INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2003/05–2–124–128
http://www.ijab.org

# Effect of Nitrogen Supply on the Response of Fababean to Salinity

MAHDI M. AL-MUTAWA
*College of Science, King Khalid University, P.O. Box 9008, Abha, Saudi Arabia*

## ABSTRACT

This study was carried out to analyze the effects of salts on symbiosis by comparing the NaCl tolerance of fababean-specific strains of Rhizobium with tolerance of the fababean plant grown either with combined N or dependent on symbiotic $N_2$-fixation. Effects of supplemental N on the salt response of symbiotic fababean were also tested. In a greenhouse pot experiment, NaCl depressed growth of fababean at 25 mM concentration and above unless mineral N was provided. With no added NaCl, seven strains of Rhizobia were all effective, producing plant yields comparable with urea control treatments, but with NaCl added at 75 mM, only one strain did significantly better than controls with no N or inoculum. Poor symbiotic performance was not due to salt limitation of growth of Rhizobia. Rhizobial growth rates determined by viable counts in yeast mannitol medium were unaffected by NaCl at 150 mM and only moderately depressed by 300 mM. The data indicated a clear need for greater salt tolerance in fababean. Since, the nature of the salt response changed markedly with N-source, selection of cultivars and testing of management procedures should be done with both N-fertilized and symbiotic plants.

**Key Words:** Nitrogen; Fababean; Salinity

## INTRODUCTION

Despite the voluminous publications dealing with the effects of salt stress on plant growth and nitrogen (N) nutrition, the literature concerning this issue on fababean-rhizobia symbiosis is scarce. In a few cases legumes-rhizobia symbiosis has been found more salt-sensitive than the legume grown with high rates of fertilizer N. Because of a wide geographical distribution and potential for high yield and protein content, fababean (*Vicia faba* L.) is among the most extensively planted grain legumes. It is cultivated in arid areas because it is adapted to low water availability. Large amounts of N are required to achieve high yields and protein (Abdulsalam *et al*., 1996) since the aboveground parts may contain as much as 250 kg N $ha^{-1}$. The exploitation of biological N fixation by appropriate strains of rhizobia for maximum yield, especially on soils of low N status is, therefore, desirable. However, fababean has been reported to fail in arid areas under salt stress (Al-Tahir *et al*., 1989) and since sensitivity was not related to germination failure (Al-Tahir *et al*., 1991), it could have been due to symbiotic failure, failure of the host, or both.

The effect of salt stress on N nutrition in plants has been studied for various plants using different methods. The results are still inconclusive; however, the change in N metabolism induced by excess salt is commonly among scientists as one of the most important factors responsible for abnormal plant metabolism and growth. In a solution-culture experiment with 31 days old barely (*Hordeum vulgare* L.) plants, total $^{15}N$ content of roots decreased (Helal & Mengel, 1979). Bernstein *et al*. (1974) found that despite decrease in total N uptake, leaf N concentration of some grain and vegetable crops increased with increasing salinity at all N fertilization levels. The uptake of $^{15}NH^{+}_{4}$ and $^{15}NO_3$ in red kidney beans (*Phaseolus vulgaris* L.) was adversely affected by both salt and water stress at – 0.4 Mpa osmotic potential (Frota & Tucker, 1978; Saad, 1979). Reduced $^{15}N$ uptake by various crops also was reported by Helal and Mengel (1979) (barely) and by Pessarakli and Tucker (1988) (tomato, *Lycopersicon hirsutum* L.) plants increased under low levels (– 0.4 Mpa osmotic potential) of NaCl salinity. Bernstein and Pearson (1956) also reported increased total N concentration of plant grown in saline substrate.

To explain these different results, a dilution or concentration effect (depending on the relative severity of salt stress on growth or N uptake) was reported a cause of the fluctuations in N content or concentration in plants (Frota & Tucker, 1978; Pessarakli & Tucker, 1985, 1988). Wilson (1970) suggested that symbiotic performance in *Clycine wightii* was limited by sensitivity of the host, not by nodule formation or function, because nodules remained remarkably resistant upon addition of sodium chloride and recovered activity soon after its removal. On the other hand, Bernstein and Ogata (1966) suggested a salt-specific effect on nodulation of soybean, since root growth was only moderately affected by a salt level that completely inhibited nodule growth. Inhibition of growth of Rhizobia has been demonstrated at sodium chloride level toxic to but not lethal to the host legume, and strains evidently differ in sensitivity (Yadav & Vyas, 1971; Steinborn & Roughly, 1975).

This paper describes the results of a study on performance of fababean under salt stress as influenced by urea and by symbiotic combination with different Rhizobia.

## MATERIALS AND METHODS

Fababean (cv. Hass-1) seed was used. The seeds were inoculated with water slurries of different local *Rhizobium leguminosarum* (Hass-1, Hass-2, Hass-3, Hass-4, Hass-5, Hass-6 and Hass-7 isolated from different regions in Al-Hassa).

**Experiment 1.** Seven local strains of Rhizobia (Hass-1, Hass-2, Hass-3, Hass-4, Hass-5, Hass-6 and Hass-7), symbiotic control and a mineral N treatment were grown with and without the addition of NaCl to give 75 mM in solution. The medium was 3 kg $pot^{-1}$ of acid washed, autoclaved loamy sand soil with a saturation paste pH of 7.5 a saturation paste $EC_e$ of 2.1 dS $m^{-1}$ and a basal fertilizer treatment of 5 mg Zn $kg^{-1}$ soil, 0.1 mg $kg^{-1}$ Mo and 175 mg $kg^{-1}$ superphosphate. The mineral N treatment received 10 mM of N as urea after three weeks from planting at the estimated end of the lag phase of N fixation and 5 mM of N after six weeks from planting. Procedures of seed sterilization and inoculation are described by Munns *et al*. (1979). There were 5 plants $pot^{-1}$ and there were three replicated pots per treatment in a completely randomized design. Plant were frequently watered to approximately 0.2 bar matric potential, twice a day if necessary, to minimize fluctuation in the soil concentration of NaCl. Sixty days after planting, tops were harvested and top dry weights were recorded.

**Experiment 2.** The ability of fababean Rhizobia to grow in salinized yeast-mannitol broth (Vincent, 1970) was tested at NaCl concentrations above those which plants received. The Rhizobia sp. cultures were transferred to separate vials containing growth medium containing 0, 150 and 300 mM NaCl. Each treatment consisted of three replications in randomized blocks. The occurrence of turbidity and the time to reach turbidity were recorded. Turbidity was an indication that populations had increased from approximately $10^4$ cells $mL^{-1}$ to $10^7$ cells $mL^{-1}$ (Keyser & Munns, 1979). Each treatment consisted of three replications in randomized blocks.

The growth of three strains (Hass-1, Hass-2 and Hass-3) was measured during 72 h period. Counts were taken of viable cells by the agar pour plate method.

**Experiment 3.** A pot experiment was conducted to evaluate the response of fababean to salts stress under four N regimes. The salt levels were 0, 25, 50 and 75 mM NaCl, maintained throughout the 60 days experiment. The N treatments were:

1- Plants were inoculated with Rhizobia (designated as $N_0$).

2- Plants inoculated with Rhizobia and received 10.0 mM urea $pot^{-1}$ between planting and one month after planting (designated as $N_1$).

3- Plants were not inoculated and received 10.0 mM of urea $pot^{-1}$ split in two equal doses one after one month and the other after a month and a half from planting (designated as $N_2$).

4- Plants were not inoculated and received 1.25 mM urea $pot^{-1}$ $week^{-1}$ throughout the experimentation period (designated as $N_3$).

The experiment was laid out in a split plot design with three replicates. The main plots were the salinity levels and the sub-plots treatments consisted of the N levels. Each experimental unit consisted of a pot containing 20 L of constantly aerated solution. The basal solution was 0.31 mM $KH_2PO_4$, 0.31 mM $K_2HPO_4$, 0.37 mM $K_2SO_4$, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 10 μM FeEDTA, 25 μM KCl, 6 μM $H_3PO_3$, 1μM $ZnSO_4$, 2.5 μM $MnCl_2$, 0.25 μM $CuSO_4$ and 0.05 μM $NaMoO_4$. Solutions were renewed after two weeks and weekly thereafter. Solution temperatures varied between 20 and 25°C, air temperature between 20 and 30°C and pH between 6 and 7.2.

Initially, there were seven plants per pot. Two plants were harvested from each pot after three weeks, when small white nodules 1 mm in diameter began to turn pink, two after five weeks when the young leaves in all treatments became dark green and the remaining three after nine weeks. Inoculated pots received approximately $10^4$ viable Rhizobia per mL of solution culture.

Acetylene reduction assay of nodule activity was done on excised roots which were rinsed, dried, and then incubated for 1 h at 25°C. For analysis, plants were separated into roots, shoots and nodules. Roots and shoots were analyzed for Na and Cl by soaking samples for 48 h in dilute nitric acid solution (Chapman & Pratt, 1961), filtering and measuring the supernatant with a chloridemeter and a flame photometer. New shoots were digested for N by Kjeldahl method and the digest was analyzed according to Cataldo *et al*. (1974). Dry weights were taken for shoots, nodulated roots and nodules. Nodules were counted. Since none of the results showed differences due to strain, data for separate strains have been pooled.

Statistical evaluation of treatment effects were determined by analysis of variance (SAS, 2001).

## RESULTS AND DISCUSSION

**Experiment 1.** At 75 mM NaCl, only the mineral N treatment and Hass-3 did significantly better than the symbiotic control, but plants nodulated and became green in all inoculant treatments except Hass-6. In the absence of NaCl, green up began three weeks after planting. It was delayed up to two weeks when salt was present. Apparently, a high level of sodium chloride delayed the onset of N fixation and prevented N fixation from resulting in significant growth, but did not eliminated nodulation or the supply of N from the nodules to the shoots.

**Experiment 2.** In salinized yeast-mannitol broth, visually estimated population of the seven strains increased by $10^3$ in a week at 75 mM NaCl or less. At 150 mM, the initiation of the turbidity was delayed by two days in strains Hass-1, Hass-5 and Hass-7. The strains Hass-4 and Hass-6 grew

very slowly at 300 mM. Growth of strains Hass-1, Hass-2 and Hass-3, estimated by plate count as affected by NaCl is presented in Table I. It is evident from these results that Rhizobial growth was not significantly affected by salinity level. It could be concluded that the poor performance of fababean plants under salt stress probably was not due to slow Rhizobia growth.

**Table I. Effect of NaCl on population growth of three Rhizobia strains**

| Time (hr) | NaCl concentration (mM) | | |
|---|---|---|---|
| | 0 | 150 | 300 |
| | log (cells mL$^{-1}$) | | |
| | **Strain Hass-3** | | |
| 1 | 4.29 | 4.29 | 4.27 |
| 24 | 4.42 | 4.42 | 4.38 |
| 48 | 4.80 | 4.78 | 4.75 |
| 72 | 6.23 | 5.97 | 5.18 |
| | **Strain Hass-2** | | |
| 1 | 4.20 | 4.20 | 4.11 |
| 24 | 4.36 | 4.32 | 4.24 |
| 48 | 4.47 | 4.39 | 4.31 |
| 72 | 5.84 | 5.93 | 5.23 |
| | **Strain Hass-1** | | |
| 1 | 3.87 | 3.71 | 3.65 |
| 24 | 4.18 | 4.07 | 3.86 |
| 48 | 4.91 | 4.83 | 4.16 |
| 72 | 5.53 | 5.48 | 5.37 |

**Experiment 3.** Under salt stress, poor N fixation and/or the absence of urea limited vegetative growth. There was no statistical difference in shoot dry weight due to the strain of Rhizobium. NaCl at or above 25 mM reduced growth. Growth was eventually inhibited by the highest salt treatment except for plants dependent on N fixation (Fig. 1). The response of Rhizobial infection of the root to salinity may have limited plant growth. Salinity stress reduced nodule number (Table II), thereby depressing total nodule activity in spite of an increase in specific nodule activity (Table III). There was a tendency of restricted nodule number before salinity seriously reduced plant growth (Table II).

Sodium chloride caused a delay in N fixation revealed by N content of the shoots (Table IV). Between day 21 and 35, there was a highly significant time x salt interaction involving the 25 mM treatment where N content increased and the two highest treatments (75 and 100 mM) where N content decreased. On day 21, salt stressed plants tended to have higher N contents because they were slower in depleting their seed N as well as N in the medium. By day 35, because of the delay in N fixation, salt stressed plant were N deficient as compared to the control. Salinity stress may have delayed the initiation of N fixation only in proportion to the delay in depletion of seed and medium N. Between day 21 and 35, the decrease in N content in salt treated inoculated plants was accompanied by an increase in Na and Cl concentrations in the shoot and root (Tables V and VI) and decreased Na retention in the root (Table VII).

**Fig. 1. Effect of salt level and nitrogen ($N_0$ = no urea added, $N_1$ = urea added up to the 35$^{th}$ day from planting, $N_2$ = urea added from the 35$^{th}$ day to the end of the experiment and $N_3$ = urea added from planting to the end of the experiment)**

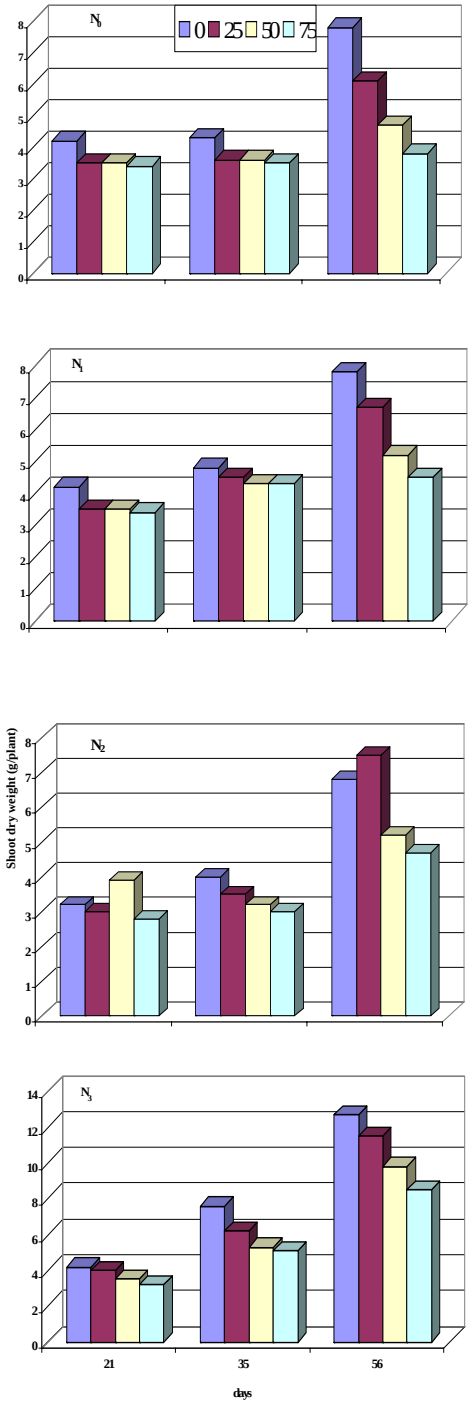

**Table II. Effect of NaCl on the number or nodules plant$^{-1}$ and the nodules weight (mg plant$^{-1}$) at different levels of N fertilization**

| Treatments | Nodule Number plant$^{-1}$ | | | Nodules weight (mg plant$^{-1}$) | | |
|---|---|---|---|---|---|---|
| | 21 | 35 | 56 | 21 | 35 | 56 |
| **25 mM NaCl** | | | | | | |
| $N_0$ | 18.8 | 19.6 | 22.7 | 0.22 | 2.18 | 2.75 |
| $N_1$ | 23.1 | 26.1 | 27.6 | 0.58 | 2.42 | 3.22 |
| **50 mM NaCl** | | | | | | |
| $N_0$ | 17.9 | 18.3 | 20.4 | 0.17 | 2.13 | 2.69 |
| $N_1$ | 21.8 | 23.4 | 24.3 | 0.54 | 2.35 | 3.05 |
| **75 mM NaCl** | | | | | | |
| $N_0$ | 15.6 | 17.1 | 18.5 | 0.16 | 1.71 | 2.04 |
| $N_1$ | 19.5 | 21.8 | 25.1 | 0.49 | 1.89 | 2.86 |
| **L.S.D. 0.05 for salt levels** | | | | | | |
| | 0.73 | 0.46 | 0.52 | 0.14 | 0.12 | 0.42 |
| **L.S.D. 0.05 for nitrogen** | | | | | | |
| | 2.18 | 3.12 | 3.41 | 0.18 | 0.12 | 0.13 |

**Table III. Effect of NaCl on the total nodule activity ($\mu$ moles $C_2H_2$ h$^{-1}$ plant$^{-1}$) and the specific nodule activity ($\mu$ moles $C_2H_2$ h$^{-1}$ g$^{-1}$) at different levels of N fertilization**

| Treatments | Total nodule activity | | | Specific nodule activity | | |
|---|---|---|---|---|---|---|
| | 21 | 35 | 56 | 21 | 35 | 56 |
| **25 mM NaCl** | | | | | | |
| $N_0$ | 1.47 | 6.18 | 10.6 | 0 | 295 | 35.7 |
| $N_1$ | 3.24 | 10.5 | 13.9 | 0 | 487 | 49.6 |
| **50 mM NaCl** | | | | | | |
| $N_0$ | 1.39 | 4.51 | 7.56 | 0 | 349 | 42.3 |
| $N_1$ | 2.13 | 5.66 | 12.6 | 0 | 569 | 64.4 |
| **75 mM NaCl** | | | | | | |
| $N_0$ | 0 | 513 | 53.7 | 1.12 | 2.74 | 5.73 |
| $N_1$ | 0 | 686 | 78.2 | 1.74 | 3.81 | 9.15 |
| **L.S.D. 0.05 for salt levels** | | | | | | |
| | 0.45 | 0.73 | 2.4 | n.s. | 46 | 9.3 |
| **L.S.D. 0.05 for nitrogen** | | | | | | |
| | n.s. | n.s. | 1.7 | n.s. | 13 | 1.3 |

**Table IV. Effect of NaCl on the shoot N content at different levels of N fertilization**

| Treatments | Shoot nitrogen (%) | | |
|---|---|---|---|
| | 21 | 35 | 56 |
| **25 mM NaCl** | | | |
| $N_0$ | 1.63 | 1.98 | 2.31 |
| $N_1$ | 2.37 | 2.75 | 2.98 |
| **50 mM NaCl** | | | |
| $N_0$ | 2.16 | 1.90 | 1.76 |
| $N_1$ | 3.06 | 2.83 | 2.52 |
| **75 mM NaCl** | | | |
| $N_0$ | 2.24 | 2.09 | 2.02 |
| $N_1$ | 3.25 | 3.12 | 2.52 |
| **L.S.D. 0.05 for salt levels** | | | |
| | 0.25 | 0.29 | 0.39 |
| **L.S.D. 0.05 for nitrogen** | | | |
| | 0.47 | 069 | 0.74 |

Sodium accumulated in the shoot and root in accordance with N treatment (Tables V & VI). Between day 21 and 56, shoot Na concentrations increased markedly in all treatments except $N_3$. The rate of increase was slightly reduced after day 35 by the late applications, but in the inoculated plants ($N_0$ and $N_1$) the sodium concentrations generally remained high despite the rising N concentrations in the plants.

**Table V. Effects of N supply on Na and Cl accumulation in shoots**

| Treatments | Na concentration | | | Cl concentration | | |
|---|---|---|---|---|---|---|
| | ---------- meq/100g ---------- | | | | | |
| | 21 | 35 | 56 | 21 | 35 | 56 |
| **25 mM NaCl** | | | | | | |
| $N_0$ | 19.9 | 20.9 | 23.8 | 26.9 | 32.0 | 34.6 |
| $N_1$ | 18.1 | 21.5 | 26.5 | 28.6 | 32.8 | 51.9 |
| $N_2$ | 18.2 | 24.3 | 29.4 | 40.3 | 57.4 | 72.4 |
| $N_3$ | 21.7 | 26.9 | 30.9 | 69.2 | 73.8 | 93.3 |
| **50 mM NaCl** | | | | | | |
| $N_0$ | 28.2 | 29.7 | 30.9 | 38.7 | 40.5 | 44.3 |
| $N_1$ | 30.0 | 33.4 | 36.5 | 40.2 | 52.8 | 62.5 |
| $N_2$ | 34.6 | 38.2 | 40.4 | 52.8 | 65.9 | 83.7 |
| $N_3$ | 39.7 | 42.3 | 45.1 | 91.3 | 93.7 | 108.2 |
| **75 mM NaCl** | | | | | | |
| $N_0$ | 38.5 | 40.2 | 43.3 | 43.9 | 52.5 | 53.7 |
| $N_1$ | 39.9 | 42.9 | 57.8 | 46.7 | 54.7 | 86.7 |
| $N_2$ | 40.2 | 51.3 | 69.4 | 52.8 | 71.4 | 98.2 |
| $N_3$ | 56.7 | 63.8 | 92.2 | 112.6 | 122.6 | 129.1 |
| **L.S.D. 0.05 for salt levels** | | | | | | |
| | 2.4 | 2.6 | 3.1 | 3.5 | 4.2 | 1.8 |
| **L.S.D. 0.05 for nitrogen** | | | | | | |
| | 1.5 | 1.8 | 2.3 | 2.8 | 3.0 | 6.5 |

**Table VI. Effects of N supply on Na and Cl accumulation in roots**

| Treatments | Na concentration | | | Cl concentration | | |
|---|---|---|---|---|---|---|
| | ---------- meq/100g ---------- | | | | | |
| | 21 | 35 | 56 | 21 | 35 | 56 |
| **25 mM NaCl** | | | | | | |
| $N_0$ | 29.4 | 28.7 | 23.1 | 43.8 | 41.8 | 29.8 |
| $N_1$ | 32.0 | 31.4 | 28.5 | 85.2 | 62.4 | 49.0 |
| $N_2$ | 41.4 | 39.5 | 32.7 | 93.7 | 73.4 | 53.7 |
| $N_3$ | 43.4 | 42.8 | 35.4 | 112.1 | 109.6 | 89.3 |
| **50 mM NaCl** | | | | | | |
| $N_0$ | 43.9 | 40.8 | 37.8 | 85.1 | 55.9 | 47.7 |
| $N_1$ | 48.9 | 44.8 | 40.3 | 87.5 | 61.9 | 56.8 |
| $N_2$ | 50.3 | 45.2 | 42.5 | 128.4 | 103.8 | 65.8 |
| $N_3$ | 64.1 | 55.1 | 52.2 | 132.7 | 120.6 | 118.1 |
| **75 mM NaCl** | | | | | | |
| $N_0$ | 62.4 | 48.2 | 43.5 | 92.8 | 58.8 | 55.6 |
| $N_1$ | 65.9 | 53.7 | 45.8 | 97.0 | 65.3 | 63.4 |
| $N_2$ | 72.6 | 57.8 | 54.4 | 151.7 | 119.3 | 91.1 |
| $N_3$ | 130.2 | 80.2 | 78.1 | 163.3 | 144.9 | 131.6 |
| **L.S.D. 0.05 for salt levels** | | | | | | |
| | 7.2 | 6.9 | 3.7 | 6.3 | 2.8 | 5.7 |
| **L.S.D. 0.05 for nitrogen** | | | | | | |
| | 3.9 | 5.3 | 2.7 | 5.7 | 7.2 | 1.5 |

The distribution of Na between roots and shoots depended on N treatment. Plants receiving N always had higher Na concentration in the roots than in the shoots (Table VII). Na retention in the roots of fababean, and to a lesser extent in the stems, has been documented (Al-Tahir *et al*., 1989, 1991). The possibility that Na retention helps to prevent Na-toxicity is lent credence by a report that photosynthesis is sensitive to external concentrations of

sodium chloride which lead to high Na and Cl ions concentration in the leaves (Downton, 1977). This effect can be attributed to Na ion and not Cl ion, since other studies have demonstrated high photosynthetic rates despite high Cl ion concentration in the chloroplasts (Luttge & Higinbotham, 1979).

**Table VII. Effects of N supply on Na distribution between the roots and shoots**

| Treatments | Na concentration | | |
|---|---|---|---|
| | meq/100g | | |
| | 21 | 35 | 56 |
| **25 mM NaCl** | | | |
| $N_0$ | 1.97 | 1.37 | 0.97 |
| $N_1$ | 1.77 | 1.46 | 1.08 |
| $N_2$ | 2.27 | 1.63 | 1.11 |
| $N_3$ | 2.00 | 1.59 | 1.01 |
| **50 mM NaCl** | | | |
| $N_0$ | 1.56 | 1.37 | 1.22 |
| $N_1$ | 1.63 | 1.34 | 1.10 |
| $N_2$ | 1.45 | 1.18 | 1.05 |
| $N_3$ | 1.61 | 1.30 | 1.16 |
| **75 mM NaCl** | | | |
| $N_0$ | 1.62 | 1.20 | 1.00 |
| $N_1$ | 1.65 | 1.25 | 0.79 |
| $N_2$ | 1.81 | 1.13 | 0.78 |
| $N_3$ | 2.30 | 1.26 | 0.84 |
| **L.S.D. 0.05 for salt levels** | 0.09 | 0.05 | 0.02 |
| **L.S.D. 0.05 for nitrogen** | 0.11 | 0.07 | 0.04 |

In the root, the highest Na and Cl concentrations mostly occurred in $N_3$ plants (Table VI). Na concentrations did not increase in the roots between days 21 and 56, and Cl concentrations increased after urea was applied (Table VI). Cl concentration in shoots increased throughout growth regardless of N source (Table V). Inability of fababean to stabilize its Cl concentration could result in increasing sensitivity to Cl with age. Studies on salt sensitive fababean (Al-Tahir *et al*., 1991) associated growth inhibition and the development of necrotic leaf tissue with high Cl content in the shoots. In interpreting these results, Greenway and Munns (1980) attributed sensitivity to excess Cl ion, reasoning that osmotic effects at the external solution-root interface would not be a factor at low external NaCl concentration. In this study, symptoms of the different salt levels appeared on $N_3$ plants as dead older leaves.

## REFERENCES


Abdulsalam, M.A., O.A. Al-Tahir and H.O. Burhan, 1996. Variation in seed size of fababean (*Vicia faba*) as influenced by cultivars, irrigation regimes and nitrogen. *Indian J. Agron.*, 41: 269–74

Al-Tahir, O.A., A.A. Al-Noaim and A.A. Al-Jasim, 1989. The effect of drainage water on nodulation nitrogen fixation and growth of faba bean. *Biological Wastes*, 29: 63–6

Al-Tahir, O.A., A.A. Al-Noaim, A.A. Al-Jasim and A.E. Musa, 1991. Studies of the effect of sodium chloride and sulphate salinity on bacterial nodulation and atmospheric nitrogen fixation efficiency in fababean (*Vicia faba* L.). *Biol. Sci*., 1: 3–7

Bernstein, L. and G.A. Pearson, 1956. Influence of exchangable sodium ions on the yield and chemical composition of plants: I. Green beans, garden beans, clover, and alfalfa. *Soil Sci.*, 82: 247–58

Bernstein, L., l.E. Francois and R.A. Clark, 1974. Interactive effects of salinity and fertility on yields of grains and vegetables. *Agron. J*., 66: 412–21

Brenstein and G. Ogata. 1966. Effects of salinity on nodulation, nitrogen fixation, and growth of soybeans and alfalfa. *Agron. J.*, 58: 201–03

Brenstein, L. and G.A. Pearson, 1956. Influence of exchangeable sodium on the yield and chemical composition of plants I. Green beans, garden beets, clover and alfalfa. *Soil Sci.*, 82: 247–58

Chapman, H.D. and P.F. Pratt, 1961. *Methods of Analysis for Soils, Plants, and Waters*, Chapter 8. University of California, USA

Downton, W.J.S., 1977. Photosynthesis in salt-stressed grapevines. *Australian J. Plant Physiol.*, 4: 183–92

Frota, J.N. and T.C. Tucker, 1978. Absorption rates of ammonium and nitrate by red kidney beans under salt water stress. *Soil Sci. Soc. Am. J.*, 42: 753–6

Gataldo, D.A., L.E. Schrader and V.L. Young, 1974. Analysis by digestion and colorimetric assay of total nitrogen in plant tissues high in nitrate. *Crop Sci.*, 14: 854–6

Greenway, H. and R.E. Munns, 1980. Mechanisms of salt tolerance in nonhalophytes. *Ann. Rev. Plant Physiol.*, 31: 149–90

Helal, H.M. and K. Mengel, 1979. Nitrogen metabolism of young barley plants as affected by NaCl-salinity and potassium. *Plant Soil*, 51: 457–62

Keyser, H.H. and D.N. Munns, 1979. Tolerance of rhizobia to acidity, aluminum, and phosphate. *Soil Sci. Soc. Am. J.*, 43: 500–03

Luttge, U. and N. Higinbotham, 1979. *Transport in Plants*, p. 146. Springer-Verlag, New York, USA

Munns, D.N., H.H. Keyser, V.W. Fogle, J.S. Hohenberg, T.L. Righetti, D.J. Lauter, M.G. Zaroug, K.L. Clarkin and K.W. Whitacre, 1979. Tolerance of soil acidity in symbioses of mung bean with rhizobia. *Agron. J.*, 71: 256–60

Oessarakli, M. and T.C. Tucker, 1988. Dry matter yield and nitrogen-15 uptake by tomatoes under sodium chloride stress. *Soil Sci. Soc. Am. J.*, 52: 698–700

Pessarakli, M. and T.C. Tucker, 1985. Uptake of nitrogen-15 by cotton under salt stress. *Soil Sci. Soc. Am. J.*, 49: 149–52

Saad, R., 1979. Effect of atmospheric carbon dioxide levels on nitrogen uptake and metabolism in red kidney beans (*Phaseolus vulgaris* L.) under salt and water stress. *Ph.D. Dissertation*, University of Arizona, Tucson

SAS Institute, 2001. *SAS User's Guide.* Statistics. 21st ed. SAS Inst., Cary, NC

Steinborn, J. and R.J. Roughley, 1975. Toxicity of sodium and chloride ions to Rhizobium ssp. in broth and peat culture. *J. Appl. Bacteriol.*, 39: 133–8

Vincent, J.R., 1970. Response to salinity to Glycine: VI Some effects of a range of short term salt stresses on the growth, nodulation, and nitrogen fixation of *Clycine wightii*. *Australian J. Agric. Res.*, 21: 571–82

Yadav, N.K. and S.R. Vyas, 1971. Response of root-nodule rhizobia to saline, alkaline and acid conditions. *Indian J. Agric. Sci.*, 41: 875–81


**(Received 10 January 2003; Accepted 10 March 2003)**