FACULTY OF AGRICULTURE

Minia J. of Agric. Res. & Develop.
Vol. (28) No. 5 pp 839-850, 2008

USE OF SALINITY AND HIGH TEMPERATURES TOLERANT TRANSFORMED STRAIN OF BRADYRHIZOBIUM ARACHIS FOR IMPROVEMENT OF PEANUT (ARACHIS HYPOGEA) PLANT

Samia F.M. Ahmed
Microbiol. Depart., Fac. of Agric., Minia Uni. Minia. Egypt

Received 1 Dec. 2008 Accepted 30 Dec. 2008

ABSTRACT

DNA extract from two *Bacillus spp.* isolates (grown under 20% NaC1 and 50°C) was used to transform salt and temperature sensitive strain of *Bradyrhizobium arachis* (specific peanut). Resulted transformants were used to infect Giza3 cultivar of peanut (*Arachis hypogea*). Results showed that the highest percentage of transformation (0.86%) were obtained when the DNA isolated from the second isolate of *Bacillus spp.* was used to transform *Bradyrhizobium arachis.* Inoculation process with transformants improved the nodules formation, fresh weight, dry weight and N-content in hosts, particularly when the transformant *Bradyrhizobium arachis* obtained at 15% NaC1 and 40°C) was used.

INTRODUCTION

The symbiotic relationship between root nodulation bacteria and legumes is one of the most important process, since successful nodulation is sufficient for supplying the leguminous plants with their nitrogen requirements during growth stages (Broughton, 1982). It is well known that, the cheapest source of nitrogen for leguminous plants is that derived from atmosphere via biological fixation with root nodulation bacteria. To achieve successful nodulation, root nodulating

bacteria are commonly used as pre-sowing inocula for seeds of legumes.

For such economically important microorganisms, knowledge of factors influencing the survival, establishment and symbiotic properties of these desired bacteria in the soil are of particular interest. Moreover, due to shortage of suitable Nile water for agricultural purpose, drainage water may be used as an alternative source for irrigation, which may lead to the accumulation of soluble sodium salts in the soil. The high concentration of salts in the soil not only affects plant growth but also inhibit the proliferation and biological activities of the soil microorganisms. Among these microorganisms, the root nodulation bacteria which are present natively or introduced as inocula.

High temperature in the Egyptian soils during the summer season which sometimes reach above 40 °C is one of the major factor affecting biological nitrogen fixation in leguminous plants. Such high temperatures may affect the survival, establishment and symbiotic properties of root nodulation bacteria (Patricia *et al.*, 1998).

The aim of the present work was to obtain highly efficient strains of *Bradyrhizoium arachis*, through transformation using DNA extracted from salt and high temperature tolerant strain of *Bacillus spp*. Growth characteristics of peanut plants after inoculation with these transformants of *Bradyrhizobium* was also examined.

MATERIALS AND METHODS

Bacterial Strains :

a- Two different thermophilic and halophilic isolates of *Bacillus spp* were used as donor parents. The first was isolated at 20% NaC1 from salted fish and the second was isolated at 50°C from the compost of the experimental farm of Faculty of Agriculture, Minia University.

Isolation of temperature and salinity tolerant strain of *Bacillus spp was carried out by taking* 0.1 ml from the suspension culture of the two strains were plated on eight nutrient agar medium plates supplemented with 20% NaC1 and

incubated at 50°C. Single colonies were isolated after 5 days for each strain.

b- *Bradyrhizobium arachis* wild type were isolated from nodules developed on roots peanut (*Arachis hypogea*) planted in Shosha Experimental Farm, Faculty of Agriculture, Minia University, and used as recipients. The isolates were identified according to Bergey's Manual (1984).

Media:

a- Nutrient agar medium was used for *Bacillus* culturing (Allen, 1959).

b- Complete medium (CM) was used for *B. arachis* culturing according to the method of Skinner and Lovelock, (1979).

DNA Isolation and purification:

DNA from the two donor isolates of *Bacillus spp.* was isolated and purified according to Ausubel *et. al.,* (1987).

Transformation Procedure:

According to Kado and Liu (1981) DNA of the donor strains was added to the recipient cultures with a final concentration of 30 mg/ml. Lithium acetate was also added (1 ml 0.3 w/v) for each culture (Ali and Hafez., 1991). The mixture was then incubated at 30°C for 4 hours. Transformants were examined by plating 0.1 ml of the mixture on CM supplemented with 10% or 15%NaCl at 40°C. Three plates without NaCl were used as a control. The WT strain of *B. arachis* as well as the donor strain of *Bacillus* was also plated under the same conditions for comparison.

Pots Experiment:

Surface soil samples (0.0-30 cm) were collected from the Faculty of Minia Agricultural Farm. The soil samples were mixed thoroughly then five samples of this soil were taken randomly for analysis. The physical and chemical properties of soil are given in Table 1. Soil was divided into four piles, salinity treatment was applied using NaCl with the four levels of salt concentration 0.0%, 0.2% 0.4 and 0.6%.

The pots experiment was conducted by growing Peanut (Giza 3) in pots containing the above mentioned soil. The pot size was 7.5cm in

diameter, 10cm in depth and was filled with 0.5kg soil. Soil and pots were sterilized by autoclaving, then three grains were planted in each of the three replicate pots. After 5 days of planting, the soil was inoculated with transformed isolates. Data were recorded after 40 days from sowing.

Table 1: Chemical and physical analysis of soil samples (depth 0-30 cm.) at beginning of the experiments.

Chemical analysis:	
O.M%	**1.910**
PH 1-2.5	**7.730**
E.C.m. mohs/cm	**1.905**
C.E.C. mg/100g.	**31.050**
Soil	**0.130%**
Total N%	
physical analysis:	
Sand	**25.71%**
Silt	**31.85%**
Clay	**41.46%**
Texture grade	**Clay loam**

The experiment was designed as a two-ways complete factorial with two replications in a completely randomized block (Snedecor, 1966). Data were recorded after 40 days from sowing. Plants of each replicate were taken and the contacted soil particles were washed out. Plant height, number of nodules/plant, fresh weight of plants, dry weight of plants, were recorded N-content was measured according to Jackson, 1958. Data were statistically analyzed according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

Using DNA isolated from the two isolates of *Bacillus spp.*, salinity and temperature tolerant transformants of *R. Bradyrhizobium* were isolated by plating on complete medium supplemented with 10% or 15% NaCl at 40°C. Data in Table 2 show that no transformants were obtained at 15% NaCl when DNA isolated from the first strain of

Bacillus spp was used. However, in the presence of 10% NaCl, *Bradyrhizobium arachis* transformants of 0.35% were obtained when the DNA isolated from the same strain of *Bacillus spp* was used for transformation. DNA of the second isolate of *Bacillus* gave the highest numbers of *B. arachis* transformants (0.86%) when plating was conducted at 10% NaCl. Number of transformants obtained at 15% NaCl using DNA of the same isolate (*Bacillus spp.* 2) was 0.21%.

Table 2: Number and percentage of salinity and high temperatures tolerant transformants of *Bradyrizobium arachis* grown at 40°C in the presence of 10 and 15% NaCl.

Donor	Control	Transforants	
		10% NaCl & 40°C	15% NaCl&40°C
Bacillus spp. 1	400.000 (100%)	1400 (0.35%)	200 (0.00%)
Bacillus spp. 2	320.000 (100%)	1800 (0.86%)	700 (0.21%)

These results indicated that, the frequency of transformants depends on the source of DNA used in transformation. The recepient strain also affects the transformation efficiency. This is possibility due to the competence of the recipient cells (Lyer and Ravian, 1962, Ali *et. al.,* 1980 and Nassif, 1992).

Fathy (2008) indicated that the two transformants of *B. Japonicum* and those of *R. meliloti* exhibited remarkable growth in the presence of different NaCl concentrations (10, 20, 30 .....up to 60 gm/L). The highest growth for transforments of *B. Japonicum* (TA and TB) and those of *R. meliloti* was recorded in media supplemented with 60 and 50 gm NaCl/L., respectively.

Moreover the obtained transformants exhibited much higher tolerance to NaCl than the wild type recipients (*B. arachis*) but were not as tolerant as the donors (*Bacillus spp* 2). This might be due to the presence of some salt tolerance and thermal stability genes on the chromsomal DNA. Such genes, if present, might not be easy to transform with those located in plasmids. On the other hand, the salt tolerance and thermal stability genes might be located on the transformed plasmids, but the highest efficiency of these genes can be

achieved in presence of other genes located on the chromosomal DNA of halophilic bacteria which are not present in the recipient. Generally, the salt tolerance degree of the transformants depends on the interaction between the plasmid and the recipient genome.

Data in Table 3 illustrate the effects of inoculation with *Bradyrhizobium arachis* transformants on plant height of peanut plants grown under different salinity levels and high temperature. The results indicated a significant decrease in plant height, fresh weight and dry weight of uninoculated plants after 40 days of sowing. Iinoculation of peanut with the wild type strain of *Brdyrizobium arachis* caused a significant increase in plant height and fresh and dry weight (Table 3) compared with the control plants under different salinity concentrations. The increase after inoculation with transformants was more than those obtained after inoculation with the wild type. Similar results were obtained on pea plants by Abdel Mageed *et al.,* (1996), using Rhizobia transformants.

Table 4 shows the effect of inoculation with some *Bradyrhizobium arachis* transformants under different salinity levels and high temperature on the number of nodules developed on peanut. This character significantly decreased with increasing salinity level. The decreasing percentage of nodules number under salinity stress (6000 ppm. salt) was about 52.8%, compared with the control plants (0.0 p.p.m. salt).

After inoculating peanut plants with transformants of *Rhizobium*, the number of nodules increased compared with plants inoculated with the wild type isolate. *Bradyrhizobium arachis* T2 isolate was the best one that showed the highest number of nodules. These results reveal that the activities of the transformants differed from one to another and might be depend on the time of inoculation (Abdel-Mageed *et al.,* 1996).

Table 4 also shows the effect of inoculation with *Bradyrhizobium arachis* transformants on N-content of peanut plants grown under different levels of salinity and temperature. N-content of plants, calculated as a percentage of dry matter, was unstable as it was fluctuated by changing salinity levels. This instability of N- content

Table 3: Plant height, fresh and dry weight of peanut plants (Giza 3) inoculated with *Bradyrhizobium arachis* WT and two transformants (T1 and T2) after 40 days of growing under different salinity levels and under summer seasons.

Salinity Levels (p.p.m)	Plant height/cm				Fresh Weight/gm				Dry Weight/gm			
	Control	WT	T1	T2	Control	WT	T1	T2	Control	WT	T1	T2
cont.(0.0)	29.00	30.08	30.50	28.93	22.08	26.58	27.70	27.15	2.21	2.82	2.89	2.78
2000	2783	30.30	30.70	30.02	19.85	25.60	28.38	28.30	1.99	2.66	2.75	2.91
4000	26.18	28.05	28.28	28.58	17.00	24.60	24.88	26.15	1.69	2.36	2.59	2.49
6000	20.50	23.03	24.73	21.15	12.25	25.50	25.40	24.35	1.59	2.57	2.65	2.51
Mean	25.88	27.86	28.22	27.17	17.79	25.43	26.59	26.48	1.87	2.60	2.76	2.67
	L.S.D.(5%)				L.S.D.(5%)				L.S.D.(5%)			
	A=0.163 B=1.194 Ab=2.390				A=1.883 B=0.971 Ab=1.940				A=0.1777 B=0.130 Ab=0.260			

Table 4: Number of nodules per plants and N-contents of peanut plants inoculated with *Bradyrhizobium arachis* WT and the two isolated transformants (T1 and T2) after 40 days of growing under different salinity levels and under summer seasons.

Nodules Number/plants				N-contents			
Control	WT	T1	T2	Control	WT	T1	T2
0.00	7.75	9.50	14.00	1.50	3.77	4.36	4.65
0.00	5.75	8.25	9.750	1.42	4.11	4.50	4.72
0.00	5.75	7.50	8.750	1.41	3.09	3.49	4.49
0.00	4.00	4.25	6.250	1.06	2.75	3.89	4.06
0.00	5.81	7.74	9.440	1.35	3.43	4.17	4.48
L.S.D.(5%)				L.S.D.(5%)			
A=1.033 B=0.987 Ab=1.973				A=0.177 B=0.130 Ab=0.260			

Where A: Temperature , B: Salinity level

with salinization may be related to the inoculation process especially inoculation with *Rhizobium* transformants, which had the ability to improve host characters against salinity effect.

Inoculating peanut plants with *Bradyrhizobium* transformants significantly increased N-content of these plants compared with the control plants. The best transformants which showed the highest N-content was *Bradyhizobium arachis* T2 in peanut plants.

Selim and Badwi (1980) found that in alfafa and Sudan grass, N-content was unaffected by salinity. On the other hand, several investigators (Paliwal and Maliwaul, 1972 and Papadopoulos and Rending, 1983) found that N-content in plant decreased with increasing salinity level in soil or irrigation water.

Dora and Hammad (1998) obtained three salt tolerant- *R. leguminosarum* transformnts via transfer plasmids from three halophilic bacterial isolates into *R. leguminosarum*. The three transformants exhibited high efficiency in nodulating the host plant of faba bean and in fixing nitrogen.

Fathy (2008) found that transferring plasmids from halophilc bacterial isolates to root nodle bacteria led to transfer the salt tolerants feature from halophilc isolates which had been used as donors to the root nodule recipients, without alteration of their efficiencies on nodulating their host plants and nitrogen fixation. Such transformants can be used as inocula for leguminous plants grown under salinity conditions to promote nodulation and nitrogen fixation also to minimize the adverse effect of salinity on the grown plants.

Improving nodulation and growth characteristics of peanut plant according inoculated with *Bradyrhizobium transformants* might suggest using this technology for obtaining highly efficient strains. Using such strains will improve plant growth specially when growing under salinity conditions as well as high temperature stress.

REFERENCES

Abdel-Mageed, Y. T, O. F. Dakhly and Samia, F.M. Ahmed (1996): Effect of some *Rhizobia* transformants and water regime on symbiotic properties, growth and yield of pea plants. Fourth Arabic Conf., Minia, Egypt, 303-315.

Ali, A. M. M., and F. M. Hafez (1991): Comparatives studies for the efficiency of different direct transformation methods using different *Saccharomyces cervisiae* genotypes as recipients. Eggypt. J. Appl Sci, 6:28-33

Ali, A. M. M., S. M. Abdel-Wahab, M. H. Hamouda, and K. A. Ahmed (1980): Transformation and conjugation analysis in *R. trifolii.* Egypt. J. Microbiol., 15: 129-142

Allen, O. N. (1959). Experimentation in soil Bacteriology, Burgess publishing Co. Manneopolis, U.S.A.

Ausubel, F., R. Brent, R Kingston, D. Moore, J. Seidman, and K. Struhl (1987): Current Protocols In Molecular Biology. Johan Wiley & Sons, New York.

Bergey's Manual of Systematic Bacteriology, (1984): Volume I, Kreig, N. R. (ed.) William & Wilkins, Baltimore, USA.

Broughton, W. J. (1982): Nitrogen Fixation. Volume 2. *Rhizobium.* Clarendon press. Oxoford. Pp. 1-34.

Dora, S. A. and Hammad, A. M. M .(1998): Transfer of salt-tolerance encoded genes of halophilic bacteria to *Rhizobium leguminosarum* via plasmid transfer technique.Egypt. J. Microbial. (33) 4: 677-691.

Fathy, Shimaa, H, (2008): Studies n some factors affecting certain root nodule bacteria. PhD Thesis, Dept. Agric. Microbiology, Fac. Agric. Minia Univ. Egypt.

Jackson, M. L. (1958): Soil chemical analysis. Prentice-Hall Inc., London pp. 134-182.

Kado, C.I-and Liu,s.T-(1981). Rapid procedure for detection and isolation of Large and small plasmids. J. Bacteriol.,145:1365-1362

Lyer, V. N., and A. W. Ravian (1962): Integration and expression of different lengths of DNA during the transformantion of Ipneumococcus of *Erythromycin* resistance. Genertics, 47: 1355-1367.

Nassif, F. A., S.M. (1992): Symbiotic properties of two Rhizobia species transformed with fungi resistance transformed with fungi resistance genes. Egypt, J. Genet. Cytol., 21(2): 115-121.

Paliwal, K. V. and G. L. Maliwaul (1972): Effects of salts on the growth and chemical composition of Okra *(Abelmoschus esculentus)* and sponge gourd *(Luffa cylindria).* Soil and Fert., 56:1095.

Papadopoulos, I., and V.V. Rending (1983): Interactive effects of salinity and nitrogen on growth and yiels of tomato plants. Plant and soil, 73: 47-47.

Patricia P. P.; Ruy R.; Andrea M. M; Lucy S.; Edilson P. Nadja M. H. S. (1998): Effects of high temperature on Survival, Symbiotic performance and genomic modifications of bean nodulating Rhizobium Strains. Rev. Microbiol. 29 (4) : 115.143.

Selim, M. H., H., and A. M. Badwi (1980): A comparative and economical study of the behaviore of both alfalfa and Sudan grass on salt affected soils. Agric. Res. Rev., 58.13

Skinner, F. A . and Lovelock, D . W. (1979). Identification methods for microbiologistis. 2nd edition. Acadimic prss London.

Snedecor, G. W. (1966): Statistical Methods. Lowa State College, press, Ames, lowa, USA, 5th. Ed. Pp.534

Steel, R. G. D. and Torrie, J. H. (1980): Principles and Procedures of statistics. A Biometrical Approach. Second Edit., Mc Grow-Hill Co., New York.

استخدام سلالة محولة وراثيا من الريزوبيم أريشص المتحملة للملوحة و الحرارة العالية تحسين صفات النمو لنبات الفول السوداني

سامية فرحات محمد أحمد

قسم الميكروبيولوجيا الزراعية – كلية الزراعة – جامعة المنيا – مصر.

فى هذه الدراسة تم عزل المادة الوراثية (DNA) من سلالتين من بكتريا الباسلس المتحملة للحرارة والملوحه أحدهما من الأسماك المملحة والأخرى من الكومبست. استخدم الـ DNA المعزول في إجراء التحول الوراثى لبكتريا العقد الجذرية المتخصصة على الفول السودانى وفول المنج. وتم عزل المستعمرات المحولة وراثياً بالتنمية على درجة حرارة ٤٠°م وفى وجود تركيزات عالية من ملح كلوريد الصوديوم (١٠ ، ١٥%) . وتم أستخدام المتحولات الوراثية الناتجة في تلقيح نباتات الفول السودانى تحت مستويات ملوحة مختلفة . أظهرت النتائج قدرة هذه المتحولات الوراثية على تحسين بعض صفات النمو مثل طول النباتات والوزن الطازج والوزن الجاف وكذلك المحتوى الكلى من النيتروجين هذا بالاضافة لعدد العقد الجذرية المتكونة على الجذور بالمقارنة بالنباتات الغير ملقحة وتلك الملقحة بالطراز البرى من بكتريا الريزوبيم أريشص. ويمكن الاستفادة من مثل هذه السلالة المحولة وراثياً للمساعدة في تثبيت النيتروجين الجوى تحت المستويات العالية من الملوحة في التربة وكذلك في أشهر الصيف الحارة وخاصة في صعيد مصر.