

## SYMBIOTIC NITROGEN FIXATION OF FABA BEAN PLANTS INOCULATED WITH SALT-TOLERANT *Rhizobium* ISOLATES

El-Khateeb, Nagwa M. M. ; M. E. Shalaby ; E. B. A. Belal and SH. M. A. El-Gremi

Dept. of Agric. Botany, Agric. Microbiology, Faculty of Agric., Kafrelsheikh Univ., Egypt

### ABSTRACT

*Rhizobium leguminosarum* biovar *viciae* isolated from salt-affected faba bean fields were *in vitro* tested. Different isolates were found to be varied in their tolerance to salinity. F<sub>1</sub> and E<sub>1</sub> were found to be the most salt-tolerant isolates even at EC-level of 12 ds m<sup>-1</sup>. Seeds of Nubaria 1 cv. were inoculated with either F<sub>1</sub> or E<sub>1</sub>, in comparison with a less salt-tolerant one (F<sub>9</sub>) in pots their salinity were adjusted at 0, 3, 6, 9 and 12 ds m<sup>-1</sup> under aseptic conditions. Number and dry weight of nodules were decreased strongly by increasing salinity levels in comparison with control. Significant enhancement of N % accumulated in the shoots was also noticed due to the biological inoculation with both *Rhizobium* isolates in presence or absence of salinity. Under non-aseptic conditions, efficacy of F<sub>1</sub> and E<sub>1</sub> with the effect of mineral N-supply (25 and 100 %) was compared. In addition to Nubaria 1 cv., Sakha 1 cv. was also used in the evaluation process for N-fixation at 0, 6, 9 ds m<sup>-1</sup> levels. Number of nodules formed on Nubaria 1 at 9 ds m<sup>-1</sup> was highly increased from 7.86 and 5.44 for 25 and 100 % N-supply to 82.33 and 93.33 for F<sub>1</sub> and E<sub>1</sub>, respectively. Accordingly, nodular dry weights were also increased to 0.40 and 0.41 g plant<sup>-1</sup> for F<sub>1</sub> and E<sub>1</sub> instead of 0.13 and 0.08 g plant<sup>-1</sup> for 25 and 100 % N, respectively. The beneficial effects of F<sub>1</sub> and E<sub>1</sub> inoculation were also extended to enhance N % of shoots and seed yield parameters. Therefore, the symbiotic N<sub>2</sub>-fixation process has a great potential to improve growth and productivity of faba bean plants in comparison with the mineral N-supply to overcome the harmful effects of salinity.

**Keywords:** *Vicia faba*, *Rhizobium*, salinity, mineral N-supply

### INTRODUCTION

Faba bean (*Vicia faba* L.) is one of the most important leguminous crops in Egypt. Due to its high nutritive value, it represents a great kind of human and animal consumption (Frag *et al.*, 2005). *Rhizobium leguminosarum* bv. *viciae* is considered specific to faba bean plants. The bacteria fix Nitrogen from the atmosphere to form ammonia, which is assimilated by the plant (Peter *et al.*, 2008). Biological N<sub>2</sub>-fixation (BNF) by specific active rhizobia plays an important role for enhancing growth and productivity of faba bean plants as well as enhancing reclamation of the marginal lands (Abo El-Soud *et al.*, 2003).

The majority of salt-affected soils are located in the northern-central part of Nile-Delta and in their eastern and western sides, in addition to the oasis of Wadi El-Natroun. About 60 % of the cultivated lands of the northern region of Delta are suffered from salt accumulation (Zahran, 1999). Under salt-stress conditions, symbiotic relationship between rhizobia and legumes

becomes more necessary and requires selecting of salt-tolerant rhizobia and plant varieties. In this subject, (Craig *et al.*, 1991) reported that host tolerance to salinity is the most important factor in determining the success of compatible *Rhizobium* strains to form successful symbiosis and N<sub>2</sub>-fixation under conditions of high soil salinity. On the other hand, Singleton and Bohlool (1984) indicated that unsuccessful symbiosis under salt-stress may be due to failure infection process, because salinity acts as limit-factor for establishment of Rhizobia. Plant growth, nutrient uptake, metabolism, and protein synthesis are all thought to be adversely affected under salt stress conditions. Therefore, evaluation of symbiotic N<sub>2</sub>-fixation of faba bean via inoculation with the most salt-tolerant isolates of *Rhizobium leguminosarum* bv. *viciae* under both sterilized and un-sterilized saline conditions was the main goal of the presented study.

## MATERIALS AND METHODS

### **Rhizobia:**

Within different salt-affected faba bean fields, isolation process of *Rhizobium* was carried out at kafr El-Sheikh Governorate. Salinity level of the soils was corresponding to electrical conductivity (EC). Samples of soil-inhabiting Rhizobia were air dried, crushed, sieved (2 cm sieve) and subjected to estimate their EC. Homogenized soil in distilled water solution (1: 5 w/v) was used to estimate EC-values (Conductance meter, Model YSI ®) according to Dewis and Freitas (1970). Soil samples were varied in their EC-values according to their locations in the studied area. Also, roots of faba bean plants were collected to isolate their *Rhizobium* nodules.

Active nodules (pink color) were dissected from roots, rinsed thoroughly in water, surface sterilized by immersion in HgCl<sub>2</sub> solution (3% v/v) for 4 min, treated with ethyl alcohol for 3 minutes, rinsed several times with sterile distilled water. Nodules were individually comminuted and the suspension was streaked onto surface-dried YMA medium (Yeast extracts Mannitol Agar) which contains 0.5 g K<sub>2</sub>HPO<sub>4</sub>, 0.5 g MgSO<sub>4</sub>, 0.1 g NaCl, 10.0 g mannitol, 1.0 g yeast extract, 15.0 g agar per one liter distilled water, pH 6.8 – 7.0, was autoclaved at 121°C for 20 min. Plates were incubated at 28 °C for 3 to 10 days. For purity tests, streaking on YMA medium with bromo-thymol blue (BTB) was done to differentiate between fast and slow growing Rhizobia on YMA medium with congo-red which was applied to differentiate between contaminants and Rhizobia. Finally glucose peptone and litmus milk media were used to differentiate between *Agrobacterium* spp. and *Rhizobium* spp. Each culture which passed through the purity tests was considered as individual Rhizobium isolate. These isolates were identified according to the key of Jordan (1984), Kersters and De Ley (1984), Ker (1992) and Don *et al.* (2005). Accordingly, the rhizobial isolates were found to be *Rhizobium leguminosarum* bv. *viciae*. The purified cultures were maintained onto slants of YMA supplemented with 0.35% CaCO<sub>3</sub> for short-term storage at 4°C and sub-cultured every 2-3 months according to Vincent (1970).

To test their salt tolerance, Rhizobial isolates were *in vitro* cultured onto YMA plates amended with NaCl to reach the required EC-levels (Hosney *et al.*, 2002). So, successive concentrations of 0.018, 0.036, 0.054 and 0.072 M of NaCl were added to obtain 3, 6, 9 and 12 dS m<sup>-1</sup> (deci-Siemens/meter). However, unsalted YMA-medium acted as control. Plates were inoculated with 3 days old rhizobial culture containing 10<sup>8</sup> CFU (Colony Forming Units). Treatments were represented in triplicates. The plates were incubated at 28°C for 3 days, and then the bacterial growth was observed. According to their tolerance to salt stress, ten isolates were found to be withstood, but F<sub>1</sub> was the most even at 12 dS m<sup>-1</sup>. Therefore, *Rhizobium leguminosarum* bv. *viciae* isolate F<sub>1</sub> (Baltim location, sandy soil in texture, EC 8.5 dS m<sup>-1</sup>) was selected to the further studies. Additionally, the presented study was supported by three well known salt-tolerant *Rhizobium leguminosarum* bv. *viciae* isolates (E<sub>1</sub>, E<sub>2</sub> and E<sub>3</sub>) as reference cultures formerly tested by El-Nady and Belal (2005). These isolates were exposed to the similar salt stress conditions to compare their efficacy with the other ten isolates. Due to its salt withstand even at 12 dS m<sup>-1</sup>, E<sub>1</sub> was also selected in addition to F<sub>1</sub> to complete the objectives of this study.

#### **Symbiotic N-fixation:**

Two pot experiments were carried out at the Dept. of Agric. Botany, Fac. of Agric., Kafrelsheikh Uni., Egypt during two winter seasons (temperature: 15 & 20 °C, humidity: 72 & 62 %, wind speed: 9 & 16 Km h<sup>-1</sup>, respectively). Trials were started at the first half of November under aseptic and non-aseptic conditions. Faba bean cultivar seeds (Nubaria 1 and Sakha 1) were kindly supplied from Field Crop Res. Inst., Agric. Res. Center, Dept. of Leg. Sakha Agric. Res. Station, Kafr El-Sheikh, Egypt.

#### **a) Under aseptic conditions:**

One loopful of 10<sup>8</sup> CFU-plates from each purified isolates (F<sub>1</sub> and E<sub>1</sub>) and from a less salt-tolerant isolate (F<sub>9</sub>) was re-cultured on 250 mL YM liquid medium in 500 mL flask. The cultures were shaken incubated at 28-30 °C, 150 rpm (revolutions per minute) for 3-5 days. Later, number of bacterial cells of each culture was counted and adjusted at 10<sup>8</sup> cell ml<sup>-1</sup> using counting chamber (Haemocytometer specialized microscopic slide).

Pots (20 cm in diameter) were sterilized by immersing in 5 % formalin solution for 15 minutes and left till complete evaporation of formalin (Hanafi, 1989). Each pot was filled with 3 kg of sterilized sand soil. Sandy soils were washed several times by 0.1 N HCl solutions and further washed with distilled water before filling them in the pots. Soils were salinized in related to sodium adsorption ratio (SAR) by using salts of NaCl and CaCl<sub>2</sub> to adjust their EC-levels. Pots trials were performed at EC-levels of 3, 6, 9 and 12 dS m<sup>-1</sup>. Each 100 g soil received 27 and 18 mg of NaCl and CaCl<sub>2</sub>, respectively to obtain EC equal 3 dS m<sup>-1</sup>. Both doses of each salt were successively doubled to get 6 (54 & 36 mg), 9 (81 & 54 mg) and 12 (107 & 72 mg) dS m<sup>-1</sup>, respectively according to Manual of Salinity Research Methods (1992).

Seeds of Nubaria 1 cv. were also surface sterilized to eliminate possible contaminations by rinsing them in ethanol (70 %) for 3-5 minutes and soaking for 4 minutes in hydrogen peroxide (3 % v/v) followed by washing in sterile distilled water several times (El-Akhdar, 2009). Four seeds per pot were sown. Seedlings were thinned to three per pot, and then inoculated with 5 mL liquid cultures of the tested rhizobial isolates ( $10^8$  CFU) each. Pots received 5 mL Rhizobium free liquid media acted as control. Trials were carried out in three replicates.

To save EC-levels, pots were covered at about one meter high with plastic sheets against rain shower and drainage water was used again. Water hold capacity of the soil was about 27.0 % under the experimental conditions. To keep the moisture content of the soil to field capacity, irrigation was regularly carried out twice weekly intervals according to weather conditions. According to Shrdleta *et al.* (1984), plants were irrigated by the free N-solution contains 0.486 g  $K_2SO_4$ , 0.200 g  $K_2HPO_4$ , 0.200 g  $MgSO_4 \cdot 7H_2O$ , 0.010 g  $FeCl_2$ , 0.376 g  $CaCl_2$ , 1.855 mg  $H_3PO_4$ , 0.280 mg  $ZnSO_4 \cdot 7H_2O$ , 2.231 mg  $MnSO_4 \cdot H_2O$ , 0.25 mg  $CuSO_4 \cdot 5H_2O$ , 0.412 mg  $NaMO_4$  per liter sterile distilled water. Solution was adjusted at pH of 6.9 using pH-meter (JENCO, 6209).

**b) Under non-aseptic conditions:**

In pot trials, symbiotic N-fixation was evaluated in the present experiment under non-aseptic conditions. Pots (30 cm in diameter) were filled with 7 kg sandy soils. As carrier for *Rhizobium*, peat moss (Agric. Res. Center, Giza, Egypt) was inoculated. Peat based cultures of Rhizobia were prepared using the method described by Thao *et al.* (2001). Cultures containing  $10^8$  CFU of the tested rhizobia were used to impregnate autoclaved peat (121°C, 30 min.) at the rate of 52 mL liquid culture per 100 g peat. Inoculated peat was well mixed and maintained at room temperature for 48 hr. Under these conditions, Sakha 1 cv. was also used in addition to Nubaria 1cv. Seeds of both faba bean cultivars were wetted with 10 % Arabic gum water solution as an adhesive agent and inoculated with rhizobial peat-based preparation (Hamdi, 1982). Seeds were allowed to air drying in the shade for 30 min. then sown immediately. Four seeds per pot were sown. Seedlings were thinned to three per pot. Soils were salinized and their EC-levels were adjusted by using similar methods previously applied under aseptic conditions.

In the present experiment, efficiency of Rhizobia was further tested to salinity, but in comparison with the mineral N-supply. Pots fertilized with 25 and 100 % N (0.22 and 0.88 g urea (45-46% N)  $pot^{-1}$ , respectively) were used. For 25 % N-supply, pots received only one dose at the sowing time. For 100 % N-supply, 0.88 g urea  $pot^{-1}$  was divided into 4 equal doses added to pots at 0, 15, 30, 45 days after sowing. Pots inoculated with rhizobia ( $E_1$  or  $F_1$ ) received also 25 % N-supply as an activation dose. All pots were also fertilized with the recommended doses of both super phosphates (1.05 g  $pot^{-1}$ ) before sowing and 0.35 g  $pot^{-1}$  of potassium sulphate at flowering phase. Trials were performed in three replicates. Irrigation and other practices were carried out when needed.

**Determinations:**

Under aseptic conditions, number, dry weight of nodules and N % of the shoots were determined 50 days after planting. For non-aseptic trials, number, dry weight, N % of nodules and dry weight of shoots were determined 70 days after sowing. On the other hand, weight of seeds per plant, weight of 100 seeds and N % of seeds were determined at harvest (135 days after planting). Dry weight values were determined using the oven at 70°C till fix weight. For determining N % and total N-content, kjeldahl methods (Barbano *et al.*, 1990) were applied. Samples of shoots or seeds were dried at 70°C and then 0.2 g of them was digested in 5 mL concentrated sulphoric acid and 1 mL concentrated Perchloric acid (in a conical flask as described by Chapman and Parker (1963). The digested samples were completed to 50 ml using distilled water. Distillation was carried out using 40 % NaOH, and ammonia was received in 4 % boric acid solution. The distillates were then titrated with 0.02 M H<sub>2</sub>SO<sub>4</sub> using a mixture consists of methyl red-and bromocrysol green as an indicator according to Black *et al.* (1965). N %, total N-content and crude proteins were calculated according to El-Akhdar (2009) as follows:  
N % = total N-content / dry weight

**Statistical analysis:**

Complete randomized block was the main design of these trials. Data were statistically tested for the analysis of variance using IRRISTAT version 3/93. Means were compared using LSD methods according to Steel and Torrie (1980) and Duncan's multiple range tests were applied for comparing means (Duncan, 1955).

## **RESULTS AND DISCUSSION**

**Rhizobia:**

In addition to the three reference isolates, screening trials of salt affected soils resulted in 10 isolates of *Rhizobium leguminosarum* bv *viciae* within faba bean fields. EC-values of soil samples in which the established isolates were found to be varied between 5.6 and 8.5 ds m<sup>-1</sup>. Therefore, the isolated and the reference rhizobia were *in vitro* tested in Petri-plates containing YMA media salted with different levels. Of these, F<sub>1</sub> (isolated) and E<sub>1</sub> (reference) were found to be the most salt-tolerant even at 12 ds m<sup>-1</sup> level. Ability of these isolates to re-infect faba bean roots was also tested in pots using successive salt doses. Rhizobia were re-isolated once again from the new formed nodules. Therefore, both isolates (E<sub>1</sub> and F<sub>1</sub>) were subjected to evaluate their efficacy to fix Nitrogen in comparison with the mineral N-supply.

**Symbiotic N-fixation:**

**a) Under aseptic conditions:**

Table (1) illustrates effect of salinity levels on number, dry weight of nodules and percentage of Nitrogen fixed in faba bean plants in pots under sterilized conditions. Number of nodules was decreased strongly by increasing salinity levels in comparison with the control. It reached 50.11 and 44.00 nodules plant<sup>-1</sup> by E<sub>1</sub> and F<sub>1</sub> at 12 ds m<sup>-1</sup>, respectively. Both isolates accumulate tiny dry matter at similar saline level compared with the control and with the less salt-tolerant isolate (F<sub>9</sub>).

**Table (1): Effect of salinity levels on number, dry weight of nodules and N % of the shoots of faba bean cultivar Nubaria 1 inoculated with the tested rhizobial isolates (F<sub>1</sub>, E<sub>1</sub> and F<sub>9</sub>) 50 days after sowing in pots under aseptic conditions.**

Treatment	Saline (EC)- level (ds m <sup>-1</sup> )				
	Control	3	6	9	12
<b>Number of nodules per plant</b>					
Un-inoculated (control)	0.00 f	0.00 e	0.00 g	0.00 e	0.00 e
R. leg. (F <sub>1</sub> )	107.89 a	92.45 a	61.78 ab	51.89 a	44.00 a
R. leg. (E <sub>1</sub> )	111.89 a	91.78 a	70.33 a	59.11 a	50.11 a
R. leg. (F <sub>9</sub> )	67.67 de	62.11 c	50.00 cd	33.00 bcd	20.89 bc
Comparison 2-S*M means	LSD 5 % = 9.13			LSD 1% = 12.06	
<b>Dry weight of nodules (g per plant)</b>					
Un-inoculated (control)	0.00 g	0.00 g	0.00 c	0.00 e	0.00 d
R. leg. (F <sub>1</sub> )	0.26 b	0.19 ab	0.14 a	0.12 ab	0.11 a
R. leg. (E <sub>1</sub> )	0.30 a	0.20 a	0.15 a	0.13 a	0.12 a
R. leg. (F <sub>9</sub> )	0.19 cde	0.12 def	0.09 b	0.08 cd	0.07 bc
Comparison 2-S*M means	LSD5 % = 0.03			LSD1% = 0.04	
<b>N % of the shoots</b>					
Un-inoculated (control)	1.69 e	1.51 f	1.37 g	1.24 e	1.09 e
R. leg. (F <sub>1</sub> )	2.31 ab	2.24 a	2.20 a	1.98 a	1.83 a
R. leg. (E <sub>1</sub> )	2.35 a	2.29 a	2.23 a	2.07 a	1.91 a
R. leg. (F <sub>9</sub> )	2.15 bcd	1.83 cd	1.64 cd	1.39 de	1.33 cd
Comparison 2-S*M means	LSD5 % = 0.18			LSD 1% = 0.23	

The numbers in the same column means followed by the same letter are not significantly different according to DMRT at 0.05 levels. R. leg. refers to *Rhizobium leguminosarum* bv *viciae*.

It reflects the relationship between mass of effective nodules and quantity of Nitrogen fixed by the endophytic rhizobia. Similar results were obtained by Badr (1984). Reduction of the symbiotic N-fixation parameters may be related to increase the free amino acids in nodules due to an increase of total Nitrogen, which alter the C/N ration consequently may reduce the normal rate of N-fixation as reported by Tu and Ford (1970). It is also noticed from the above mentioned results a deleterious effect of salinity on un-inoculated plants. This effect was higher than those of rhizobial inoculated plants, in which N % data were increased due to rhizobial inoculation in presence or absence of salinity. This phenomenon is more pronounced in case of higher salinity levels. Inoculation with F<sub>1</sub> or E<sub>1</sub>

rhizobial isolates attained the highest N %. It was in accordance with Matiru and Dakora (2004), who stated that rhizobia produce plant growth promoters such as vitamins and phytohormones (auxins, cytokinins, gibberellins and abscisic acid required to cell division, cell elongation and photosynthetic pigments formation) which play an important role for enhancing the plant growth. Due to their biological impacts under sterilized conditions, the salt-tolerant isolates of rhizobia (F<sub>1</sub> and E<sub>1</sub>) were selected to the next step to inoculate faba bean plants in pot trials under natural conditions.

**b) Under non-aseptic conditions:**

In addition to Nubaria 1, another cultivar (Sakha 1) was also inoculated for evaluating *Rhizobium* isolates in pot trials. Pots inoculated with either E<sub>1</sub> or F<sub>1</sub> were compared with 25 and 100 % mineral N-fertilization. Due to its usual and common supply during cultivation of faba bean under all conditions, 25 % N-supply alone could be considered as control. **Table (2)** illustrates effect of salinity levels on number and dry weight of nodules, as well as N % of shoots under 0, 6 and 9 ds m<sup>-1</sup> saline levels.

**Table (2): Effect of salinity levels on number and dry weight of nodules and shoot N % of faba beans (Nubaria 1 and Sakha 1 cultivars) inoculated with rhizobia isolates (F<sub>1</sub> and E<sub>1</sub>) in comparison with 25 and 100 % N-supply 50 days after sowing in pots under non-aseptic conditions.**

Treatment	Number of nodules plant <sup>-1</sup>			Dry weigh of nodules g plant <sup>-1</sup>			N % of the shoots		
	Control	6 ds m <sup>-1</sup>	9 ds m <sup>-1</sup>	Control	6 ds m <sup>-1</sup>	9 ds m <sup>-1</sup>	Control	6 ds m <sup>-1</sup>	9 ds m <sup>-1</sup>
<b>Nubaria 1</b>									
25% N	14.56 c	9.11 c	7.86 d	0.22 b	0.19 b	0.13 b	1.91 e	1.61 c	1.48 c
100% N	10.00 c	6.89 c	5.44 d	0.19 b	0.11 b	0.08 b	2.09 d	1.87 b	1.75 b
R. leg. (F <sub>1</sub> )	125.11 b	96.78 b	82.33	0.56 a	0.47 a	0.40 a	2.36 ab	2.23 a	2.11 a
R. leg. (E <sub>1</sub> )	132.67 a	114.67 a	bc 93.33 a	0.59 a	0.49 a	0.41 a	2.40 a	2.25 a	2.13 a
<b>Sakha 1</b>									
25% N	13.78 c	8.748 c	7.44 d	0.21 b	0.17 b	0.12 b	1.95 e	1.62 c	1.47 c
100% N	9.11 c	6.89 c	4.78 d	0.18 b	0.10 b	0.06 b	2.08 d	1.82 b	1.74 b
R. leg. (F <sub>1</sub> )	119.45 b	96.33 b	81.11 c	0.56 a	0.46 a	0.39 a	2.27 c	2.21 a	2.06 a
R. leg. (E <sub>1</sub> )	122.67 b	110.33 a	88.33 ab	0.58 a	0.46 a	0.38 a	2.30 bc	2.23 a	2.08 a
Comparison 2-S*M means	LSD 5 % 6.31	LSD 1 % 8.42		LSD 5 % 0.06	LSD 1 % 0.08		LSD 5 % 0.08	LSD 1 % 0.11	

The numbers in the same column means followed by the same letter are not significantly different according to DMRT at 0.05 levels. R. leg. Refers to *Rhizobium leguminosarum* bv *viciae*.

These results show decreases in the tested parameters due to an increase of salinity concentrations. Significant increases of these data due to F<sub>1</sub> and E<sub>1</sub> inoculation were also found compared with the plants treated with 25 and 100 % N. Numbers of nodules existed with the non-saline pots sown

with Nubaria 1 were highly increased from 14.56 and 10.00 using 25 and 100 % N, respectively to 125.11 and 132.67 via F<sub>1</sub> and E<sub>1</sub> rhizobial isolates, respectively. However, lower number of nodules was obtained due to salinity with 6 and 9 ds m<sup>-1</sup>, similar trend was obtained with both cultivars. Due to use 6 and 9 ds m<sup>-1</sup>, lower nodules number was obtained than control. Using 9 ds m<sup>-1</sup>, numbers of nodules were highly increased from 7.86 and 5.44 by 25 and 100 % N, respectively to 82.33 and 93.33 by F<sub>1</sub> and E<sub>1</sub>, respectively of Nubaria 1, indicating a remarkable activity of the native Rhizobia. Correspondingly, dry weight of nodules formed under 9 ds m<sup>-1</sup> reached 0.40 and 0.41 g plant<sup>-1</sup> for F<sub>1</sub> and E<sub>1</sub>, respectively instead of 0.13 and 0.08 g plant<sup>-1</sup> for 25 and 100 % N, respectively. Similar behavior was reflected on nodule dry weight of both Nubaria 1 and Sakha 1 cvs. These results were in accordance with the data of Nitrogen in the shoots.

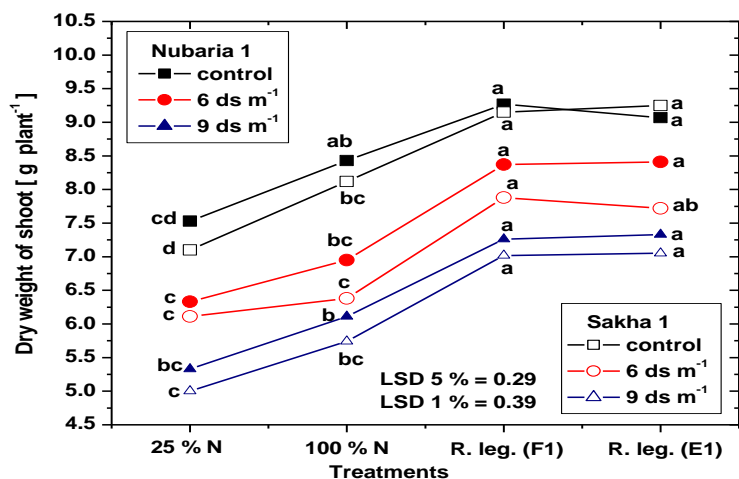
So, the significant increase of nodular numbers due to inoculation with E<sub>1</sub> and F<sub>1</sub> comparing with N-supply could be explained by formation of large varied sized nodules due to the biological nitrogen fixation (BNF). Similar findings were stated by Gaballah and Gomaa (2005), who found a massive increase of nodulation due to *Rhizobium* inoculation compared with the control. Lower magnitudes of N % were obtained due to 6 and 9 ds m<sup>-1</sup> in comparison with control, indicating reduction of shoot dry matter. This reflects the effective role of nitrogen formation via Rhizobia in comparison with N-supply. It means that both E<sub>1</sub> and F<sub>1</sub> rhizobial isolates have massive potentials to fix the atmospheric Nitrogen even under saline stress conditions, but overall lower than the un-saline plants (control). Ghobrial *et al.* (2009) found that the nitrogen fixing parameters of three faba bean cultivars (Masr-1, Sakha-1 and Giza-843) inoculated with Rhizobia were increased due to enhance the physiological state and defensive capacity in the treated plants. These results were also in accordance with Yousef and Sprent (1983), who found weakly nodulation of faba bean roots due to salinization with NaCl which may cause failure infection with rhizobia. Delgado *et al.* (1993) found lower contents of leghemoglobin in the nodules affecting by salinity. However, Borucki and Sujkowska (2008) summarized that salinity may cause (1) loss of turgor of the nodule peripheral cells, (2) changing nodule zonation, (3) stimulated infection thread enlargement and expansion; (4) disturbances in bacterial release form the infection threads and (5) induced synthesis of electron dense material (EDM) and its deposition in vacuoles. These results were confirmed by Marcar *et al.* (1991) who stated that the symbiotic properties of legumes (N-fixation) were more sensitive under salt stress conditions. Similarly, El-Sheikh and Wood (1995) reported that the salt-tolerant strains of *Rhizobium* spp. became more effective under saline conditions. These were agreed with Zou *et al.* (1995), who suggested that inoculation with a salt-tolerant *Rhizobium* strain improved biological N-fixation under saline conditions.

#### **Growth and yield parameters:**

To indicate efficacy of the salt-tolerant rhizobial inoculations, different plant growth and yield parameters in pots trials under saline-stress conditions were evaluated. Data of shoot dry weight of both faba bean cultivars 70 days



after sowing were determined and plotted in Fig. (1). Data showed similar behavior of both varieties against all treatments. Majority of the dry weight was achieved in control plants, followed by 6 and 9 ds m<sup>-1</sup>, respectively. Regardless salinity, both rhizobial inoculations recorded better dry weight formation than the N-supply even under saline conditions. Accordingly, biological N-fixation (BNF) caused a great potential for enhancing dry matter values in comparison with N-fertilization. The results were in agreement with Peter *et al.* (2008), who found that biomass of faba bean, was decreased with increasing salt concentrations. El-Nady and Belal (2005) stated that the salt-tolerant rhizobium isolates could fix nitrogen and enhance plant growth and yield parameters of faba bean due to supply plants with IAA in its tissues. Hussain *et al.* (2002) found also decreasing in the dry matter of Berseem (*Trifolium alexandranum*) plants (shoots and roots) with increasing of salinity level. At harvest, seed yield index data (dry weight of seeds plant<sup>-1</sup>, dry weight of 100 seeds and N % of the seeds) were shown in Table (3). Results showed significant decreases in the tested parameters with increasing salinity levels. These parameters were increased due to inoculate with rhizobia (F<sub>1</sub> or E<sub>1</sub>) in comparison with N-supply. Using salt-tolerant isolates of *Rhizobium leguminosarum* bv. *viciae*, tolerant of faba bean plants to salinity was improved compared with N-fertilized plants under saline conditions. Similar results were obtained by Cordovilla *et al.* (1999).



**Fig. (1):** Shoot dry weight of faba bean cultivars (Nubaria 1 and Sakha 1) inoculated with rhizobial isolates (F<sub>1</sub> and E<sub>1</sub>) in comparison with 25 and 100 % N-supply 70 days after sowing under saline-stress conditions.

Similarly, Hammouda *et al.* (1990) reported that the seed yield of broad bean was increased due to rhizobial inoculation. Seed yield parameters were further enhanced clearly by inoculation with effective strains of *R. leguminosarum* bv. *viciae* (on pea) and *Bradyrhizobium japonicum* (on soybean) under salt stress conditions (Borucki and Sujkowska, 2008 and Miransari and Smith, 2009). As well as, Zou *et al.* (1995) stated that N % of faba bean were also increased in their seeds due to enhance biological N<sub>2</sub>-fixing (BNF) process by rhizobium inoculation in comparison with the uninoculated controls. Therefore, we suggest inoculation of salt-tolerant faba bean varieties with salt-tolerant rhizobial isolates such as F<sub>1</sub> or E<sub>1</sub>, in particular in the new reclaimed lands instead of the mineral N-fertilizers.

**Table (3): Seed yield index parameters of faba bean cultivars (Nubaria 1 and Sakha 1) inoculated with rhizobia (F<sub>1</sub> and E<sub>1</sub>) in comparison with 25 and 100 % N fertilization at harvest under saline-stress conditions.**

Treatments	Dry weight of seeds plant <sup>-1</sup>			Dry weight of 100 seeds (g)			N % of the seeds		
	Control	6 ds m <sup>-1</sup>	9 ds m <sup>-1</sup>	Control	6 ds m <sup>-1</sup>	9 ds m <sup>-1</sup>	Control	6 ds m <sup>-1</sup>	9 ds m <sup>-1</sup>
<b>Nubaria 1</b>									
25% N	10.68 f	8.04 f	5.85 e	91.70 c	84.15 b	73.83 c	3.08 c	2.93 c	2.72 c
100% N	12.86 d	10.07 d	7.56 c	94.84 b	86.61 b	83.06 b	3.95 ab	3.52	3.12 b
R. leg. (F <sub>1</sub> )	14.57 b	11.29 b	8.76 a	100.45 a	90.77 a	86.71 a	4.03 a	ab	3.40 a
R. leg. (E <sub>1</sub> )	15.05 a	11.86 a	9.06 a	101.79 a	91.98 a	88.23 a	4.07 a	3.57 a	3.40 a
<b>Sakha 1</b>									
25% N	9.85 g	7.44 g	5.68 e	79.21 f	72.11 e	64.79 e	3.08 c	2.90 c	2.67 c
100% N	11.68 e	8.88 e	6.71 d	81.53 e	76.11 d	68.65 d	3.80 b	3.38 b	3.08 b
R. leg. (F <sub>1</sub> )	12.96 d	10.16 cd	7.86 bc	85.13 d	77.49 cd	72.93 c	3.92 ab	3.53	3.32 a
R. leg. (E <sub>1</sub> )	13.42 c	10.55 c	8.05 b	86.29 d	78.46 c	74.23 c	4.02 a	ab	3.32 a
Comparison	LSD 5 %		LSD 1	LSD 5 %		LSD 1 %	LSD 5 %		LSD 1
2-S*M means	%		0.60	1.71		2.28	%		0.21
	0.45						0.16		

The numbers in the same column means followed by the same letter are not significantly different according to DMRT at 0.05 levels. R. leg. Refers to *R. leguminosarum* bv *viciae*.

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### التثبيت التكافلي للنيتروجين في نباتات الفول المملحة بعزلات ريزوبيوم تتحمل الملوحة

نجوى محمد محمد الخطيب ، مصطفى السيد شلبي ، السيد بلال عبد المنطلب بلال و  
شكري محمد على الجريمي  
قسم النبات الزراعي - ميكروبيولوجيا زراعية - كلية الزراعة - جامعة كفر الشيخ - مصر

نتيجة الإختبار المعملى لبكتيريا *Rhizobium leguminosarum* biovar *viciae* المعزولة من حقول فول متأثرة بالملوحة ، وجد أن عزلاتها المختلفة قد تباينت في تحملها للملوحة . وقد وجد أن العزلات  $F_1$  و  $E_1$  كانت الأكثر تحملا للملوحة حتى درجة توصيل كهربى ( EC ) تعادل ١٢ ديسيسيمنز لكل متر. وقد لقحت بذور نباتات الفول (صنف نوبارية ١) بالعزلات  $F_1$  و  $E_1$  مقارنة بإحدى العزلات الأقل تحملا للملوحة ( $F_0$ ) في أصص أضيف إليها ملح كلوريد الصوديوم وضبطت درجة توصيلها الكهربى عند صفر، ٣، ٦، ٩، ١٢ ديسيسيمنز متر<sup>-1</sup> تحت ظروف معقمة. وقد وجد تناقص شديد في أعداد والوزن الجاف للعقد البكتيرية الجذرية نتيجة الزيادة في مستويات الملوحة مقارنة بالكنترول. وقد لوحظ تحسن معنوى في النسبة المئوية للنيتروجين المتراكم في المجموع الخضرى بسبب التلقيح البيولوجى بكتنا عزلتى الـ *Rhizobium* فى وجود أو غياب الملوحة. كما قورنت كفاءة العزلات  $F_1$  و  $E_1$  بالتسميد الأزوتى المعدنى (٢٥ و ١٠٠ %) فى أصص تحت ظروف طبيعية غير معقمة. وقد أستخدم صنف الفول سخا ١ بالإضافة إلى الصنف نوبارية ١ فى عملية تقييم التثبيت الأزوتى عند مستويات ملوحة صفر و ٦ و ٩ ديسيسيمنز لكل متر . ولقد تزايدت أعداد العقد البكتيرية المتكونة على جذور الصنف نوبارية بشكل كبير عند معدل ٩ ديسيسيمنز متر<sup>-1</sup> من ٧.٨٦ و ٥.٤٤ عقدة لكل نبات عند التسميد النيتروجينى بـ ٢٥ و ١٠٠ % إلى ٨٢.٣٣ و ٩٣.٣٣ بواسطة  $F_1$  و  $E_1$  على التوالى. وتبعاً لذلك ، أزدادت الأوزان الجافة للعقد البكتيرية من ٠.٤٠ و ٠.٤١ جم لكل نبات لـ  $F_1$  و  $E_1$  بدلا من ٠.١٣ و ٠.٠٨ لـ ٢٥ و ١٠٠ % تسميد نيتروجينى ، على التوالى. ولقد أمتد التأثير النافع للعزلات البكتيرية  $F_1$  و  $E_1$  الى زيادة مقياس النسبة المئوية للنيتروجين فى كل من المجموع الخضرى ومحصول البذور. وعلى ذلك فعلمية التثبيت التكافلي للنيتروجين التى تتم بواسطة  $F_1$  و  $E_1$  كمسمدات حيوية ذات قدرة كبيرة على تحسين مقاييس النمو والمحصول فى نباتات الفول مقارنة بالتسميد المعدنى النيتروجينى من أجل التغلب على الآثار الضارة للملوحة.

قام بتحكيم البحث

كلية الزراعة - جامعة المنصورة  
كلية الزراعة - جامعة كفر الشيخ

أ.د / ساميه محمد مرسى بيومى  
أ.د / محمد كمال القزاز