

PJN

ISSN 1680-5194

PAKISTAN JOURNAL OF
NUTRITION

ANSI*net*

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Nutritional Influence of Salt Stress on the Growth and Nodule Formation of *Vicia faba*

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Abstract: Seedlings of *Vicia faba* were germinated and grown on moist filter paper in Petri dishes for about ten days. Seedlings of uniform size were then transplanted into pots containing garden soil and different NaCl treatments/EC values i.e 0.2 (control), 1.3, 2.4, 4.6, 9.1, 13.6, 18.0 and 22.5 mS/cm. Root and shoot lengths, their fresh and dry weights and root nodulation were recorded at different intervals after transplantation. Fresh and dry weight of shoot generally decreased with increasing NaCl levels in *Vicia faba*. Plants of treatment (control), 0.1, 0.2, 0.4 remain alive, their weight and length of shoot increased with the passage of time and the weight first increased and then decreased with increasing NaCl levels. Similar results were obtained for roots, but other die. In *Vicia faba* flowering and pod formation was negligible during first, second and third harvest at any treatment. Nodule formation was observed and the number of nodules per plant decreased with increasing NaCl levels. It was that increasing trends of salinity levels in *Vicia faba* caused a reduction in the growth of morphological characters and nutritional stress by decreasing in nodulation number and weight age.

Key words: *Vicia faba*, salinity stress, morphological characters, nodulation

INTRODUCTION

In Pakistan out of 34 million acres of arable land, 14 million are affected by salinity. Out of total salty area 4.7 million acres are saline. 7.2 million acres are saline sodic (Anonymous, 2010). Saline and saline sodic soils with their relatively low pH (usually less than 8.5) have detrimental influence on plants, when water solution with large amount of dissolved salts comes in contact with root hairs. It causes the shrinkage of its protoplasm by plasmolysis (Ahmed and Ghaffar, 1987). This is due to the movement of water from the root hair to the more concentrated soil solution by exosmosis, the cell then collapse (Borucki and Sujkowska, 2011). The nature of salts, the species of the plants as well as other factors determine the salt concentrations at which the plant would not grow (Nawaz, 2007; Bouhmouch *et al.*, 2005). Saline soils are characterized by the presence of high level of neutral salts in the surface layer resulting from the capillary rise of water when evaporation exceeds precipitation. Considering the proportion of soils in the world which are saline remarkable little effort has been expended to examine the effect of salinity on nitrogen fixation (Sprent, 1984).

Generally nodulated crop plants do not like saline conditions (Mirza and Tariq, 2011). Yousaf and Sprent (1983) found that under saline conditions nodules partially compensated by producing larger rather than more nodules. Balasubramaniam and Sinha (1976) found that at 0.3% sodium chloride, nodule ignition was

severely reduced in beans but nodule development was not affected. Adverse effects of increasing salinity on nodules number and nodule weight have been well reported (Lauter *et al.*, 1981; Predeepa and Ravindran, 2010).

Vicia faba cultivated as vegetable is used green or dried, fresh or canned and for stock feed. Feeding value of broad bean is high and considered in some areas superior to field peas or other legumes (Anthraper and Dubois, 2003). Broad bean has been considered as a meat extender (Duke, 1981; Soussi *et al.*, 1998).

Salts in soil contribute to lower the fertility and productivity status of the soil. In Pakistan, a vast agricultural area is adversely affected by salinity every year. That is why, more and more farm houses are turned into barren plains and every year yield is reducing at an alarming rate. A lot of work has been done to study the effect of salinity on plants growth. However, little efforts have been made to study the effect of salinity on root nodulation. Present work was therefore carried out to investigate the effects of NaCl on growth and nodulation of a leguminous crop *i.e. Vicia faba* that is commonly known as Broad bean and is widely cultivated.

MATERIALS AND METHODS

Physic-chemical characteristics of soil: Soil samples from each treatment were collected in labeled polythene bags and brought in laboratory. They were crushed, air

dried and passed through a 2 mm sieve before analysis for saturated paste. 250 g soil was taken in 10 pots for each treatment; distilled water was added by a stirring with a spatula. At saturation, the soil paste glistened as it reflected light, flow slightly when the container was tripped and the paste slid freely of the spatula. Saturated soil paste was then put in Buchner funnel having a filter paper and attached to a suction pump. The saturated soil extract was collected in suction flask. Electric pump was operated for about 15 min for collecting soil extract until a definite end point reached. Saturated soil extract was then used to determine pH (pH meter, HM-10K) and EC_e (conductivity meter, CM-30EF). The EC_e was determined at about 34°C. Later on it was converted in a standard reference temperature. i.e. EC using the formula:

$$EC_{25} = EC_t, EC_t = EC_t \text{ at } 34^\circ\text{C}$$

and

$$t = \text{Temperature conversion factor}$$

Regression of EC_{25} on salt percent in soil was then computed. [The salty % of the controlled soil (with SP = 50) was determined from Fig. 7 given in an Agri. Handbook No. 60 USDA, 1968, P17]. The salty % of the control was then added to each of the NaCl gradient (treatment) accordingly. The SP saturation percentage was determined by the following two methods in Agri. Handbook no.60USDA, 1968.P17 Saturation percentage (sp) from oven dried saturated soil paste was prepared as given above. A portion of saturated soil paste was subjected to 105°C and weighted again. Saturation percentage was then calculated by the formula:

$$Sp = \text{Loss in dry weight/Oven dry weight of soil} \times 100$$

The volume and weight of a cup was determined. Cups filled with saturated pastes. Jar it during filling, exclude the air and level the paste with the help of knife. It was weighed and the cup weight was subtracted to get the net weight of the paste (Table 1).

SP of soil was calculated by using the formula:

$$Sp = 100 (2.65 V - W) / 2.65 (W - V)$$

To know the soil texture, the soil sample (control) was crushed, air dried and sieved through a 2 mm sieve. The soil was taken in a 1000 ml beaker. Then 200 ml tap water and 20 ml saturated solution of sodium oxalate (as a deflocculating agent) was added. This soil suspension was stirred with the help of the electric stirrer for about 10 min. The soil suspension was transferred into 1000 ml graduated glass cylinder. The volume of suspension was made up to 1000 ml by adding more tap water. The suspension was washed thoroughly before keeping it undisturbed for minutes.

After 5 min a Bonyoucos Hydrometer was lowered down slowly and carefully into the suspension. The reading of hydrometer was noted that proved it to be clay slit. Temperature of the suspension was also noted by lowering a thermometer into cylinder. The cylinder was reshaped end over end and left undisturbed for 5 hr. After 5 hr hydrometer and thermometer readings were again noted. This reading was for clay (Table 2) for temperature correction.

If the temperature was above 20°C, units were added in hydrometer reading for every one degree rise in temperature, if temperature was less than 20°C then 0.3 units were subtracted for every one degree fall in temperature. Percentage of sand, clay and slit was calculated. The texture class of soil sample was determined by comparing with soil sample triangle.

Plant material: Seeds were sown in Petri dishes containing wet filter paper. The Petri dishes were placed on a table filled with a bank of fluorescent tubes. After 10 days, the seedlings were transplanted into pots (5 seedling/pots). The pots contained eight NaCl treatments i.e. 0.1%, 0.2%, 0.4%, 0.8%, 1.2%, 1.6%, 1.6%, 2.0% (w/w) and controlled. For 0.1% NaCl treatment, 5 gm of NaCl dissolved in 900 ml tap water was added into the pot constraining 5 kg garden soil. This amount of salt solution was efficient enough to soak the soil up to the bottom of the pot. For 0.2% NaCl treatment 10 g of NaCl was dissolved in 900 ml of tap water. Similarly, other NaCl treatments were prepared accordingly and pots were then left undisturbed for about two days to dry the soil. After this, the soil of each pot was mixed thoroughly. There were 10 replicate pots for each NaCl treatment and the Completely Randomized Design (CRD) was used. The observations were recorded as given below.

Plant length (cm): Plants were harvested and separated into root and shoot. A scale (in centimeter) was used to measure the length of plant i.e. root and shoot length.

Plant fresh and dry weights (g): Fresh and dry weights of root and shoot were done with digital, pitted electric balance (Chyo, M.K.200B). The dry weights were measured after drying in oven at 80°C for 48 hr.

Nodule frequency and weight (g): Nodules from each plant were separated and weighed freshly. Dry weight was recorded after drying in an oven at 80°C for 48 hr. Nodules at each plant's root were counted.

Number of pods per plant: Counted the number of pods formed on each plant.

Number of flowers per plant: Total number of flowers was counted on each plant.

Table 1: pH and ECe of various soil samples with different NaCl percentage (w/w)

Salt percentage in soil	pH	EC ₃₄ (ms/cm)	EC ₂₅ 9(ms/cm)	EC ₂₅ from regression equation (ms/cm)
0.05 (Control)	7.40	1.30	1.09	0.2
0.15	7.40	1.35	1.12	1.3
0.25	7.04	2.90	2.44	2.4
0.45	6.90	5.12	4.31	4.6
0.85	6.93	7.24	6.10	9.1
1.25	6.84	19.50	16.43	13.6
1.65	6.59	22.80	19.22	18.0
2.05	6.60	24.90	20.99	22.5

The percent salt in control soil (SP = 6, EC₂₅ = 1.09 mS/cm) was determined from the Fig. 7 (agricultural hand book no. 60, USDA, 1968, p. 17). EC₂₅ = Ect x fl agricultural hand book no. 60, USDA, 1968, p. 89).

Note: pH and ECe of soil saturation extract was determined for the soil sample 9 from the root zone) taken midway through the growing season

Table 2: Some physical characteristics of control soil (ECe = 0.2 ms/cm)

Sand	68.4%
Silt	11.4%
Clay	20.2%

Sandy clay loam textural class

Statistical analysis of data: The data for plant length, number of nodules, fresh and dry weight of nodules, fresh and dry weight of roots and shoots were subjected to Analysis of Variance (ANOVA) by using MS Excel 2003 and MSTATC (Anonymous, 1986) in order to determine the effect of various NaCl treatments on the various growth attributes (Steel and Torrie, 1984). Duncan's Multiple Range Test (Duncan, 1955) was applied to compare the treatment means of various variables.

RESULTS AND DISCUSSION

Table 1 show the pH and ECe25 (From saturated paste extract) in soil samples with increasing NaCl percentage. The pH of the soil remained around 7.0 in all the treatments, however, ECe25 of the soil samples taken from the root zone increased proportionally with increasing NaCl percentage. It can be seen that the control and the first two NaCl treatments have ECe25 values below saline soils (saline soils have ECe25>4), where as higher NaCl treatments have ECe25 values above saline soils. Sea water 200°C has ECe of 46.6 mS/cm (Singleton *et al.*, 1982). Mean Saturation Percentage (SP) of the control soil was 46 and texture of the soil was "sandy clay loam" (Table 2).

It is quite obvious from Table 7 that number of flowers per plant was the highest in the third harvest. Among treatments 0.2mS/cm maximum number of flowers per plant (3.33a±0.43) were observed, while it was minimum at 4.6 mS/cm. It means there was gradual decrease in number of flowers with increase in salinity (as shown in Table 5 and 3).

Data (Table 3) showed that number of pods per plant were absent in first harvest, while maximum number of pods per plant (3a±0.29) were observed in third harvest (Table 7) and same is the case with flowers. In

second harvest, among treatment 0.2 mS/cm maximum number of pods per plant were observed, while the least number was observed at 4.6 mS/cm. It means numbers of pods per plant were greatly influenced with increase in salt concentration. Correspondingly, nodules per plant were the highest in first harvest. Like wise fresh weight of nodule per plant was highest in first harvest and decreased with increase in salinity.

In *Vicia faba* at 12 and 15 weeks shoots and whole plants grow well as compared to growth after 8 weeks with increasing NaCl levels up to 1.3 and 4.6 mS/cm respectively. At higher salinity levels the shoot growth was decreased. At 8, 12 weeks and 15 weeks after sowing, however, the root growth was not enhanced but was reduced with increasing salinity stress. Number of flowers were negligible at 8th week and appeared at 12 and 15 weeks. Yield was low as pods were observed at 15 week. This suggested that with age, the inhibitory effect of NaCl was not only decreased but there was also a positive response at lower NaCl levels. Shoot growth was enhanced at lower levels at 12 and 15 weeks as compared to root growth which was highest at 12 week while moderate at 8 week and lowest at 15 week. Moreover plants grown at high salt concentrations i.e. 9.1, 13.6, 18 and 22.5 mS/cm died at 15 weeks because of inability to compete such a high salinity.

These trends have been confirmed by Borucki and Sujkowska (2011), Khadri *et al.* (2006), Mirza and Tariq (2011) and Predeepa and Ravindran (2010) that number of pods per plant, number of flowers per plant, number of nodules per plant and fresh weight of nodules tends to decrease with the increase in concentration of salt in the soil solution in other legume crops, too and *vice versa*.

Table 8 showed that fresh weight of shoots/plant, fresh weight of roots/plant and fresh weight of whole plant (in terms of grams) were the highest at 0.2 mS/cm (control) while it gradually decreased at higher levels. In second (Table 6) and third harvest (Table 8) there was increase in growth but gradual decline in growth with increase in salt concentration.

Table 3: Effect on the number of flowers, number of nodules, fresh weight of nodules and number of pods in *Vicia faba* at 1st harvest. Each value represents mean±SEM of about 5 plants

Ece25 (mS/cm)	No. of flowers per plants	No. of nodules per plants	Fresh weight of nodules per plants (g)	No. of pods per plants
0.2	2.53a±0.43	20a±0.58	0.013a±0.003	0
1.3	2.00ab±0.36	14.66b±1.48	0.011a±0.004	0
2.4	1.56bc±0.17	10c±0.58	0.1ab±0.003	0
4.6	1.36c±0.07	9c±1.17	0.004b±0.03	0

Mean sharing same letters do not differ significantly from each other while means having different letters differ significantly from each other at 5% probability level using Duncan's Range Multiple Tests

Table 4: Effect of NaCl on fresh and dry weight of shoot, root and plant, root, shoot and pant length in *Vicia faba* at 1st harvest. Each of values represents mean±SEM of about 5 plants

Ece25 (mS/cm)	Fresh weight of shoot per plant (g)	Fresh weight of root per plant (g)	Whole fresh weight (g)	Dry weight of shoot per plant (g)	Dry weight of root per plant (g)	Whole dry weight (g)	Stem length (cm)	Root length (cm)	Whole length (cm)
0.2	2.73a±0.13	0.50a±0.03	3.23a±0.17	0.71a±0.07	0.46a±0.08	0.87a±0.08	49.8a±17.14	18.46a±0.88	68.26a±16.32
1.3	1.24b±0.26	0.22b±0.05	1.46a±0.27	0.51b±0.08	0.41a±0.32	0.92a±0.07	19.36b±0.41	17.4a±1.35	36.76b±1.16
2.4	0.68c±0.01	0.10c±0.008	0.79a±0.02	0.43b±0.12	0.26b±0.06	0.69a±0.03	17.36b±0.64	9.43b±0.71	26.8bc±1.35
4.6	0.4c±0.04	0.02d±0.008	0.42a±0.03	0.03c±0.008	0.21b±0.01	0.24b±0.03	15.66b±0.78	5c±0.58	20.66bcd±1.33
9.1	0.36cde±0.08	0.02d±0.005	0.38a±0.006	0.02c±0.003	0.20b±0.33	0.22b±0.01	9b±0.58	3.03d±0.42	11.6cd±0.70
13.6	0.2de±0.05	0.01d±0.003	0.21a±0.05	0.02c±0.005	0.04c±0.005	0.06c±0.06	6.23b±0.51	2.53d±0.29	7.83d±0.86
19.0	0.10de±0.05	0.01d±0.003	0.11a±0.05	0.01c±0.003	0.02c±0.003	0.03c±0.005	5.53b±0.80	2.3d±0.36	6.86d±1.29
22.5	0.01e±0.003	0.01d±0.003	0.02a±0.006	0.009c±0.003	0.03c±0.031	0.39c±0.03	4.96b±1.03	1.86d±0.35	5.9d±1.29

Mean sharing same letters do not differ significantly from each other while means having different letters differ significantly from each other at 5% probability level using Duncan's Range Multiple Tests

Table 5: Effect on the number of flowers, number of nodules, fresh weight of nodules and number of pods in *Vicia faba* at 2nd harvest. Each value represent mean±SEM of about 5 plants

Ece25 (mS/cm)	No. of flowers per plants	No. of nodules per plants	Fresh weight of nodules per plants (g)	No. of pods per plants
0.2	1.11a±0.07	21.00a±0.58	0.010a±0.003	1.00a±0.29
1.3	0.62b±0.11	15.66b±1.79	0.009a±0.005	0.63b±0.08
2.4	0.27c±0.03	12.00c±0.58	0.009a±0.001	0.26c±0.03
4.6	0.15c±0.02	9.00d±1.17	0.013a±0.003	0.08c±0.03

Mean sharing same letters do not differ significantly from each other while means having different letters differ significantly from each other at 5% probability level using Duncan's Range Multiple Tests

Table 6: Effect of NaCl on fresh and dry weight of shoot, root and plant, root, shoot and pant length in *Vicia faba* at 2nd harvest. Each of values represents mean±SEM of about 5 plants

Ece25 (mS/cm)	Fresh weight of shoot per plant (g)	Fresh weight of root per plant (g)	Whole fresh weight (g)	Dry weight of shoot per plant (g)	Dry weight of root per plant (g)	Whole dry weight (g)	Stem length (cm)	Root length (cm)	Whole length (cm)
0.2	3.18a±0.44	0.48a±0.06	3.46a±0.50	0.66a±0.06	0.03a±0.01	0.4a±0.05	26.9a±1.51	16.1ab±0.30	43a±1.81
1.3	2.55ab±0.09	0.40a±0.02	2.95ab±0.11	0.6a±0.30	0.10b±0.005	0.49a±0.08	21.8b±0.60	20.3a±4.74	42.1a±4.14
2.4	2.21b±0.47	0.34a±0.04	2.58abc±0.52	0.53a±0.33	0.09b±0.005	0.76a±0.06	22b±3.03	14.95ab±0.45	36.95a±0.34
4.6	1.84bc±0.59	0.35a±0.04	2.03bcd±0.611	0.38a±0.07	0.05abc±0.05	0.38a±0.02	15.75c±0.25	11.35bc±0.85	27.1b±1.11
9.1	1.56cd±0.26	0.46a±0.34	1.62de±0.611	0.37a±0.04	0.04c±0.025	0.21a±0.005	12.8c±0.20	11.05bc±2.57	23.85b±2.37
13.6	0.79de±0.09	0.31a±0.18	1.11def±0.09	0.33a±0.05	0.04c±0.2	0.64a±0.32	7.9d±1.71	6.85cd±0.35	14.75c±1.36
19.0	0.28e±0.08	0.3a±0.10	0.58ef±0.18	0.25a±0.05	0.04c±0.010	0.57a±0.34	7.05d±0.45	4.5d±0.50	11.55cd±0.50
22.5	0.09e±0.005	0.15a±0.05	0.24f±0.05	0.17a±0.02	0.01c±0.005	0.26a±0.05	4.75d±0.25	2.5d±0.50	7.25d±0.75

Mean sharing same letters do not differ significantly from each other while means having different letters differ significantly from each other at 5% probability level using Duncan's Range Multiple Tests

Dry weight of shoot/plant, dry weight of root/plant and dry weight of whole plant (in terms of grams) exhibited the highest weight at 1.3 mS/cm. Hence root, shoot and plant length tended to decrease with increase in salinity first, second and third harvest, respectively. It means the increase in salt concentration had adversely affected the whole length or growth including fresh weight of root shoot and plant as well as dry weight of shoot root and plant. Comparable trends were observed in case of root, shoot and plant length.

Root nodulation was completely inhibited at NaCl levels higher than 4.6 mS/cm. Nodulation was greater at 8 week as compared to 12 week. However Nodules were absent at 15 week because of intolerable salt concentrations. The inhibitory effect of salinity on nodulation is well documented (Cooper *et al.*, 1986; Sprent, 1984). Epstien and Norlyn (1997) reported that salinity effects the growth of plants by decreasing rate of water up take due to osmotic effect through ion specific toxic effect or through a nutritional imbalance caused by

Table 7: Effect on the number of flowers, number of nodules, fresh weight of nodules and number of pods in *Vicia faba* at 3rd harvest. Each value represent mean±SEM of about 5 plants

Ece25 (mS/cm)	No. of flowers per plants	No. of nodules per plants	Fresh weight of nodules per plants (g)	No. of pods per plants
0.2	3.33a±0.43	18.66a±0.89	0.02a±0.005	3.00a±0.29
1.3	1.28b±0.11	13.00b±1.17	0.18a±0.007	1.23b±0.14
2.4	0.69c±0.06	12.00b±1.56	0.02a±0.01	0.43c±0.122
4.6	0.27c±0.03	7.33c±1.48	0.01a±0.003	0.33c±0.08

Mean sharing same letters do not differ significantly from each other while means having different letters differ significantly from each other at 5% probability level using Duncan's Range Multiple Tests

Table 8: Effect of NaCl on fresh and dry weight of shoot, root and plant, root, shoot and pant length in *Vicia faba* at 3rd harvest. Each of values represents mean±SEM of about 5 plants

Ece25 (mS/cm)	Fresh weight of shoot per plant (g)	Fresh weight of root per plant (g)	Whole fresh weight (g)	Dry weight of shoot per plant (g)	Dry weight of root per plant (g)	Whole dry weight (g)	Stem length (cm)	Root length (cm)	Whole length (cm)
0.2	7.66a±0.85	3.17a0.16	10.83a±2.70	1.01ab±0.28	0.66a±0.04	1.19bc±0.32	36.10b±3.30	25.33a±2.38	61.4b±3.04
1.3	7.54a±2.41	0.66ab±0.6	10.20a±2.84	0.64b±0.05	0.54a±0.19	2.87a±0.86	52.38a±13.3	24.66a±1.48	77.5a±13.17
2.4	4.84ab±0.35	2.45abc±0.35	7.29ab±0.56	0.55a±0.05	0.52ab±0.23	1.26bc±0.18	31.66bc±2.71	23a±1.55	54.66bc±3.91
4.6	3.68abc±0.44	1.61bcd±0.31	5.29bc±0.73	0.49a±0.08	0.29ab±0.15	1.92ab±0.24	28.3bcd±3.98	15b±0.58	43.3cd±4.12
9.1	2.68bc±0.19	1.46cde±0.30	4.15bcd±0.32	0.46a±0.08	0.25ab±0.03	2.03ab±0.12	19.66cde±0.33	11.16bc±0.44	30.83de±0.61
13.6	0.58bc±0.22	1.28de±0.16	187cd±0.22	0.23c±0.13	0.06b±0.008	0.29cde±0.13	15de±2.12	10.33c±0.89	25.33e±2.37
19.0	0.26c±0.01	0.37ef±0.31	0.64cd±0.31	0.05c±0.003	0.02b±0.01	0.08cd±0.008	9e±0.58	7.66c±0.89	16.66ef±0.89
22.5	0.20c±0.15	0.02f±0.003	0.23d±0.15	0.007c±0.003	0.01b±0.003	0.02c±0.003	6.33e±1.35	2.33d±0.33	8.66f±1.69

Mean sharing same letters do not differ significantly from each other while means having different letters differ significantly from each other at 5% probability level using Duncan's Range Multiple Tests

ion antagonism. Likewise, Anthraper and Dubois (2003) observed reduced nodulation under salt stress. This is confirmed from the present studies in beans. By summarizing the results and growth of beans due to salt stress is associated with toxic effect of NaCl and inhibition of nodule formation due to low interaction between rhizobium and plant root for nodule formation under salt stress.

This was further inveterate by Bouhmouch *et al.* (2005), Eaglesham and Anyaba (1984) and Okon and Hardy (1983) that fresh weight of shoot, root per plant and whole plant as well as dry weight of shoot, root per plant and dry weight of whole plant tended to decrease with the increase of salinity levels in various other legumes and *vice versa*.

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