalfa could be very detrimental. In fact pulse crops are reported not to nodulate on salt-affected land even though native rhizobia are known to be present (Bhardwaj, 1974). The major point to investigate is whether symbiotic nitrogen fixation is more sensitive to salinity than host plant growth.

This paper reports the effect of salinity on the growth, nitrogen fixation, yield and nutrient uptake in mungbean. In addition, *in vitro* salt tolerance of five strains of *Rhizobium* spp. was also assessed.

Materials and methods

The seeds of mungbean (cv. 20–121) were obtained from Mutation Breeding Division, Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad. Three experiments were conducted in this study. The effect of different salt concentrations on the host (Mungbean) was studied in experiment A and B and on the endophyte (Rhizobium) was studied in experiment C.

Experiment A

This experiment was conducted in sterilized sand flushed with nutrient solution contained in Leonard jars. Seeds were inoculated with locally prepared carrier (gamma irradiated, filter mud amended with heavy loam and sucrose) based Rhizobium inoculum at a dose of 20 g100 g^{-1} seeds. Inoculum was mixture of five strains (M-11, M-17,

TAL 441, TAL 420, and TAL 169). For ensuring firm association of Rhizobium to the seeds, the seeds were successively coated with gum arabica, Rhizobium inoculum and rock phosphate. The number of viable cells at sowing time were $1.7 \times 10^{10} g^{-1}$ of inoculum and $7 \times 10^6 seed^{-1}$. Sand cultures were flushed daily with 1/4 strength nitrogen free Hoagland solution. At first leaf stage, thinning was done to leave uniform seedlings in each jar. At this stage NaCl, $CaCl_2$, Na_2SO_4 and $MgCl_2$ were added in ratio of 4:5:10:1 to the flushing nutrient solution to produce the salt concentrations of 1.4, 5.0, 7.5 and 10.0 dS m^{-1}. Each treatment was replicated six times. Plants were grown for 30 days. At harvesting shoot and root dry weight were recorded and roots were studied for nodulation and nitrogen fixation as estimated by acetylene reduction technique (Hardy *et al.*, 1968). Nitrogenase activity was measured by incubating excised nodulated root systems in 200-ml plastic bottles tightly closed with screwed caps fitted with suba seals. A 10:90 acetylene air atmosphere was created inside the bottle. After incubation for 1 hour at room temperature the gas samples (100 μl) were analysed on a gas chromatograph (Carlo-Erba Model 180) fitted with a 1 m × 2 mm steel column filled with Porapak R (80–100 mesh) and a H_2 flame ionization detector (FID). Nitrogen was used as a carrier gas at a flow rate of 30 ml min^{-1}. Two controls, one bottle with C_2H_2 but without nodules and the other with nodules but without addition of acetylene were also included during each assay to check indigenous production of ethylene.

Experiment B

This experiment was conducted in soil in 8 kg capacity plastic buckets. The soil used in the experiment was sandy clay loam, non-saline (EC_e = 1.4 dS m^{-1}; pH 7.8) and had available N and P of 0.039 and 2 ppm respectively. Available nitrogen in soil was determined according to Bremner (1965). Available phosphorus in the soil was assayed according to the Olsen method (Watanabe and Olsen, 1965). Artificial salinization of the soil was achieved with NaCl, $CaCl_2$, Na_2SO_4 and $MgCl_2$ mixed in ratio of 4:5:10:1 to produce salinity levels of EC_e of 1.4, 5.0, 7.5 and 10 dS m^{-1} (Qureshi *et al.*, 1977). Urea (20 kg ha^{-1}) and KH_2PO_4 (60 kg ha^{-1}) wer applied at rates of 0.17 g and 1.7 g per pot respectively. Ten seeds were sown in each plastic pot. The pots were not drained and water was given as needed to keep the soil around field capacity. Seeds were inoculated as in experiment A. Each treatment was replicated twelve times. At first leaf stage the seedlings were thinned out to four uniform plants, in each pot.

Plants were sampled for nodulation and acetylene reduction assay at preflowering, 33 days after sowing (DAS) and flowering (45 DAS) stages and for yield at 65 DAS. Oven dried plant samples as well as air dried grains were ground and thoroughly homogenized. Such samples were used for nitrogen, phosphorus, potassium and sodium assay. For the estimation of total nitrogen concentration in plant, samples were digested in concentrated H_2SO_4 and measured by micro-Kjeldahl method (Bremner, 1965). For phosphorus, sodium and potassium assay, plant material was digested in a mixture of HNO_3 and $HClO_4$ (Richards *et al.*, 1954). Phosphorus concentration in plant was determined colorimetrically (Jackson, 1962). Potassium and sodium concentrations were measured by flamephotometry. Each value was the mean of three replicates.

Experiment C

The salt tolerance of five Rhizobium strains (Cowpea group) was studied in yeast mannitol (Vincent, 1970) cultures containing 1.7, 5, 10, 25, 50, 100, 150 and 200 mol m^{-3} NaCl. The strains were chosen on the basis of their efficiency to nodulate mungbean. Several strains were studied and most effective were included in the experiment for further studies. Cells were grown for three days in the dark at 29 °C in shaken Erlenmeyer flask containing 50 ml medium. The initial cell density was 10^6 viable cell ml^{-1}. Among the five Rhizobium strains, two strains (M-11, M-17) were of local origin while the rest (TAL 441, TAL 420, TAL 169) were obtained from NIFTAL Hawaii USA. Viable cells were counted by using standard serial dilution and plated by spread plate count method (Vincent, 1970).

Table 1. Effect of salinity and inoculation on dry matter yield of mungbean grown in soil. Plants were harvested at different stages of its growth (Experiment B)

EC_e of soil dS m^{-1}	Dry weight (g $plant^{-1}$)		
	Preflowering	Flowering	Maturity
1.4 Uninoc.	0.8	2.1b	2.4ab
Inoc.	1.1	2.7a	3.1a
5.0 Uninoc.	0.6	1.0d	1.9bc
Inoc.	1.0	1.6c	2.5ab
7.5 Uninoc.	0.4	0.6e	1.0d
Inoc.	0.6	1.1d	1.3cd
10.0 Uninoc.	0.3	0.3e	
Inoc.	0.3	0.4e	
	NS		

Means followed by same letter are not significantly different at 5% probability level.
Uninoc. Uninoculated.
Inoc. Inoculated.
NS: Non-significant.
Values are mean of three readings.

Results and discussion

Germination and plant survival

The germination was 100 percent in both soil and sand cultures at all salinity levels; only a delay of three days in germination was noted in the case of 7.5 and 10.0 dS m^{-1}. In fact in most plant species, moderate levels of salinity delay germination and not the germination percentage (Salim *et al.*, 1979). In experiment B the survival of plants was decreased with increasing salinity levels. The survival was 96% at 7.5 dS m^{-1}, at an EC_e level of 10 dS m^{-1} the survival of plants were decreased from 80% to 58% at flowering stage and all plants had died by maturity. At high salt concentration death of seedlings after germination has also been reported in case of *Sesbania aculeata* (Salim *et al.*, 1979) and *Leucaena leucocepheala* (Niazi *et al.*, 1985).

Plant growth and yield

Dry matter yield per plant (Table 1, 2) decreased significantly with increase in salinity levels regardless of the stages of plant growth. Data also showed that soil salinity of 7.5 dS m^{-1} was quite detrimental to cause a 50–60% decline in dry matter yield of mungbean plant, whereas, the soil EC_e level of 10.0 dS m^{-1} was almost toxic. Reduction in plant growth and dry matter accumulation was observed at moderate salinity levels in mungbean. This is in agreement with salt sensitivity reported in other leguminous plant species *Vicia faba*, *Phaseolus vulgaris* grown at the same salinity levels as reported in our experiments, (Abdel Ghaffar *et al.*, 1982) and *Glycine wightii* (Wilson, 1970).

An inverse correlation was found between salinity and grain weight. Higher the soil salinity the lower were the weight of grains (Table 3). However, the number of pods per plant and grains per pod were not significantly affected up to a salinity level of 7.5 dS m^{-1}. The grain yield of mungbean was about 60% lower at salinity levels of 5 dS m^{-1} and 7.5 dS m^{-1} as compared to 1.4 dS m^{-1} while it was completely depressed at 10 dS m^{-1}. Similar results

Table 2. Effect of salinity and inoculation on dry matter yield and nodulation on mungbean grown in nutrient solution. Plants were harvested after 30 days of growth (Experiment A)

EC_e dS m^{-1}	No. of nodule $plant^{-1}$	Frequency of nodulation (%)	Dry weight of nodules (mg $plant^{-1}$)	μmole C_2H_4 g^{-1} dry nodule h^{-1}	Dr matter mg $plant^{-1}$ (whole plant)
Uninoc.					
1.4	0	0	0	0	753 ± 33
Inoc.					
1.4	7	85	18 ± 0.3	22 ± 2.2	901 ± 17
5.0	4	40	8 ± 0.2	18 ± 2.5	732 ± 13
7.5	2	10	4 ± 0.5	3 ± 1.6	417 ± 12
10.0	0	0	0	0	77 ± 6

Uninoc. Uninoculated.
Inoc. Inoculated.
Values are the mean of three readings ± standard deviation of the mean.

Table 3. Effect of salinity and inoculation on some components of grain yield of mungbean grown in soil (Experiment B)

EC_e of soil $dS\,m^{-1}$	Yield components			
	No. of pod $plant^{-1}$	No. of grain pod^{-1}	Wt of grain $plant^{-1}$ (mg)	Wt of 1000 grain (g)
1.4 Uninoc.	2.7	4.1	506a	45.7a
Inoc.	4.0	4.7	816b	43.4a
5.0 Uninoc.	2.3	3.7	214d	25.2b
Inoc.	3.7	4.3	345c	21.7b
7.5 Uninoc.	1.7	5.3	195d	21.6b
Inoc.	2.3	5.3	330c	27.0b
	NS	NS		

Means followed by the same letter are not significantly different at 5% probability level.
Uninoc. Uninoculated.
Inoc. Inoculated.
NS: Non-significant.
Values are the mean of three readings.

have been reported on other leguminous plant species *Vicia*, *Phaseolus*, *Glycine* and *Medicago* (Abdel Ghaffar *et al.*, 1982; Bernstein and Ogata, 1966; Wilson, 1970).

Nodulation and nitrogen fixation

The reponse of mungbean to inoculation is shown in Table 2 and 4. In Experiment A, uninoculated plants bore no nodule and had a 16% lower dry matter yield than the inoculated plants at salinity level of EC_e 1.4 $dS\,m^{-1}$. In case of Experiment B, not a single nodule was observed in uninoculated plants, therefore, soil is either devoid of Rhizobium or indicates the ineffectiveness of local strains to infect mungbean roots. Similar results were obtained by Idris *et al.* (in press) in field grown mungbean plants. The frequency of nodulation on mungbean at flowering stage was 88%. In this experiment inoculation with mixture of five Rhizobium strains clearly benefitted *V. radiata* as indicated with increased nodulation, nitrogen fixation, dry matter production and grain weight per plant. However, the effect of inoculation was not significant on the number of pods per plant and number of grains per pod as compared to controls.

The effect of salinity on nodulation and nitrogen fixation in mungbean is presented in Table 3 and 4. Nodulation and nitrogen fixation of inoculated plants were highly sensitive to salt. Frequency of nodulation, weight of nodules and nitrogen fixation was decreased by increasing salinization. Nodulation was reduced to about half at salinity level of EC_e 5.0 $dS\,m^{-1}$ as compared to 1.4 $dS\,m^{-1}$. The nodulation was completely depressed at EC_e 10 $dS\,m^{-1}$ regardless of plant growth stages. The effect of salinity was more severe on the number of nodules per plant than on the specific nitrogenase activity. A noteable decline in acetylene reduction activity occurred when salinity level increased to 7.5 $dS\,m^{-1}$. These results corroborate the earlier findings on *Glycine*, *Macroptilium*, *Neonotonia*, *Medicago*, *Vicia* and *Phaseolus* species (Abdel Ghaffar *et al.*, 1982; Bernstein and Ogatam, 1966; Lakshmi *et al.*, 1974; Wilson, 1970, 1985).

Nutrient uptake

The concentration of nitrogen in plant tissue as affected by soil salinity was in harmony with those of dry matter at maturity stage (Table 5). With the rise of salinization the total nitrogen concentration in pod grain and whole plant significantly decreased. The concentration of nitrogen significantly

Table 4. Effect of salinity and inoculation[a] on nodulation and nitrogen fixation of mungbean grown in soil (Experiment B)

EC_e of soil $dS\,m^{-1}$	No. of nod. $plant^{-1}$		Frequency of nodulation (%)		Dry weight of nodule (mg)		μmole $C_2H_4\,g^{-1}$ dry nodules h^{-1}	
	Pre-flowering	Flowering	Pre-flowering	Flowering	Pre-flowering	Flowering	Pre-flowering	Flowering
1.4	14	20	66	88	12 ± 2.0	15 ± 5.4	38 ± 2.4	32 ± 6.6
5.0	4	9	33	38	8 ± 2.1	11 ± 2.3	35 ± 1.3	24 ± 2.9
7.5	2	1	33	8	4 ± 1.1	5 ± 2.0	23 ± 1.4	4 ± 1.5
10.0	0	0	0	0	–	–	–	–

[a] Uninoculated plants had no nodules. Therefore, data are given only for inoculated plants.
Values are the mean of three readings.

Table 5. Effect of salinity and inoculation on total nitrogen concentration[a] at maturity (Experiment B)

EC_e of soil $dS\,m^{-1}$	Pod shell	Grains	Whole plant
1.4 Uninoc.	11.1ab	27.9b	50.7bc
Inoc.	13.6a	32.9a	59.4a
5.0 Uninoc.	8.1bc	24.7cd	45.6cd
Inoc.	9.2abc	26.4bc	53.0ab
7.5 Uninoc.	4.7c	23.0d	44.1bcd
Inoc.	5.0c	26.3bc	50.1d

Means followed by the same letter are not significantly different at 5% probability level.
Uninoc. Uninoculated.
Inoc. Inoculated.
[a] $mg\,N\,g^{-1}$ dry matter.
Values are the mean of three readings.

increased in grains and whole plant at all levels of salinity when inoculated with Rhizobium. The results are in agreement with those reported earlier on *Phaseolus vulgaris*. Inoculation of *P. vulgaris* markedly enhanced nodulation, N_2-fixation plant dry matter N content and final yield (Abdel Ghaffar *et al.*, 1982). The P concentration was similar to N concentration in plant (data not presented) so is not discussed separately.

In general K^+ concentration was similar in different plant parts at preflowering and flowering stage of growth of mungbean grown at various salinity levels. In contrast higher the EC_e level in the soil, higher was the concentration of Na^+ in the shoot and plant as a whole at both preflowering and flowering stage. However, there was only a slight increase in concentration of Na^+ in pods and grain with increase in EC_e level of the soil (Table 6). The ions to the pods and grains have been mainly supplied through phloem. Therefore, the exclusion of Na^+ in the pods and grains may have been due to efficient exclusion of Na^+ in phloem vessels.

Experiment C

In this experiment five Bradyrhizobium strains 'Cowpea group' were checked for their relative salt tolerance. Out of these five strains, two strains M-11, M-17 were local isolates while TAL 441, TAL 420 and TAL 169 were obtained from NIFTAL, Hawaii, USA. There was no significant difference in the growth of all the strains tested as even

Table 6. Effect of salinity on concentration[a] of Na^+ and K^+ in mungbean grown in soil. Plants were harvested at different stages of growth (Experiment B)

EC_e of soil $dS\,m^{-1}$	Flowering		Maturity					
			Pod shell		Grain		Shoot	
	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+
1.4	23.9	85.7	4.8	77.4	3.6	66.4	28.5	83.8
5.0	23.0	86.3	5.8	58.6	4.6	–	43.0	86.4
7.5	50.0	85.0	7.6	75.4	5.9	67.0	61.0	88.0
10.0	75.6	76.4	–	–	–	–	–	–

[a] $meq\,100\,g^{-1}$ dry matter.
Values are the mean of three readings.

$200\,mol\,m^{-3}$ NaCl did not effect the growth. Strain M-17 however showed a slight decrease (13%) in its cell numbers at $200\,mol\,m^{-3}$ NaCl. Similar results showing relatively high salt tolerance of Rhizobium strains have also been reported by other workers while working with *Rhizobium meliloti* (Douka *et al.*, 1984; Kassem *et al.*, 1985; Singleton *et al.*, 1982).

This study suggests that salinity has an indirect effect on biological nitrogen fixation in mungbean. The rhizobia are generally more capable to cope with salinity than their host legumes. However, the effect of salinity was more pronounced on the number and weight of nodules per plant than on their specific nitrogenase activity. It is clear from Table 4 that at preflowering stage as a result of the increase in salinity, a reduction of 75% in nodule dry weight was estimated as compared to only 40% in case of nitrogenase activity. These observations indicate that in addition to indirect effects, salinity affects nodule formation. However, it seems that when a nodule is formed, then subsequently there is little influence of salinity on its functioning provided the plant can maintain a reasonable photosynthetic activity as in evident from the biomass yields at different salinity levels and the physiological age of the plant. More work is needed to explore the reasons why *Rhizobium* species fail to successfully infect mungbean roots at high salt concentrations inspite of their high *in vitro* tolerance to NaCl.

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