Middle East Journal of Agriculture Research
ISSN 2077-4605

Volume : 07 | Issue : 02 | April-June | 2018
Pages:618-624

Effect of Plant Growth Promoting Rhizobacteria (PGPR) on growth and leaf chemical composition of date palm plants *cv.* Bartamuda under salinity stress

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Received: 11 Mar. 2018 / Accepted: 07 June 2018 / Publication date: 25 June 2018

ABSTRACT

This investigation was carried out during the two successive seasons (six months for each season) in open field at the nursery of Central Laboratory for Research and Development of Date Palm, Agriculture Research Centre (ARC), Giza on 4 years old date palm plants cv. Bartamuda (produced by tissue culture). The main purpose of this study was to use plant growth promoting rhizobacteria (PGPR) to increase the plant resistance against salinity stress. Four bacterial strains were used; *Azotobacter chrococcum* with *Azospirillum brasilense*, *Bacillus megaterium* and *Bacillus circulans* at rate 1:1:1, 1:1/2:1/2 and 1:1/4:1/4 (v/v), respectively under salinity stress (15000 ppm NaCl). In general, all PGPR treatments increased number of bacteria in the used soil as compared to the control treatment, the highest values were obtained from the soil which received bio fertilizer at rate 1:1/2:1/2 (v/v) in both seasons. Number of leaves/plant, leaves length, trunk length and trunk diameter significantly increased in most cases as a result of treatment with the various rate of bio fertilizer than that of the control, with the superiority of bio fertilizer at rate 1:1/4:1/4 (v/v) which recorded the highest values in this respect. Chlorophyll a, b and carotene were significant increased at different rates of bio fertilizer; the highest values were recorded in the leaves of plants which treated with bio fertilizer at rate 1:1/2:1/2 (v/v) as compared with the other tested rates and the control treatment. This was true in both seasons. Moreover the application of bio fertilizer at rate 1:1/4:1/4 (v/v) induced significant increases in the Leaf nutrient elements content (N, P, K, Fe, Mn, Zn, and Cu over control treatment. So, it can be recommended to use plant growth promoting rhizobacteria (PGPR) as a source of nitrogen (*Azotobacter chrococcum* and *Azospirillum brasilense*), phosphorus (*Bacillus megaterium*) and potassium (*Bacillus circulans*) at rate 1:1/4:1/4 (v/v) to improve the vegetative growth, increase chemical compositions in leaves and improved nutrients uptake of date palm plants grown under saline stress conditions.

Key words: PGPR, Date palm, Bio fertilizer, Salinity

Introduction

Date palm (*Phoenix dactylifera.* L.) belongs to Areaceaceae family, which contains about 21 genera and 4000 or more species. The members of this family are essentially found in the tropical and subtropical regions. Soil salinity is a major biotic environmental stress prevalent throughout the Arabian Peninsula and an important source of income and nutrition in a number of countries, it is considered a salt tolerant plant species (Al- Mansoori *et al.,* 2007). Moreover, Date palm are able to grow under divers biotic stress conditions including saline soils (Yaish *et al.,* 2016). Despite the fact that date palm is a relatively salt tolerant plant, nevertheless suffers from high levels of salt in soil (Yaish and Kumar, 2015). Marasco *et al*. (2012) reported that plant growth promoting rhizobacteria (PGPR) naturally associated with plants, have been shown to be essential partners for improving plant tolerance to stressful conditions. Cherif *et al*. (2015) and Yaish (2016) found that endophytic bacteria species cultured from date palm roots had positive effects on plants growing under saline and /or drought conditions. These organisms may facilitate plant growth in a variety of ways including improving the availability of some nutrients such as nitrogen, phosphorus, potassium, iron and calcium or modulating plant hormone levels, providing plants with phytohormones such as auxins,

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cytokinins or gibberellins or by lowering plant ethylene levels (Ryan *et al.,* 2008 and Glick, 2012). Root-associated bacterial (rhizosphere) communities in date palm have previously been studied under saline conditions (Ferjani *et al.,* 2015). In this work; several strains belonging to *Enterobacteria* and *Bacillius* genera were cultured and tested for their growth promoting capacity on the growth parameters and chemical content in leaves of date palm plant grown under salinity stress.

Materials and Methods

The main purpose of this study was to evaluate the ability of plant growth promoting rhizobacteria (PGPR) to reduce of salinity stress or to increase the plant resistance against salinity. This study was consummated in open field at the nursery of Central Laboratory for Research and Development of Date Palm, Agriculture Research Centre (ARC), Giza, in two successive seasons 2016-2017(six months for each) on 4years old date palm plants cv. Bartamuda (produced by tissue culture) with average 46.0-50.0cm in height, 5.0 leaves/plant, and 3cm for trunk diameter, planted in 50cm diameter plastic bag (one plant/bag) filled with 35.0kg of soil mixture (sand and clay 2:1 v/v).Some physical and chemical properties of the used soil were determined according to the standard methods described by Jackson (1973) and illustrated in Table (1).

Four active strains of diaztrophs via, *Azotobacter chrococcum* and *Azospirillum brasilense* as a source for nitrogen, *Bacillus megaterium* as a source for phosphorus and *Bacillus circulans* as a source for potassium were obtained from Biofertilizers production unit, Agricultural Microbiology, Dept., Soils, water and Environmental Research Institute (SWERI), Agric. Res. Center (ARC), Giza Egypt. The enrichment culture technique was adapted by using the nitrogen deficient semi solid malate medium (Dobereiner *et al*., 1976), modified Ashby,s medium (Abdel- Malek and Ishac,1968) for *Azotobacter chroococcum* Dobereiner semi solid agar medium (Dobereiner, 1976) for *Azospirillum brasilense,* modified *Bunt and* Rovira medium (Abdel-hafez,1966) for *Bacillus megaterium* and modified Aleks and Rovs medium (Zahra, 1969) for *Bacillus circulans*. These strains of bacterial; *Azotobacter chrococcum* with *Azospirillum brasilense*; *Bacillu smegaterium* and *Bacillus circulans* (PGPR) were applied at three rates; 1:1:1, 1:1/2:1/2 and 1:1/4:1/4 (v/v), respectively in addition to control treatment under salinity stress (15000 ppm NaCl) as follow:

1. Control under saline stress conditions (15000 ppm NaCl).
2. 15000 ppm NaCl + liquid culture of PGPR at rate 1:1:1 (v/v).
3. 15000 ppm NaCl + liquid culture of PGPR at rate 1:1/2:1/2 (v/v).
4. 15000 ppm NaCl + liquid culture of PGPR at rate 1:1/4:1/4 (v/v).

The PGPR treatments were applied after 30, 60 and 120 days and mixture of PGPR was applied as a soil drench at the rate of 10L fed^{-1} (= 100 ml/L for each plant) All experiment plants were received 2 g/l NPK and irrigated with 8 liters of saline water (15000 ppm NaCl)/bag one time for two weeks, three replicates were used for this experiment and three plants for each replicate. At the end of each season (after six months from treatments, September, $30^{th}$the following data were recorded:

1. Total count of *Azotobacter chrococcum*, *Azospirillum brasilense*, *Bacillus megaterium* and *Bacillus circulans* in the used soil (Page *et al.,* 1982).
2. Vegetative growth; number of leaves/plant, leaf length (cm), length and diameter of trunk (cm).
3. Photosynthetic pigments; chlorophyll a, b and carotene content of leaves as mg/gm Fw were determined according to Moran (1982).
4. Leaf nutrient elements content: nitrogen content in dry leaves was determined using micro-kjeldahel method described by Jakson (1973), while phosphorus calorimetrically as recommended by Hucker and Catroux (1980), potassium using flame photometer as explained by Cottenie *et al.* (1982). Zn, Fe, Cu and Mn were determined using operation chart of Shimactzu atomic absorption.

Randomized complete block design was used and data were subjected to analysis of variance, separation of means among treatments was determined using L.S.D. test at 5% according to Steel and Torrie (1980).

Table 1: Some physical and chemical properties of the used soil

Analysis	Value
Particle size (%)	
Coarse sand (%)	12.17
Fine sand (%)	37.81
Silt (%)	39.77
Clay (%)	10.25
Soil texture (%)	Silty clay soil
Physical properties	
S.P. (%)	37.88
E.C ($ds^{-1}m$)	2.79
PH	7.8
O.M. (%)	0.9
O.C. (%)	0.52
T.N. (%)	0.18
Chemical properties	
Soluble cations ($meql^{-1}$)	
Ca^{++}	13.65
Mg^{++}	4.55
Na^{+}	20.17
K^{+}	2.04
Soluble anions ($meql^{-1}$)	
CO_3^{--}	----
HCO_3^{-}	2.83
Cl^{-}	14.7
SO_4^{--}	22.88

Results and Discussion

Microbial status:

Results presented in Table (2) showed that, all PGPR treatments increased number of bacteria in soil as compared to the control treatment, the highest values were obtained in the soil which received bio fertilizer at rate 1:1/2:1/2 (v/v) in both season, and recorded 210 ,310 $x10^6$ & 95,120 $x10^6$ & 19 ,32 x 10^5 and 12 ,15 $x10^4$ cfu/ml, whereas control treatment recorded few values 3, 11 x 10^6 & 2, 9 $x10^6$ & 8 , 12 $x10^5$ and 6, 9 x 10^4 cfu /ml for *Azotobacter chrococcum* , *Azospirillum brasilense , Bacillus megaterium and Bacillus circulans* at the end of the first and second season, respectively. This can be explained by the ability of growth promoting **(**PGPR**)** in various rates to increase microbial counts in soil.

Table 2: Effect of PGPR at various rates on total count of microorganisms in the used soil for planted of date palm plants cv Bartamuda under saline conditions at first and second season.

Parameter	Total count of *Azotobacter* ($x10^6$ cfu/ml)		Total count of *Azospirillium* ($x10^6$ cfu/ml)		Total count of *B.megaterium* ($x10^5$ cfu/ml)		Total count of *B.circulans* ($x10^4$cfu/ml)	
Treatments	First season	Second season	First season	Second season	First season	Second season	First season	Second season
Control	3	11	2	9	8	12	6	9
PGPR at rate 1:1:1	170	230	60	80	17	21	9	11
PGPR at rate 1:1/2:1/2	210	310	95	120	19	32	12	15
PGPR at rate 1:1/4:1/4	80	110	75	95	13	28	8	10

These data are in agreement with those of Pondy *et al*. (1998); Abo Taleb *et al*. (2002) and Mahmoud *et al*. (2006) who reported that inoculation with diazotrophic bacteria had an activation effect on the population of rhizospheric microorganisms and increased their numbers by more than 50% at the end of the experiment compared with the numbers recorded before planting. Ferjani *et al*. (2015) and Yaish *et al.* (2016) reported a positive relationship was found between inoculation with PGPRs and date palm root system as well as rhizospheric microorganisms.

Vegetative growth:

Data presented in Table (3) indicated that in both seasons all bio fertilizers (PGPR) treatments employed in this study significantly increased the number of leaves per plant as compared with the control, Bartamuda plants which received PGPR at rate 1:1/4:1/4 (v/v) recorded highest value (6.67and 9.00 leaves/plant) as compared to plants of the control treatment which recorded lowest value (4.67 and 6.67 leaves/plant in the first and second season, respectively). Data also show the differences between treatments PGPR at rate 1:1:1 and 1:1/2:1/2 in the first season and between PGPR at rate 1:1/2:1/2 and 1:1/4:1/4 in the second season did not reach the level of significance.

In regard to leaves length the obtained data in Table (3) in both seasons show significant differences in leaves length between PGPR treatments at various rate. Application of PGPR at 1:1/4:1/4 had a marked significant increasing of leaves length of Bartamuda plants (93.23 and 100.46 cm) as compared to control plants which recorded the shortest leaf length (64.82 and 74.14 cm in the first and second season, respectively).

Table 3: Effect of PGPR at various rates on number of leaves, leaves length, trunk length, and diameter of date palm plants cv Bartamuda under salinity stress at first and second season.

Treatments	No. of leaves/plant)		Leaf length (cm)		Trunk length (cm)		Trunk diameter (cm)	
	First season	Second season	First season	Second season	First season	Second season	First season	Second season
Control	4.67	6.67	64.82	74.14	11.17	12.03	3.49	3.63
PGPR at rate 1:1:1	5.67	7.67	80.47	88.26	12.05	12.42	3.77	4.08
PGPR at rate 1:1/2:1/2	6.00	9.00	89.48	95.64	11.47	12.55	4.37	4.50
PGPR at rate 1:1/4:1/4	6.67	9.00	93.23	100.46	13.47	14.03	4.64	4.92
L.S.D at 0.05	0.640	0.551	0.364	0.432	0.499	0.350	0.254	0.139

As for trunk length and trunk diameter data of Table (3) reveal the same trends as observed in leaves length, i.e. treatment of PGPR at 1:1/4:1/4 significantly increased both trunk length and diameter (13.47,4.03 and 14.03 and 4.92 cm, respectively in the first seasons) as compared with the control treatment (11.84 and 3.55cm, respectively in the second one). No significant differences were detected in trunk length between, the control and treatment PGPR at rate 1:1/2:1/2 in the first season and between PGPR at rate 1:1:1 and 1:1/2:1/2 in the second season. These may indicate that application of bio fertilizer, *Azotobacter chrococcum* and *Azospirillum brasilense* (as a source for nitrogen) and decreasing both *Bacillus megaterium* (as a source of P) and *Bacillus circulans* (as a source of K) had a appositive effect on growth of plants it may be stimulation growth as well as increase the plant resistance against salinity. These data are in agreement with Sperling *et al.* (2014); Ferjani *et al*. (2015); Yaish and Kumar (2015) and Cherief *et al*. (2015) who reported that , numerous plant growth promoting rhizobacteria (PGPR) and/ or bio fertilizers had appositive effects on date palm plants that were growing under both saline and drought conditions. Ali *et al*. (2014) added that, inoculation of PGPRs endophytic bacteria promotes salinity tolerance and increases the productivity of various plant species.

Chemical composition:

Chlorophyll and carotene contents:

Results presented in Table (4) showed the effects of PGPR at various rates on leaf content of chlorophyll a, b and carotene in Baratmuda plants. Data indicated that Chlorophyll a, b and carotene were significant increased at different rates of bio fertilizer; the highest values of chlorophyll a (0.80 and 0.84 mg/g fw), chlorophyll b (0.98 and 0.99 mg/g fw) and carotene (0.64 and 0.67 mg/g fw in both seasons, respectively) were recorded in the leaves of plants which treated with bio fertilizer at rate 1:1/2:1/2 (v/v) as compared with the other tested rates. This was true in both seasons. On the other hand, the control treatment recorded the lowest values of chlorophyll a (0.33and 0.34 mg/g fw), chlorophyll b (0.16 and 0.19 mg/g fw) and carotene (0.18 and 0.19 mg/g fw) for both seasons respectively. These results are in harmony with Zelicourt *et al.* (2013) and Sperling *et al.* (2014) who reported that, rhizospheric microorganisms as essential partners for plant stress tolerance as well as enhance plant growth and may had an indirect effect on physiological processes.

Table 4: Effect of PGPR at various rates on chlorophyll a, b and carotene content in leaves of date palm plants c.v. Bartamuda under salinity stress at first and second season..

Treatments	Chlorophyll a (mg/g f.w)		Chlorophyll b (mg/g f.w)		Carotene (mg/g f.w)	
	First season	Second season	First season	Second season	First season	Second season
Control	0.33	0.34	0.16	0.19	0.18	0.19
PGPR at rate 1:1:1	0.61	0.62	0.31	0.36	0.33	0.37
PGPR at rate 1:1/2:1/2	0.80	0.84	0.98	0.99	0.64	0.67
PGPR at rate 1:1/4:1/4	0.36	0.39	0.53	0.54	0.35	0.37
L.S.D at 0.05	0.025	0.018	0.031	0.036	0.040	0.044

Nutrient elements content:

According to data in Table (5), it is evident that application of bio fertilizers induced significant increases in the leaf content of nitrogen (N) over control treatment. The highest value (2.95%) resulted by bio fertilizers at rate 1:1/4:1/4 as compared with the other tested rates and control, whereas the lowest values of nitrogen content (2.22 and 2.44%) were obtained by application of PGPR at 1:1:1and 1:1/2:1/2, respectively as compared to control which recorded 2.63%.

As for Phosphorus (P) content data of Table (5) reveal the same trends as observed on nitrogen content, i.e. there are a positive effect of different rates of bio fertilizers, the highest value of Phosphorus content in Baratmuda leaves (0.210%) was obtained by treatment of PGPR at 1:1/4:1/4 followed by PGPR at1:1/2:1/2 (0.137%), while PGPR at rate 1:1:1 was the lowest which gave the same value as the control (0.043%).

In regard to potassium (K) content the obtained data in the same Table (5) show that PGPR at1:1/2:1/2 and 1:1/4:1/4 significantly increased the leaf content of potassium (1.22% for each) as compared with PGPR treatment at 1:1:1 (0.98) and control which gave the lowest potassium content (072%).

Regarding Fe, Mn, Zn and Cu content data of Table (5) reveal a nearly similar trend as discussed for K content, PGPR treatment at1:1/4:1/4 was the most effective in increasing Fe, Mn , Zn and Cu leaf content (244.0, 87.67, 17.00 and 4.00) as compared with control treatment (163.0,53.00,9.00and 2.00 ppm, respectively). No significant differences were detected in Mn content between, PGPR treatment at 1:1/2:1/2 and 1:1/4:1/4 and between PGPR treatments at 1:1/2:1/2 and 1:1:1 in Zn leaf content, it was also found between PGPR treatments at 1:1/4:1/4 and 1:1:1 and between PGPR treatments at 1:1/2:1/2 and control. Worth to mention that leaf mineral content increased with decrease *Bacillus megaterium and Bacillus circulans*.

This may indicate the synergistic effect of PGPRs that had positive effects on growth parameter, improved nutrients uptake, increase chemical compositions and increase the plant resistance against salinity stress. Generally, these results were in harmony with Zelicourt *et al.* (2013); Sperling *et al*. (2014); Ferjani *et al*. (2015) and Yaish *et al.* (2016) who reported that date palms were able to grow under divers biotic stress conditions where microbial communities may help in plants salinity tolerance. Moreover, using PGPR able to produce specific growth promoting substances can enhanced date palm growth under saline stress conditions (Cherif *et al*., 2015 and Yaish, 2016) In addition Marasco *et al.* (2012) reported that plant growth promoting rhizobacteria (PGPR) naturally associated with plants, have been shown to be essential partners for improving plant tolerance to stressful conditions; Cherif *et al*. (2015) and Yaish (2016) found that endophytic bacteria species cultured from date palm roots had positive effects on plants growing under saline and /or drought conditions.

Table 5: Effect of PGPR at various rates on nutrient elements content in leaves of date palm plants cv Bartamuda.

Treatments	N	P	K	Fe	Mn	Zn	Cu
	%			ppm			
Control	2.63	0.043	0.72	163	53.00	9.00	2
PGPR at rate 1:1:1	2.22	0.043	0.98	188	63.00	10.00	4
PGPR at rate1 :1/2:1/2	2.44	0.137	1.22	227	88.00	9.67	2
PGPR at rate 1:1/4:1/4	2.95	0.210	1.22	244	87.67	17.00	4
L.S.D.at 0.05	0.080	0.018	0.082	1.312	0.929	0.512	0.080

Conclusion

In conclusion, could be recommended to use plant growth promoting PGPR (*Azotobacter chrococcum* and *Azospirillum brasilense* as a source for nitrogen, *Bacillus megaterium* as a source for phosphorus and *Bacillus circulans* as a source for potassium **)** at rate 1:1/4 :1/4 (v/v) to improve the vegetative growth, increase chemical compositions in leaves and improved nutrients uptake of date palm plants grown under saline stress conditions .

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