

BIOLOGICAL NITROGEN FIXATION AND THE ASSOCIATED IRON, MANGANESE,
PHOSPHORUS AND SODIUM ABSORPTION BY SOYBEAN UNDER SALINITY STRESS.

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ABSTRACT

Soybean (*Glycine max* L. Merr.) variety "Clark" was raised in sand on a free-nitrogen nutrient solution containing different amounts of sodium chloride up to 6000 ppm. The number and dry weight of nodules, root nitrogenase activity, vegetative vigour and contents of nitrogen phosphorus, iron, manganese and sodium were determined twice during growth and at maturity. Also, flowers number, at maximum flowering stage, and pods number and grain yield at maturity were recorded.

The results showed that all the tested parameters were depressed by increasing salinity. Grain yield was reduced to 10% at salinity of +6000 ppm. Nitrogen fixed per plant was reduced to 0.398 gm at a salinity of +1000 ppm, to 0.111 gm at a salinity of +4000 ppm and to 0.018 gm at a salinity of +6000 ppm. A positive correlation was found between nitrogen percent in plant and that of iron, manganese and phosphorus. The last three elements were negatively correlated to sodium percent.

INTRODUCTION

Problems of soil salinity are critical constraints to improve and develop grain legumes production (Ibrahim et al. 1978 and Mannus and Franco, 1981). Soybean is relatively a new crop to Egyptian Agriculture and occupies about 40,000 ha. annually (Hamdi and Alaa El-Din, 1981).

Previous studies showed that salinity reduced the growth of soybean (Abel and Mackenzie, 1964 and Shabassy et al. 1972) and affected the uptake of phosphorus (Mattson, 1967 and Metwally et al. 1977) iron, manganese and sodium (Dahiya and Singh, 1976). Also, sodium accumulation resulted in plant failure to utilize nitrogen in protein synthesis (Richards, 1954, Dahiya and Singh, 1976 and Frota and Tucker, 1978). However, information concerning absorption of nutrients in association with biological nitrogen fixation by soybean under salinity stress is lacking.

The present work aimed to find out the biologically fixed nitrogen by soybean under salinized free-nitrogen conditions and the iron, manganese, phosphorus and sodium absorbed in association.

MATERIALS AND METHODS

The experiment was conducted at Soil Salinity Laboratory, Alexandria.

Pots of 28 cm. diameter, 36 cm. height provided with a hole at bottom for drainage were filled with salt-free sand. Soybean (*Glycine max.*, L. Merr.), variety "Clark" was sown at a rate of 10 plants/pot on 25/3/1985. Seeds were treated with local rhizobial strain inoculum provided by the Department of Agricultural Microbiology, Ministry of Agriculture, Egypt. For the first week after sowing, the pots were irrigated every 2 days with Bond's modified Corné's free-nitrogen nutrient solution (Allen, 1949) with additional doses of the inoculum. Eight days after sowing, the pots received irrigations with Bond's solution (Control), Bond's solution +500, +1000, +2000, +4000 and +6000 ppm of NaCl. Irrigation was applied every 2 days with the proper leaching requirement to avoid salt accumulation. Twelve replicates were used, 4 of which removed 45 days after sowing, 4 after 80 days (maximum flowering), and the last 4 left to maturity (118 days after sowing).

At the 45, 80 and 118 days after sowing, the plant was carefully uprooted and the root was rinsed in tap water then in distilled water excised and subjected to nitrogenase activity measurement by the acetylene reduction technique (Hardy et al. 1973). Effective nodules were counted and their dry weight at 66-68°C determined. Roots and shoots were dry weighed then analyzed for total nitrogen, phosphorus, iron, manganese and sodium (Cattienis, 1980). Flowers were counted 80 days from sowing and yield of pods and grains were taken at maturity.

The obtained data were analyzed statistically (Snedecor and Duncan, 1967).

RESULTS AND DISCUSSION

The results obtained are summarized in Tables 1, 2 and 3. It is clear from the data in Table 1 that presented as either number or dry weight of nodules, nitrogenase activity of root and vegetative vigour, all declined gently as salinity increased up to 1000 ppm then rather steeper with further salinity increase. This occurred at all plant ages examined. With age, for all salinity levels, the number and dry weight of nodules, nitrogenase activity increased up to a maximum (at about 80 days) then declined towards harvest. Vegetative vigour, however, proceeded progressively until harvest (Table 1). The average weight of a single nodule (dry weight of nodules per number of nodules) and its nitrogenase activity (nitrogenase activity of root/number of nodules) tended to increase slightly as the number of nodules decreased (Calculated from Table 1).

Maximum flowers produced/plant, as seen in Table 2, showed the same trend as above with increasing salinity. From the same table, it is seen that flowers set was the same (about 37%) for control and 500 ppm NaCl, but declined to about 30% at 4000 ppm, then to 17% at 6000 ppm NaCl treatment. On the other side, grain yield decreased progressively with salinity until reached almost about 10% of that

of the control at 6000 ppm NaCl. Nitrogen fixed as shown in Table 1 was 0.567 gm/plant for control, then decreased progressively with NaCl increase. This is due to the reduction in plant growth (Table 1) and in nitrogen percent of plant (Table 3). Figure 1 shows the relative decline with salinity of grain yield, dry weight of nodules, N_2 -ase activity and of total nitrogen fixed by a plant. The decline in dry weight of nodules and in N_2 -ase activity with salinity was nearly of the same order while that in grain yield was more steeper. The decline in nitrogen fixed took an intermediate position except at the higher salinity because of sharp decline in nitrogen percent.

Table 1: Effect of salinity in Bond's solution on nodulation, nitrogenase activity and vegetative vigour of soybean plant at different ages*.

NaCl added to Bond's solution, ppm.	Nodules/plant		Nitrogenase activity μ mole C_2H_4 /root/hr. ⁴	Vegetative vigour (dry weight), g./whole plant
	Number	Dry weight, gm.		
45 days				
0	13.4	240.5	3.7	7.4
500	12.3	222.3	3.4	7.0
1000	10.1	189.7	2.9	5.4
2000	6.5	127.2	2.0	4.0
4000	4.2	84.6	1.4	2.7
6000	1.2	24.1	0.4	1.6
L.S.D. at (5%)	0.8	6.4	0.5	0.6
80 days (maximum flowering)				
0	16.3	306.2	5.2	12.5
500	15.0	293.5	4.8	12.0
1000	13.5	280.7	4.4	10.4
2000	9.1	198.8	3.3	7.3
4000	5.4	123.6	2.1	4.8
6000	1.2	26.2	0.5	2.1
L.S.D. at (5%)	0.9	5.6	0.6	0.8
118 days (maturity)				
0	14.6	271.4	4.6	18.7
500	11.8	231.6	3.5	18.0
1000	9.3	192.3	2.8	14.7
2000	7.4	153.9	2.5	10.5
4000	3.1	63.0	1.0	6.2
6000	0.5	9.8	0.2	3.5
L.S.D. at (5%)	1.0	6.8	0.4	0.5

* Each value is the mean of 40 plants (10 plants/pot x 4 replicates).

Table 3 shows the percentages of nitrogen, phosphorus, iron, manganese and sodium in dry plant materials at different stages of growth as salinity increased up to +6000 ppm in Bond's solution. In young plants N% was not affected up to +4000 ppm, if not slightly stimulated at +1000 ppm. At 6000 ppm, NaCl treatment it dropped almost to half its value at lower concentrations. Drop in phosphorus percent started at NaCl higher than 1000 ppm, while that in iron and in manganese were at concentrations higher than 2000 ppm. Sodium percent increased steadily with salinity.

Table 2: Number of flowers, flowers set, grain yield and nitrogen fixed by soybean plant as affected by salinity in Bond's nutrient solution.

NaCl added to Bond's solution, ppm	Number of flowers ^x /plant	Flowers set ^{xx}	Grain yield, g./plant	Biologically ^{xxx} fixed, nitrogen mg/plant
0	61.2	0.368	10.7	567.4
500	53.3	0.370	8.0	504.6
1000	49.0	0.322	6.3	397.7
2000	36.7	0.278	4.4	274.3
4000	19.5	0.303	2.4	111.0
6000	7.6	0.171	1.1	18.3
L.S.D. at (5%)	1.6	0.024	0.6	4.8

Each value within the table is the mean of 40 plants (10 plants/pot x 4 replicates).

^x Accounted at 80 days

^{xx} Flowers set = Number of pods at maturity/Number of flowers.

^{xxx} Determined at 118 days.

Table 3: Effect of salinity in Bond's solution on percentages^x of nitrogen, phosphorus, iron, manganese and sodium in soybean plant at different growth stages.

NaCl added to Bond's solution, ppm.	Nitrogen %	Phosphorus x 10 ⁻³ %	Iron x 10 ⁻³ %	Manganese x 10 ⁻³ %	Sodium %
45 days age.					
0	2.343	98.9	13.0	9.1	0.274
500	2.410	97.5	13.1	9.2	0.289
1000	2.802	98.5	14.3	9.5	0.306
2000	2.655	74.2	13.3	9.4	0.318
4000	2.615	68.9	8.6	5.4	0.316
6000	1.256	48.8	6.7	5.6	0.324
L.S.D. at (5%)	0.160	12.0	1.6	0.6	0.003
80 days age (maximum flowering)					
0	2.603	89.9	11.3	7.4	0.283
500	2.613	86.0	11.5	7.7	0.285
1000	2.712	72.8	11.7	7.6	0.298
2000	2.626	50.4	12.3	7.7	0.306
4000	2.465	50.1	6.8	7.3	0.313
6000	1.286	49.4	6.1	6.9	0.337
L.S.D. at (5%)	0.284	10.8	1.2	0.2	0.003
118 days age (maturity)					
0	3.034	70.0	10.0	6.4	0.275
500	2.803	67.2	10.1	6.5	0.277
1000	2.705	71.0	11.2	6.5	0.307
2000	2.612	53.9	10.8	7.1	0.309
4000	1.790	52.8	9.9	7.0	0.318
6000	0.523	45.2	7.0	6.2	0.328
L.S.D. at (5%)	0.204	10.0	1.0	1.0	0.004

Each value is the mean of 40 plants (10 plants/pot x 4 replicates).

^x Based on the oven dry weight of the plant.

In middle age (80 days) N% was almost the same for treatments up to 4000 ppm then decreased almost to half this value at 6000 ppm NaCl. Phosphorus % declined all through with increasing salinity. Iron was the same for treatments up to 2000 ppm NaCl then declined to half this value with further increase in salinity. Manganese had reduced only

at higher salinity (6000 ppm NaCl). Sodium increased steadily with increasing NaCl concentration.

At full maturity, nitrogen percent took on decline with rising salinity all through, phosphorus percent at concentrations higher than +1000 ppm and iron percent at concentrations higher than +2000 ppm were declined, manganese percent did not vary and sodium percent increased still with salinity.

With age, in saline media (Table 3), nitrogen percent appreciably increased, phosphorus and iron percentages slightly decreased, manganese percent appreciably decreased but sodium percent attained the same value allthrough. Correlation coefficients (r) between nitrogen percent on one side and P, Fe, Mn and Na percentages on the other side were +0.5319, +0.6530, +0.3359 and -0.672, respectively.

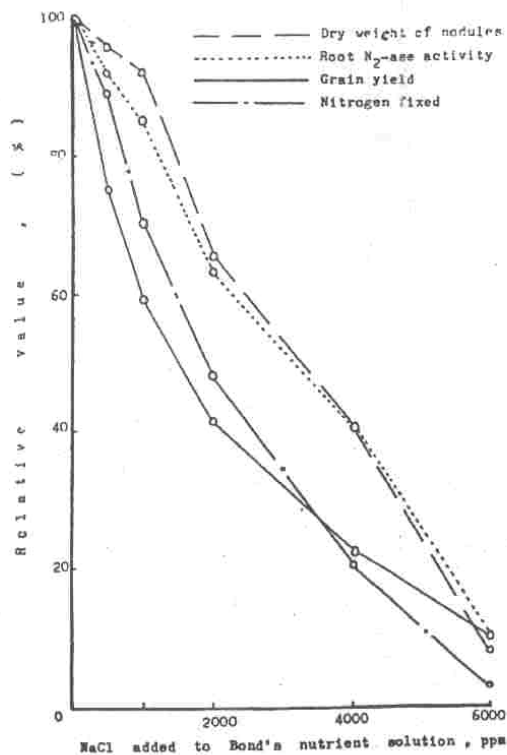


Fig. 1: Relative depression in dry weight of nodules and root nitrogenase activity at maximum flowering age and grain yield and biologically nitrogen fixed at maturity of soybean plants as affected by salinity in Bond's solution.

The results obtained in the present study with respect to the effect of salinity on soybean yield are in general agreement with that reported by Richards (1954) and Ayers and Westcot (1976). With respect to the nitrogen fixed by soybean plant, the obtained results confirmed previous investigation of El-Shakweer and Barakat (1984) for faba bean on sand treated with nitrogen-free nutrient solution. Also, with respect to absorption of nutrients by soybean plant, the obtained results are in parallel with previous investigations of Shabassy et al. (1972) for phosphorus, of Dahiya and Singh (1976) for phosphorus, iron, manganese and sodium and of El-Shakweer and Barakat (1984) for phosphorus and potassium.

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تأثير الملوحة على التثبيت الحيوى للنيتروجين و امتصاص كل من الحديد والمنجنيز والفوسفور والصوديوم
فى فول الصويا .

عثمان القيمى وم .أ . الشقير .

معهد ملوحة الاراضى باسكندرية و كلية الزراعة بالفيوم - مصر .

فى هذه التجربة تم زراعة نبات فول الصويا (صنف كلارك) فى ارض رملية رويت بمحلول
منذى بدون نيتروجين مع تركيزات متفاوتة من كلوريد الصوديوم حتى ٦٠٠٠ جزء فى المليون
أخذت النباتات عند اعمار ٤٥ و ٨٥ و ١١٨ يوم حيث قدر بها كل من عدد العقد
الجزرية ووزنها الجاف ، النشاط النيتروجينى للجذور ، الوزن الجاف للنباتات ، تركيز
كل من النيتروجين والفوسفور والحديد والمنجنيز والصوديوم وكذلك تم
تقدير كل من عدد الأزهار عند مرحلة التزهير القصوى وعدد القرون
ومحصول الحبوب عند مرحلة النضج .

هذا وقد اوضحت النتائج انخفاض كل التقديرات السابقة بزيادة الملوحة
حيث انخفض محصول الحبوب الى ١٠ ٪ عند تركيز ٦٠٠٠ جزء فى المليون
من كلوريد الصوديوم وكذلك انخفض تركيز النيتروجين المثبت لكل نبات الى ٣٦٨ .
١١١ و ١٨٥ و ٠ ر . جم عند تركيزات ١٠٠٠ و ٤٠٠٠ و ٦٠٠٠ جزء فى المليون من
كلوريد الصوديوم على التوالي . كذلك اظهرت النتائج وجود ارتباط ايجابى
بين النسبة المئوية للنيتروجين فى النبات والنسبة المئوية لكل من الحديد والمنجنيز
والفوسفور بينما كان الارتباط سلبى بين العناصر الثلاثة الأخيرة والنسبة المئوية
للصوديوم .