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TRANSFERE OF SALINITY TOLERANCE PLASMID FROM HALOPHILIC BACTERIA TO *RHIZOBIUM LEGUMINOSARUM* BV. *VICIAE.*

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ABSTRACT

In this study an isolate of *Rhizobium leguminosarum* bv. *viciae* was isolated from a root nodule of the host plant (faba bean), growing at the experimental farm of Faculty of Agriculture, Minia University. The isolated *R. leguminosarum* bv. *viciae* was grown in liquid media containing different concentrations of NaCl (0%, 1%, 2%... upto 8%). The root nodule bacterial isolate exhibited high sensitivity to NaCl, since the highest growth value was recorded in the normal medium (without NaCl). A halophilic bacterial isolate was isolated from saline soil sample collected from Kafr El-Sheikh Governorate. The isolated halophilic bacterium exhibited high tolerance to salinity, since the highest growth value was recorded in medium containing 8% NaCl. Plasmid DNA was isolated from both root nodule bacteria and halophilic bacterial isolate and separated on agarose gel electrophoresis. *R. leguminosarum* bv. *viciae* was found to contain one plasmid of about 800-bp which designated pR1. Halophilic bacterial isolate contained two plasmids. These two plasmids were designated pH1 and pH2, their sizes were found to be about 2300-bp and 850 bp, respectively. Transformation was carried out using halophilic bacterial isolate as a donor and *R. leguminosarum* as a recipient. The transformed root nodule bacteria exhibited high tolerance to NaCl. The highest growth value of the transformed bacteria was recorded at 4% NaCl. In a pot experiment, faba bean plants were inoculated with the transformant and irrigated with salinized water (0.5% NaCl). No significant differences were observed in number of nodules, fresh, dry weight/plant and N% in plants

inoculated with the transformant and irrigated with salinized water (0.5% NaCl) as compared to plants irrigated with tap water and inoculated with *R. leguminosarum* which was used as a recipient. Whereas, in plants inoculated with *R. leguminosarum* which was used as a recipient and irrigated with salinized water (0.5% NaCl), significant decrease in the values of the above mentioned parameters was observed as compared to those of plants irrigated with tap water.
Key words: *Rhizobium leguminosarium*, halophilic bacteria, plasmid DNA, transformation.

INTRODUCTION

The symbiotic relationship between root nodule bacteria and legumes is one of the most important processes, since successful nodulation is sufficient for supplying the leguminous plants with their nitrogen requirements during the different growth stages **(**Broughton, 1982**)**. In addition, the symbiotic functions (*i.e.* nodulation and nitrogen fixation) in *Rhizobium* and *Bradyrhizobium* species are encoded by genes located on plasmids (symbiotic plasmids) not on chromosomal DNA (Hammad and Dora, 1994).

Due to shortage of suitable Nile water for agriculture purpose, drainage water may be used as an alternative source for irrigation, leading to accumulation of soluble sodium salts in the soil. The high concentration of salts in the soil does not only affect plant growth, but also inhibits the proliferation and biological activities of the soil microorganisms. Among these microorganisms, the root nodule bacteria which are natively present or introduced as inocula (Zahran, 1991)**.** Salt stress may inhibit the initial steps of the symbiosis (root colonization and nodule development), it also has a depressive effect on nitrogen fixation **(**Zahran, 1999).

Upon the above mentioned information, salinity could affect the denisity and activity of such important nitrogen fixing bacteria (rhizobia) in the soil and hence nodulation of legumes can be affected as well.

Therefore, this investigation was carried out to study the depressive effect of high salinity on root nodule bacteria. Moreover, transformation technique was carried out to transfer plasmid DNA from halophilic bacteria into *R. leguminosarum* bv. *viciae* to obtain transformant tolerant to high salinity to be used as inoculum under saline soil conditions.

MATERIALS AND METHODS

Root nodule bacteria**:** An isolate of root nodule bacteria of *Vicia faba,* was isolated from root nodule of faba bean plants collected from the Experimental Farm of the Faculty of Agriculture, Minia University, Egypt, using congo red yeast extract mannitol (YEM)-agar medium **(**Skinner and Lovelock, 1979) as described by Marques Pinto *et al.*, (1974).

Halophilic bacteria: An isolate of halophilic bacteria was picked from a

saline soil sample collected from Kafr-El-Sheikh Governorate.

For isolation of the halophilic bacteria, serial dilutions of the saline soil suspension were prepared. Drops from each dilution were spread on sodium chloride agar medium plates (containing 5% NaCl) (Irshad *et al*., 2013**)**, and were incubated at 30°C for 24 – 48 h. One single colony was individually transferred onto slant surface of nutrient agar medium (Allen, 1959**)** in test tubes. The slant was incubated at 30°C for 48h. then maintained at 4°C.

A smear from the halophilic bacterial isolate was prepared on a glass slide and Gram stained. The prepared smear was microscopically examined. Cell morphology and Gram reaction of the isolated bacteria were recorded.

Isolation of plasmid DNA**:** Plasmids of both halophilic- and rood nodule bacteria were isolated using the method discribed by Kado and Liu (1981). Plasmid DNA of each isolate was separated by agarose gel electrophoresis. Agarose gel electrophoresis was carried out using the tris-borate EDTA buffer (TBE) as described by Peacock and Dingman (1968).

Transformation of root nodule bacteria: The method described by Kiss and Kalman (1982) was used to transfer plasmids isolated from the halophilic bacteria into root nodule bacteria as below:

Preparation of the competent cells**:** The liquid culture of root nodule bacteria (24-48h. old) was cooled at 4°C and centrifuged at 4000 rpm for 5 min. The pellet was resuspended in 1/2 culture volume of cold $CaCl_2$ buffer (100 mM $CaCl_2$, 10mM tris-Cl) and was kept on ice for 20 min. Cells were precipitated again as mentioned above. The pellet was resuspended in 1/15 culture volume of cold $CaCl_2$ buffer and was kept for overnight at 4°C.

Transfer of Plasmid: Two hundred µl of the prepared suspension of competent cells were mixed with 1 µg of plasmid DNA which isolated from the donor bacteria (halophilic bacterial isolate) and chilled on ice for 40 min, then heated at 38°C for 2 min. The mixture was chilled again on ice for 5 min. One ml of YEM-broth was added to the mixture and incubated at 30°C for 4h. The treated cells were collected by centrifugation, and resuspended gently in 100 µl of YEM-broth and then spread on plates of YEM-agar medium containing 4% NaCl. Plates were incubated at 30°C for 3 days, and examined for the presence of transformed bacterial colonies. The transformants were separately transferred onto slant surfaces of YEM-agar medium in test tubes, then incubated at 30°C for three days and finally maintained at 4°C for further tests.

Stability of bacteria to high salinity:

***In vitro* study**: The root nodule bacterial isolate of *Vicia faba* (as a recipient) and halophilic bacterial isolate (as a donor) as well as the transformant isolate were subjected to saline stress and growth was recorded. Erlenmayer flasks (100 ml) containing YEM-broth (50 ml/flask) in case of

root nodule bacteria and transformant as well as nutrient broth medium **(**Allen, 1959) in case of halophilic bacterial isolate were prepared. The media were salinized with NaCl at concentrations of 0%, 1%, 2% , 3%, 4%, 5%, 6%, 7% and 8%. The prepared flasks were inoculated with root nodule bacteria, transformant or halophilic bacterial isolate by transferring 1ml of 24h-old liquid culture to every flask. Flasks were incubated at 30°C and was estimated at intervals of 24 h upto 120 h as Optical density at wave length of 608 nm (OD_{608nm})

***In vivo* study:** The efficiency of salt-tolerant transformant in nodulating the host plants and fixing nitrogen was tested in a green house experiment.

Preparation of YEM-broth for 4 days at 30°C (giving 1.5-2.8 x 10^9 cell/ml). These liquid cultures were used for inoculation of plants.

Soil used: A clay loam soil which used for cultivation of plants were collected from the 15 cm surface layer of the Experimental Farm of Faculty of Agric. Minia university, Minia-Egypt. The collected soil was autoclaved at 121°C for 1h before placing in plastic bags (2 kg/bag). The bags containing soil were cultivated with faba bean plants (*Vicia faba*). The following inoculation treatments were employed:

a- Inoculation with root nodule bacterial isolate (which used as a recipient)

b- Inoculation with transformed root nodule bacteria

The cultivated seeds in each bag were received 10-ml of the prepared inocula (root nodule bacterial isolate or the transformant) just after sowing. Plants were subjected to irrigation with salinized water containing 0.5% NaCl (w/v).

Sampling and determinations: Plants of 50 days-age were carefully uprooted. Number of root nodules-, plant fresh- and dry weights as well as N% were estimated at each sampling time.

Plant nitrogen content was determined by modified macro-kjeldahl's method (A.O.A.C., 1980). Data were statistically analyzed according to Duncan's multiple range test (Duncan, 1955).

RESULTS

Root nodule bacterial isolate: An isolate of root nodule bacteria of *Vicia faba,* was isolated from root nodules of faba bean plants collected from the Experimental Farm of Faculty of Agriculture, Minia university, Minia, Egypt.

The isolated root nodule bacteria formed circular, elevated, milky and opaque colonies when was grown on YEM-agar medium.

A smear from the isolated root nodule bacteria was prepared, Gram stained and was examined by oil immersion lens of a light microscope. The bacterial isolate was found to be Gram-negative, short and thin rods (Figure 1).

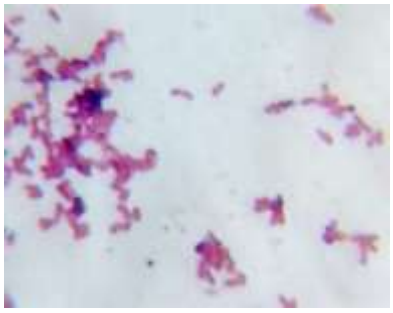

Figure (1): Light micrograph of the isolated root nodule bacteria.

Figure (2): Light micrograph of the **halophilic** bacterial isolate.

Halophilic **bacterial isolate:** An isolate of halophilic bacteria was picked up from a saline soil sample collected from Kafr-El Shikh Governorate. As shown in Figure (2) the light microscope examination indicated that it is Gram positive, long rods and sporeforming bacteria. The cells are arranged in chains.

Growth of root nodule bacteria in salinized medium:

Effect of salinity on the isolated root nodule bacteria of faba bean (*Vicia faba*) was examined *in vitro* in liquid cultures. Eight concentrations of NaCl (*i.e*. 1%, 2%, 3% upto 8%) were used and the OD_{608nm} was taken as a measure for bacterial growth.

Table (1): Effect of different concentrations of NaCl on growth (at OD_{608nm}) of root nodule bacterial isolate of faba bean (*Vicia faba*) after different incubation periods.

Incubation period (hr.)	Concentrations of NaCl (%)								
	0	1	2	3	4	5	6	7	8
24	22.4	17.1	16.6	15.2	8.3	0	0	0	0
48	41.3	25.1	19.8	17.2	10.0	0	0	0	0
72	48.5	33.5	28.2	18.5	13.3	0	0	0	0
96	88.9	55.0	38.1	20.3	15.5	0	0	0	0
120	80.1	71.2	40.2	25.0	18.9	0	0	0	0

The highest growth values

As shown in Table (1), the highest growth of the tested root nodule bacterial isolate was recorded in the normal medium with no added salt (control). Whereas, in the salinized media, the growth was gradually decreased with increasing the salt concentration. Growth of root nodule bacteria of *Vicia faba* was detected in salinized medium upto 4% of NaCl. Whereas, no growth was detected in salinized medium which contained 5% or higher.

Moreover, with increasing the incubation period, gradual increase in the growth of the tested root nodule bacteria was observed in the normal medium (0% NaCl) as well as in

media contained NaCl at concentrations of 1, 2, 3 and 4%. Whereas, the growth values were higher in the normal medium as compared to the values recorded in the salinized media.

Growth of the isolated halophilic bacteria in salinized media:

The halophilic bacterial isolate was grown in midia containing different concentrations of NaCl (*i.e.* 1%, 2% , 3% upto 8%). The growth was measured as OD_{608nm} at different incubation periods at 30°C.

As shown in Table (2), the lowest growth values were recorded in the normal medium with no added salt. The growth values increased with increasing NaCl concentration. The highest growth was achieved in medium containing 8% NaCl. Moreover, at any salt concentration, the growth values increased with increasing the incubation period. *i.e*. the highest growth was achieved after 120 h in saline broth containing 8% NaCl.

Table (2): Effect of different concentrations of NaCl on growth (at OD_{608nm}) of the halophilic bacterial isolate, after different incubation periods.

Incubation period (h)	Concentration of NaCl (%)								
	0	1	2	3	4	5	6	7	8
24	13.1	15.2	17.2	21.2	26.3	31.0	33.3	35.3	36.0
48	21.2	25.5	27.3	30.3	35.o	38.0	41.2	43.3	48.3
72	28.5	30.2	33.3	40.1	48.8	55.5	60.3	69.3	77.8
96	80.2	83.3	84.2	87.0	88.8	89.8	91.0	93.3	94.0
120	85.2	88.6	89.4	89.7	90.0	92.0	93.2	94.6	96.1

The highest growth values

Plasmids of root nodule bacteria and halophilic bacterial isolate:

Plasmid DNA was isolated from root nodule bacterial isolate of *Vicia faba* and halophilic bacterial isolate then separated by 1% agarose gel electrophoresis.

Data presented in Figure (3) and Table (3) indicated that the plasmid DNA isolated from root nodule bacteria of *Vicia faba*, was detected on the agarose gel as one band. This result indicates that the root nodule bacterial isolate contains one plasmid of about 800-bp. This plasmid was designated pR1

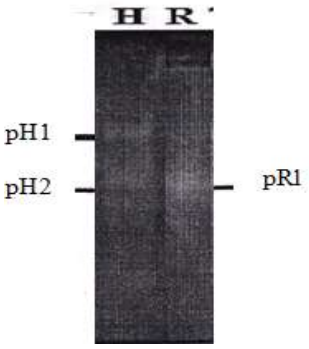

Figure (3): Agarose gel electrophoresis of plasmids pH1 and pH2 isolated from halophilic bacterial isolate (lane H) and pR1 isolated from root nodule bacterial isolate (lane R).

Moreover, as shown in Figure (3) the halophilic bacterial isolate was found to contain two plasmids. These two plasmids were designated pH1 and pH2. The sizes of pH1 and pH2 plasmids were found to be of about 2300 bp and 850 bp, respectively.

Table (3): The detected plasmids in the root nodule bacterial isolate and halophilic bacterial isolate.

Bacterial isolate	Name of plasmid	Size (bp)
R. leguminosarum	pR1	~ 800
Halophilic bacteria	pH1	~ 2300
	pH2	~ 850

Construction of salt tolerant-root nodule bacteria:

Transformation was carried out using halophilic bacterial isolate as a donor and root nodule bacteria as a recipient. Plasmid DNA was isolated from transformant and separated by 1% agarose gel electrophoresis.

As shown in Figure (4), the transformant contained two plasmids: i) pR1, which was previously detected in the root nodule bacteria, and ii) pH1, of about 2300-bp, which was detected in halophilic bacteria.

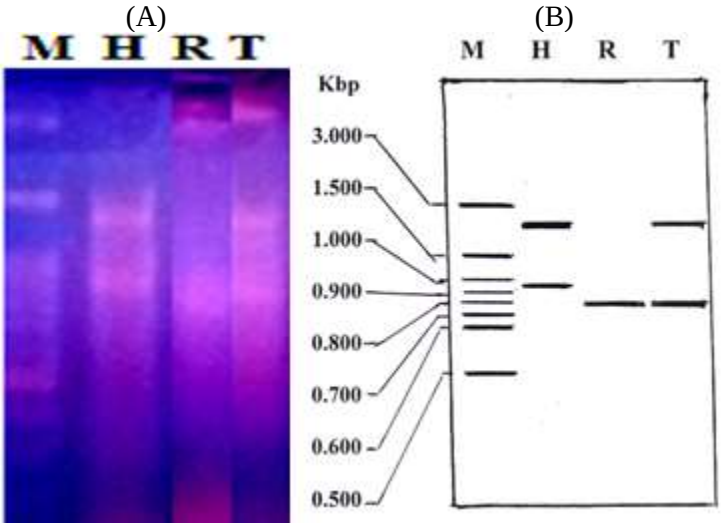

Figure (4): A photograph (A) and a diagram (B) of agarose gel electrophoresis of the detected plasmids in root nodule bacterial isolate of *Vicia faba* (lane R) which was used as a recipient; halophilic bacterial isolate (lane H) which was used as a donor and transformant (lane T). Marker DNA (lane M).

Tolerance of transformant to salinity

a- *in vitro* study: As a result of transferring the plasmid DNA of the halophilic bacteria into the root nodule bacteria of *Vicia faba*, a transformed isolate was obtained. Data presented in Table (4) indicate that the transformant exhibited remarkable growth in presence of the different NaCl concentrations (*i.e*. 1%, 2%, 3% upto 8%). The highest growth of this transformant was recorded in the medium supplemented with 4% NaCl.

Table (4): Effect of different concentrations of NaCl on growth (at OD_{608nm}) of transformed root nodule bacterial isolate of *vicia faba*, after different incubation periods.

Incubation period (h)	Concentrations of NaCl%								
	0	1	2	3	4	5	6	7	8
24	21.2	22.1	24.3	28.1	30.1	30.1	30.1	17.0	15.1
48	44.3	47.8	50.3	55.1	60.1	55.2	35.5	22.1	20.1
72	54.5	59.6	61.1	64.1	68.5	57.5	51.3	39.1	36.4
96	73.3	78.3	84.5	88.4	92.3	88.3	80.1	59.5	51.2
120	81.0	87.5	90.0	93.0	98.0	96.4	94.0	92.5	87.0

The highest growth values

***In vivo* study**

Since, the obtained transformant of the root nodule bacteria of *Vicia faba* exhibited much higher tolerance to salinity than the recipient one, it was of a particular interest to study its efficiency in nodulating its host plants and fixing nitrogen under saline conditions.

Liquid cultures of both root nodule bacteria of *Vicia faba* and the transformant were prepared to be used as inocula for the host plant (*Vicia faba*). The inoculated plants were subjected to irrigation with salinized water (0.5 % NaCl). Control plants inoculated with root nodule bacteria of *Vicia faba* and irrigated with tap water were also involved.

As shown in Table (5), under every treatment, formation of root nodules was observed when plants were 45-days old. Inoculation of *Vicia faba* plants with non-transformed root nodule bacteria (recipient) and irrigation with salinized water (0.5% NaCl) resulted in significant reduction in values of both fresh and dry weight of plants, number of the formed nodules and nitrogen percent as compared to those irrigated with tap water.

On the other hand, no significant differences in the values of the tested measurement were detected in plants inoculated with the salt tolerant transformant and irrigated with saline water (0.5% NaCl) as compared to those inoculated with the non-transformed bacteria and irrigated with tap water.

Table (5): Growth, nodulation and nitrogen content of *Vicia faba* plants inoculated with root nodule bacterial isolate or salt tolerant transformant under saline condition.

Treatments	Days after sowing											
	45				60				75			
	F.W	D.W	No.. nod	N%	F.W	D.W	No.. nod	N%	F.W	D.W	No.. nod	N%
Recipient + 0.5%NaCl	6.10	3.80	5.80	16.62	8.27	5.20	8.00	19.0	10.67	6.20	9.90	40.00
Transforment+ 0.5% NaCl	15.20	8.00	10.50	21.00	17.00	9.20	11.22	25.20	24.63	14.11	13.55	58.80
Recipient (control) + Tap water	15.03	7.80	10.40	20.00	16.97	9.20	10.85	24.90	24.33	14.30	13.50	59.50
L.S.D												
5%	0.898	0.311	0.334	1.16	0.511	0.088	0.230	0.608	0.859	0.237	0.179	1.57
1%	1.37	0.473	0.508	1.76	0.777	0.134	0.350	0.925	1.31	0.360	0.272	2.39

F.W = Fresh weight (gm/plant) D.W = Dry weight (gm/plant)
No. nod = Number of nodules/plant N = Nitrogen percent

DISCUSSION

Rhizobium-legume symbiosis is the most efficient of all biological nitrogen fixing systems. This symbiotic association is highly influenced by various environmental factors. Salinity of the soil is one of the most important factors which may affect persistence and efficiency of the root nodule bacteria.

In this study root nodule bacterial isolate (*Rhizobium leguminosarum*) was isolated from root nodule of faba bean plants (*Vicia faba*). The isolated bacteria exhibited high sensitivity to the saline conditios, hence the highest growth of the root nodule bacterial isolate of *Vicia faba* was recorded in the normal medium with no added salt (control). Whereas, in the salinized media, the growth was gradually decreased with increasing the salt concentration. Similar results were reported by Ferreras *et al*. (2006) who found that high salt concentrations may have a detrimental effect on rhizobial populations as a result of direct toxicity, as well as through osmotic stress. Moreover, Abdel Wahab and Zahran (1979) stated that tolerance of root nodule bacteria to different levels of NaCl, varied from strain to another. In addition, Bolan˜os *et al*. (2003) stated that salt stress (0.45 %) inhibited N_2 fixation and the development of *Pisum sativum* cultivar Argona plants. Furthermore, Brı´gido *et al*. (2012) observed a considerable inhibition of nodulation in *Cicer arietinum* plants grown at 0.15 % NaCl.

In this study a halophilic bacterial isolate was successfully isolated from saline soil sample collected from Kafr El- Sheikh Governorate. The light microscope examination of this isolate indicated that it is Gram positive, long rods and sporeforming bacteria. The cells are arranged in chains. Accordingly, this isolate could be belonging to genus *Bacillus*.

The lowest growth values of the halophilic bacterial isolate was

recorded in the normal medium with no added salt. The growth values increased with increasing NaCl concentration. Such results are in agreement with those obtained by Fawaz *et al.* (1972) who found that virgin saline soil contained high numbers of halophilic bacteria and numbers decreased upon leaching. Moreover, the growth values increased with increasing the incubation period for the halophilic bacterial isolate. Similar results were obtained by Hammad (1983) who stated that number of halophilic bacteria in the soil increased gradually with increasing the accumulated salts during the gradual salinization of the cultivated and uncultivated soils.

As a result of transferring the plasmid DNA of the halophilic bacteria into the root nodule bacteria of *vicia faba*, the resulting transformant was found to contain two plasmids: i) pR1, which was derived from the root nodule bacteria in addition to ii) pH1, which was detected in halophilic bacterial isolate. This transferred plasmid of the halophilic bacteria may carry genes involved in the salt tolerance mechanism.

The obtained transformant exhibited much higher tolerance to NaCl than the recipient (root nodule bacteria of *Vicia faba*) but was not as tolerant as the donor (halophilic bacterial isolate). This may indicate that the salt tolerance can be highly explained by the presence of certain plasmids. Shamseldin (2008) found that all salt-tolerant strains of broad bean rhizobia contained a 250 kbp plasmid with the exception of one strain, suggesting that this plasmid may play a role in the salt tolerance mechanism. Moreover, Domı´nguez-Ferreras *et al.* (2006) obtained evidence that salt stress and hyperosmotic stress have similar effects on gene transcription in *Sinorhizobium meliloti,* causing induction of a large number of genes (mainly on plasmids). On the other hand, Brı´gido *et al.* (2012) reported that no relationship between salt tolerance and the presence of plasmids in *Mesorhizobium.*

The transformant exhibited much higher tolerance to NaCl than the recipient (root nodule bacteria of *Vicia faba*), but was not as tolerant as the donor (halophilic bacterial isolate). This may be due to one of the following hypothesis:

1- Not all the salt tolerance genes are located on the transferred plasmid, but there are some others located on the chromosomal DNA or on the other plasmid (pH2) of the halophilic bacteria which was not transferred to the recipient one.

2- All the salt tolerance genes are located on the transferred plasmid, but the highest efficiency of these genes can be achieved in presence of other genes located on the chromosomal DNA of the halophilic bacteria or located on the other plasmid (pH2) which was not transferred from the halophilic bacteria into the recipient. These results are in agreement with those obtained by Farahat (2016**).**

Since, the obtained transformant of the root nodule bacteria of *Vicia faba* exhibited much higher tolerance to salinity than the recipient one, it was of a particular interest to study its efficiency in nodulating their host plants and in fixing nitrogen under saline conditions.

Liquid cultures of the transformant and the root nodule bacterial isolate of *Vicia faba* (which was used as a recipient) were prepared to be used as inocula for the host plant (*Vicia faba*). The results indicated that values of plant growth, number of nodules/plant and N% in *Vicia faba* plants inoculated with the root nodule bacterial isolate (which was used as a recipient) decreased with increasing the salinity of the irrigation water. Such results may reflect the high sensitivity of the root nodule bacteria of *Vicia faba* to salinity. These results are in agreement with those obtained by Abdel-Rahim (1992); Cordovilla *et al*. (1994); Dora and Hammad (1998); Gama *et al.* (2007); Fathy (2008) and Farahat (2016**).**

On the other hand, no significant differences in the values of the tested measurements were detected in plants inoculated with the salt tolerant transformant and irrigated with saline water (0.5% NaCl) as compared to those inoculated with the non-transformed bacteria and irrigated with tap water.

Along the whole experimental period, number of root nodules in *Vicia faba* plants inoculated with transformant was significantly higher than in plants inoculated with non-transformed bacteria in case of irrigation with salinized water (0.5% NaCl). The high number of nodules resulted in higher values of N% and consequently higher values of plant growth were achieved (expressed as fresh and dry weight of plants) *i.e.* the transformation process did not affect the efficiency of the root nodule bacteria neither in nodulating their host plants nor in fixing nitrogen. Similar results were obtained by Farahat (2016). Moreover, Dora and Hammad (1998) obtained three salt tolerant-*R. leguminosarum* transformants via transfer plasmids from three halophilic bacterial isolates into *R. leguminosarum*. The three transformants exhibited high efficiencies in nodulating the host plant and fixing nitrogen.

Dadarwal and Sen (1974) found that both nodulation and yield increased significantly due to inoculation of leguminous plants under stress conditions with stress tolerant rhizobia. Moreover, EL-Nady and Belal (2005) stated that inoculation with stress tolerant rhizobia may enhance nodulation and nitrogen fixation ability of plants under stress condition.

CONCLUSION

On the basis of the obtained results, it can be concluded that soil salinity is one of the most adverse environmental factors which may affect the survival and efficiency of root nodule bacteria in nodulating the host plants and fixing nitrogen. Transfer of plasmid DNA

from halophilic bacteria into root nodule bacteria resulted in construction of salt tolerance transformant of root nodule bacteria which can be used as inocula in saline soil to improve the efficiency of nodulation and nitrogen fixation under salinity stress.

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نقل بلازميد تحمل الملوحة من البكتيريا المحبة للملوحة الى ريزوبيم الفول البلدى

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في هذه الدراسه تم عزل عزله من بكتيريا العقد الجذريه *Rhizobium leguminosarium* bv.*viciae* من المزرعه البحثيه لكلية الزراعه –جامعة المنيا . تم تنميه هذه العزله علي تركيزات مختلفه من كلوريد الصوديوم (0%و1%و2% حتي تركيز 8%). لقد اظهرت عزلة بكتيريا العقد الجذريه حساسيه عاليه للملوحه واعطت اعلي نمو لها في البيئة العاديه الغير محتويه علي كلوريد الصوديوم .ولقد تم عزل عزله واحده من بكتيريا المحبه للملوحه من عينه تربه ملحية من محافظة كفر الشيخ .واظهرت عزلة البكتيريا المحبه للملوحه تحمل عالي للملوحه حيث كانت اعلي قيمه للنمو لها في البيئه المحتويه علي تركيز 8% كلوريد صوديوم. تم عزل بلازميد DNA من عزلة بكتيريا العقد الجذريه وعزلة البكتيريا المحبه للملوحه وتم عزل وفصل البلازميد بواسطة جهاز التفريد الكهربي على جل اجاوز.وجد ان بكتيريا *Rhizobium leguminosarium* bv.*viciae* تحتوي علي بلازميدة واحدة حجمها 800 زوج من القواعد وتم تسميتها PR1 بينما تحتوي عزلة البكتيريا المحبه للملوحه علي زوج من البلازميدات احجامهم 2300 زوج من القواعد و850 زوج من القواعد تم تسميتهما PH1 ,PH2 على التوالى. تم اجراء تحول وراثي باستخدام بكتيريا المحبه للملوحه (بكتيريا معطيه للبلازميد) وبكتيريا العقد الجذريه (بكتيريا مستقبله للبلازميد). أظهرت بكتيريا العقد الجذريه المتحوله وراثيا تحملا عالي للملوحه واعلي قيمه لنموها عند تركيز4% كلوريد صوديوم. في تجربة اصص تم تلقيح نباتات الفول البلدي بالعزله المتحوله وراثيا وتم ريها بماء مملح بتركيز 0,5% كلوريد صوديوم. لم يلاحظ اي اختلافات واضحه في عدد العقد والوزن الجاف والرطب /للنبات الواحد ونسبة النيتروجين في نباتات الفول البلدي الملقحه بالعزله المتحوله وراثيا والتى تم ريها بماء مملح بتركيز (0,5% كلوريد صوديوم) وذلك بالمقارنه بالنباتات التى تم ريها بماء الصنبور والملقحه ببكتريا *Rhizobium leguminosarium* (البكتيريا المستقبله). بينما النباتات الملقحة *Rhizobium leguminosarium* والمروية بماء مملح بتركيز 0,5% كلوريد الصوديوم اعطت انخفاض ملحوظ في القيم بالنسبه للمعاملة المذكوره اعلاه والمقارنة بتلك النباتات التى تم ريها بماء الصنبور.