Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo, 11(1), 87 - 96, 2003

NODULATION, NITROGEN FIXATION AND YIELD OF SOYBEAN IN SALINE SOIL AS AFFECTED BY SALT TOLERANCE OF BRADYRHIZOBIA

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ABSTRACT

The effects of increased concentrations of NaCl, ranged from 0.05 to 14 %, on growth of 3 strains of *Bradyrhizobium. japonicum*, i.e, USDA110, UF201 and UF305 were studied *in vitro*. The growth, nodulation, nitrogen fixation and yield of soybean were also evaluated when inoculated with those strains under saline soil conditions. Data of this study, showed that while the 2 strains UF201 andUF305 tolerated NaCl up to 10 and 12%, respectively, the USDA110 was quite sensitive. This observation was also reflected on the effects of those strains on soybean grown in saline soil. In many cases, the 2 former strains effectively stimulate all above-mentioned parameters with insignificant differences between them. The USDA110 strain proved to be ineffective in the saline soil due to its lower nodulating frequency under this condition. This finding was also conjugated with reduced levels of nitrogen fixation and productivity of plants inoculated with that strain.

Key words: Soybean, Bradyrhizobia, Salt tolerance, Nodulation, Nitrogen fixation, Yield

INTRODUCTION

Soybean (*Glycine max* L.) crop is widely grown as a low economic input crop and has the potential for balancing the protein deficiency in cereal-rich diets of the rural population in arid and semi-arid regions. In Egypt, a very poor nodulation in soybean under field conditions was attributed mainly to the absence of bradyrhizobia in Egyptian soils (**El-Haddad *et al* 1984 and El-Fayoumy *et al* 1996**). Other environmental factors such as salinity, high temperature and soil

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(Received October 20, 2002)
(Accepted December 18, 2002)

moisture stress may also affect nodulation and biological N_2 fixation (**El-Essawi *et al* 1982; El-Haddad *et al* 1984; Attia, 1989; El-Sheikh and Wood, 1990; El-Essawi and Abali, 1990; El-Essawi *et al* 1993 and El-Fayoumy *et al* 1996**). However, the performance of different legumes was generally shown to be negatively affected under saline conditions (**Lauter *et al* 1981; Yousef and Sprent, 1983; Singleton and Bohlool, 1984; Marcar *et al* 1991 and Zou *et al* 1995**), and those symbiotic plants were found to be more sensitive to salts in the root medium than plants received mineral nitrogen (**Wilson, 1970 and Lauter *et al* 1981**). The poor symbiotic performance of legumes was mainly attributed to: a) the salt intolerance of the host plants, and b) the *Rhizobium* and *Bradyrhizobium* strains were not specifically selected for their ability to form nodules and fix nitrogen under saline conditions. The finding that any symbiotic nitrogen fixing strains being similarly effective but differed in their salt tolerance (**Zou *et al* 1995; El-Sheikh and Wood, 1995 and Shabeb *et al* 1999**) has opened the possibility of improving the salt tolerance of the *Rhizobium*-legume symbiosis through inoculation with salt tolerant *Rhizobium* strain.

This work has been carried out to evaluate the effects of inoculation with three *Bradyrhizobium japonicum* strains varied in their salt tolerance on nodulation, nitrogen fixation and yield of soybean plants under saline soil conditions.

MATERIAL AND METHODS

Soil

A pot experiment was conducted during the summer season of 2000 in El-Fayum Governorate. The soil was obtained from El-Fayum and analyses showed that it was a loamy sand (79.4% sand, 12.8% silt and 7.8% clay) with EC of 9.93 dSm^{-1} , total nitrogen of 0.15%, organic carbon of 2.2% and $CaCO_3$ of 5.36%

Seeds

Soybean seeds (*Glycine max* cv. Giza 82) were kindly provided from the Department of Soybean Research, Agricultural Research Center, Giza, Egypt.

Bradyrhizobium japonicum strains

Three effective strains of *B. japonicum* i.e., USDA110, UF210 and UF305 were obtained from the Culture Collection of the Unit of Biofertilizers, Faculty of Agriculture, Ain Shams University, Cairo, Egypt. They were grown and maintained using yeast extract mannitol broth (**Somasegaran and Hoben, 1985**) at 28±2°C.

Experimental techniques

Salt tolerance of *B. japonicum* strains

In this experiment, 250-ml conical flasks contained yeast extract mannitol broth (50-ml $flask^{-1}$) supplemented with either of nine concentrations of NaCl, i.e., 0.05, 1, 2, 4, 6, 8, 10, 12 or 14% were inoculated with 2 ml inoculum $flask^{-1}$. The inoculum contained about 10^6 cells ml^{-1} of the active culture of *B. japonicum* USDA110, UF201 or UF305. Inoculated flasks were incubated at 28±2°C for 7 days. The growth of the three above-mentioned strains was recorded as positive and negative.

Performance of soybean inoculated with bradyrhizobia under saline soil conditions

Inoculum preparation

The three *Bradyrhizobium* strains were separately grown in yeast extract mannitol broth (**Somasegaran and Hoben, 1985**) for 7 days at 28±2°C. The broth cultures were then mixed with $CaCO_3$ neutralized peat moss to give a density of about 10^6cells g^{-1} carrier.

Seed inoculation and planting

One hundred milliliter (10^6 cells ml^{-1}) of the inoculant prepared from each strain were mixed with one kilogram of soybean seeds using gum arabic 16% as an adhesive agent. Eight kilograms of the soil were packed in pots (30 cm in diameter) and planted with five inoculated seeds, with either of the 3 strains and then watered to provide suitable moisture for the inocula and to maintain the water holding capacity at the suitable rate (approximately 60%). After complete germination, seedlings were thinned to two plants per pot. Ten replicates were made for each treatment. Developed plants were carefully uprooted after 45 days of cultivation to recored different parameters related to growth (shoot height, number of branches $plant^{-1}$, number of leaves $plant^{-1}$ and leaf dry weight $plant^{-1}$) and nodulation (nodulation frequency, number and dry weight of nodules $plant^{-1}$). Dry weights of plant materials were determined after oven drying at 70°C to a constant weight. Nitrogenase activities in nodules were measured by acetylene reduction assay as described by **Hardy *et al* (1973)**.

Timing of flowering and pod filling of soybean plants grown under different inoculation treatments was also recorded. Ninety days after cultivation, plants were harvested to record total pod yield (kg fed^{-1}), seed yield (kg fed^{-1}), straw yield (kg fed^{-1}) and biological yield (kg fed^{-1}). Three other yields related parameters were also calculated according to **Wallace *et al* (1972)** as follows:

$$\textit{Crop index} = \frac{\textit{Seed yield (kg fed}^{-1}\textit{) X 100}}{\textit{Straw yield (kg fed}^{-1}\textit{)}}$$

$$\textit{Harvest index} = \frac{\textit{Seed yield (kg fed}^{-1}\textit{)}}{\textit{Biological yield (kg fed}^{-1}\textit{)}}$$

Seed index = weight of 100 seeds (g)

Representative samples of soybean seeds of each treatment were analyzed for protein % and oil % according to **A.O.A.C (1990)**. The results of those two parameters were used to calculate both protein and oil yield (kg fed^{-1}). Data were statistically analysed according to **Snedecor and Cochran (1969)**

RESULTS

Resistance of *B. japonicum* strains to increased NaCl concentrations

Data in Table (1) indicated that the tested *B. japonicum* strains have the capability to tolerate increased concentrations of NaCl in different degrees. While, the UF201 and UF305 strains seemed to be quite tolerant to increased NaCl concentrations, the USDA110 strain was very sensitive to high salt cocentrations. In this respect, the two former strains

Table 1. Resistance of *B. japonicum* strains to NaCl concentrations in yeast extract broth

B. japonicum strains	NaCl Concentrations (%)								
	0.05	1	2	4	6	8	10	12	14
UF201	+	+	+	+	+	+	+	-	-
UF305	+	+	+	+	+	+	+	+	-
USDA110	+	+	+	-	-	-	-	-	-

+ = Growth. - = No growth.

tolerated up to 10 and 12% NaCl, respectively, and the latter only tolerated 2%. However, the 14% concentration of NaCl inhibited the growth of the three tested strains.

Growth, nodulation status and yield components of inoculated soybean plants grown in saline soil

Growth

Records of growth characters obtained after 45 days of cultivation showed that the two strains UF201 and UF305 are generally good halotolerant enhancing soybean development under saline soil conditions (Table, 2). This finding was clear in the records of shoot height, number of branches plant^{-1} and number of leaves plant^{-1}. No significant differences were observed between these parameters of plants inoculated with either of the two above-mentioned strains. Only plants inoculated with strain UF201 produced significantly greater amounts of leaf dry weight (g plant^{-1}) than those inoculated with strain UF305. However, the two groups of plants showed significant growth enhancement in comparison to those inoculated with the strain USDA110.

Nodulation and nitrogen fixation

While all soybean plants inoculated with strain UF201 or UF305 developed root nodules, only 30% of those inoculated with USDA110 strain did so in the tested saline soil (Table, 3). This observation was also conjugated with significantly lower records of the number and dry weight of nodules as well as acetylene reduction activity. In this respect, plants inoculated with strain UF305 showed significantly higher records of all the above mentioned criteria

Yield and yield components

Soybean plants inoculated with strain USDA110 showed a late flowering and pod filling compared to those inoculated with strain UF201 or UF305 (Table, 4). The latter two groups of plants were nearly similar in their timing of growth development.

Results presented in Table (5) confirmed the effectiveness of strain UF201 and UF305 in enhancing the productivity of soybean plants compared to

Table 2. Growth of soybean plants cultivated in a saline soil and inoculated with *B. japonicum*.different strains

B. japonicum strains	Shoot height (cm)	No. of branches plant^{-1}	No. of leaves plant^{-1}	Leaf dry weight (g plant^{-1})
UF201	67.6 cd	1.3 e	14.5 a	52.3 d
UF305	71.2 c	1.2 e	13.2 ab	46.4 e
USDA110	55.9 f	1.1 f	10.9 cd	43.2 g

For each parameter, means not followed by the same letter are not significantly different by Duncan's LSD test (P 0.05).

Table 3. Nodulation and nitrogen fixation of soybean plants cultivated in a saline soil and inoculated with *B. japonicum*.different strains

B. japonicum strains	Nodulation frequency (%)	No. of nodules plant^{-1}	Dry weight of nodules mg plant^{-1}	Nitrogenase activity (n moles C_2H_4 h^{-1} g dry nodules^{-1})
UF201	100	24.4 el	217 gh	37.2 c
UF305	100	26.5 d	223 d	39.2 a
USDA110	30	08.0 g	098 i	10.7 g

For each parameter, means not followed by the same letter are not significantly different by Duncan's LSD test (P 0.05).

Table 4. Timing of flowering and pod filling of soybean plants cultivated in a saline soil inoculated with *B. japonicum*.different strains

B. japonicum strains	Days to flowering	Days to pod filling
UF201	50*	81*
UF305	51	83
USDA110	55	88

*: Average of ten replicates.

Table 5. Yield and yield characteristics of soybean plants cultivated in a saline soil inoculated with *B. japonicum*.different strains

B. japonicum strains	Total pod yield (kg fed^{-1})	Seed Yield (kg fed^{-1})	Straw yield (kg fed^{-1})	Biological yield (kg fed^{-1})	Crop index	Harvest index	Seed index
UF201	1696.6 b	1290.3 d	2987 fg	4277.3 de	43.2	30.1	41.1
UF305	1880.7 ab	1298.1 d	2889 fg	4187.1 f	44.9	31.0	13.9
USDA110	1090.8 d	0501.2 e	1270 j	1771.2 hj	39.5	28.3	13.4

For each parameter, means not followed by the same letter are not significantly different by Duncan's LSD test ($P > 0.05$).

strain USDA110. This finding was clear in insignificant differences between plants inoculated with either of the two strains with respect to total pod yield, seed yield and straw yield. These results were also reflected on crop index, harvest index and seed index. In contrast, the lowest records were obtained from plants inoculated with strain USDA110. While nearly similar contents of protein and oil were determined in seeds of plants inoculated with either of the three strains, the yields (kg fed^{-1}) of these inoculated with strain USDA110 were markedly low (Table, 6).

DISCUSSION

Salinity has been reported to reduce shoot and root weights, nodulation and total nitrogen in legumes such as chickpea (**Lauter *et al* 1981**), soybean (**Singleton & Bohlool, 1984 and Grattan & Mass, 1988**) and faba bean (**Yousef & Sprent, 1983 and Zahran & Sprent, 1986**). Plants grow under salinity stress show considerable differences in physiological and biochemical activities, as a result of adaptive mechanisms during evolution. **Grattan and Grieve (1992) and Amer (1999)** mentioned that salinity disrupted the nutrient acquisition by plants in two ways. The first, through ionic strength of substrate, regardless its composition, which can influence uptake and translocation. The second, and the more common mechanism is reducing the nutrient availability through ionic competition with ions. However, the process of root hair infection of legumes was shown to be particularly sensitive to saline stress, perhaps due to the common cessation of root hair growth under these conditions (**Sprent, 1984**). A reduction in 50% of soybean nodulation, compared to maximum nodules number and weight was reported by **Singleton & Bohlool (1984) and El-Sheikh & Wood (1995)** with 26.6 and 34.2 mM NaCl in solution culture, respectively. Also, **Hafeez *et al* (1988)** reported that nodulation of cowpea was reduced about 50% by 5.0 dS m^{-1} when compared to 1.4 dS m^{-1} with a complete depression at 10.0 dS m^{-1}.

Table 6. Seed protein, oil content and their yield of soybean plants cultivated in a saline soil inoculated with *B. japonicum*. different strains

B. japonicum strains	Seed protein		Seed oil	
	Content (%)	Yield (kg fed^{-1})	Content (%)	Yield (kg fed^{-1})
UF201	39.9*	514.8*	24.7*	318.7*
UF305	40.9	530.9	25.4	329.7
USDA110	39.8	199.5	22.9	117.8

*: Average of ten replicates.

Salinity may also interfere with nodule initiation due to its effect on the bacterial partner. There are marked differences between rhizobia strains in adaptation to saline conditions and the host legumes which are much more sensitive to salinity than the bacteria. **Subba Rao *et al* (1990)** showed that rhizobia strains were markedly different in their ability to infect and form nodules on pigeon pea under saline conditions. This finding was confirmed in this study, where the salt tolerant *B. japonicum* UF201 and UF305 were quite effective in nodulating soybean under saline soil conditions compared to the sensitive strain USDA110. This variation in nodulating ability was reflected on all measured parameters of growth, nitrogen fixation and soybean yield. In contrast to these data, **Yelton *et al* (1983)** found no difference between *R. fredii* strain USDA 191 (salt tolerant) and *B. japonicum* strain USDA 110 (salt sensitive)., in terms of the total nodules number $plant^{-1}$, nodules weight and total plant weight for soybean grown under non-saline conditions. However, the salt sensitive strain fixed more nitrogen than the salt tolerant strain under those conditions. This finding may indicate that the cultivar used may also have an important effect on the performance of salt tolerant strain **(Raovelagaleti and Marsh, 1989)**. Overall, the obtained data indicate that the use of salt tolerant strain of *Bradyrhizobium* to inoculate soybean plants under saline conditions can produce an increase in biological nitrogen fixation when compared to a salt sensitive strain. Similar conclusion was also reported by **El-Sheikh and Wood (1995)**.

ACKNOWLEDGMENT

The authors would like to express their sincere gratitude and appreciation to Prof. Dr. Magdi I. Mostafa, Department of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University, Cairo, Egypt for his sincere help during this work.

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مجلة اتحاد الجامعات العربية للدراسات والبحوث الزراعية ، جامعة عين شمس ، القاهرة ، ١١(١) ، ٨٧ - ٩٦ ، ٢٠٠٣

التعقيد وتثبيت النيتروجين الجوى وإنتاجيه فول الصويا فى التربه الملحيه تحت تأثير تحمل الملوحه للبرادى ريزوبيا

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تم فى هذا البحث دراسه أثر التركيزات المتزايده من كلوريد الصوديوم فى مدى من ٠.٥ إلى ١٤% على نمو ثلاثه سلالات من برادى ريزوبيم جابونيكم هى USDA110، UF201 ، UF305 ثم إستخدمت هذه السلالات فى تلقيح نباتات فول الصويا . ولقد تم تقدير النمو ، وتكوين العقد الجذريه، وتثبيت النيتروجين الجوى ومحصول فول الصويا تحت ظروف التربه الملحيه وقد أظهرت النتائج أنه بينما تتحمل السلالتان UF201، UF305 تركيزات من كلوريد الصوديوم تصل إلى ١٠ ، ١٢ % على الترتيب فإن السلاله USDA110 كانت أكثر حساسيه .ولقد انعكست هذه الملاحظه على تأثير هذه السلالات على نمو نباتات فول الصويا فى التربه الملحيه . فقد أعطت السلالتان السابقتان زيادة ملحوظه للقياسات سابقه الذكر مع اختلافات غير معنويه بينهما فى حالات كثيره. فى حين أظهرت السلالهUSDA110 عدم فعاليتها فى التربه الملحيه نتيجة انخفاض معدل تكوينها للعقد الجذريه وتثبيت النيتروجين الجوى وإنتاجيه النباتات الملقحه بهذه السلاله .

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