Egypt. J. Bot., **Vol. 56**, No, **3**, pp. 925-943 (2016)

Effectiveness of the Isolated Rhizobacteria From the Fields on Growth Promotion and Antioxidant Capacity of Maize Plant

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THE REPLACEMENT of chemical fertilizers by rhizobacteria (PGPR) for promoting maize plant growth and productivity was in the aim of the present study. In the current study, rhizobacterial strains were isolated from the rhizosphere of *Trifolium alexandrina* L. and *Trifolium aestevum* L. plants cultivated in Nubaria, Egypt., The isolated bacteria were purified and were then identified as *Bacillus subtilis*, *P. pseudoalcaligenes*, *Kocuria marina*, *Bacillus cereus*, *Kocuria rhizophila*, *Bacilus subtilis* subsp. *qingdao*, *Pseudomonas putida*, *Bacillus licheniformis*, *Bacillus thuringiensis*, *Bacillus subtilis* subsp. *Spizizenii*. The rhizobacterial strains were then applied at 6 different combinations, for evaluation of their effects on maize seedling growth. All bacterial combinations increased the total fresh weight of the plant shoot while only combinations 1, 3, 4 and 6 increased that of root. The results obtained showed that combination 6 was the best followed by combination 4 in inducing the extension growth and weight of shoots and roots of maize seedlings (14-day-old). Both combinations included two *Bacillus* spp. two *Pseudomonus* spp. and one *Kocuria* sp. The photosynthetic pigment efficiency showed remarkable increases by four bacterial combinations (1, 3, 4 and 6). The protein to carbohydrate ratio showed appreciable increases by those bacterial combinations. Proline content in maize shoot increased by 5 bacterial combinations (1, 3, 4, 5, and 6) with a maximum increase by combination 6. The shoot antioxidant capacity increased by treatment 3 but that of root was induced by all the bacterial combinations. Protein profiles indicated marked a variation in the number of the newly formed bands, in response to the applied bacterial combinations.

Keywords: Maize Rhizobacteria (PGPR), Photosynthetic pigments, Carbohydrates, Protein, Proline, Antioxidant capacity, Protein profiles.

Plant Growth Promoting Rhizobacteria (PGPR) are a group of bacteria that enhances plant growth and yield via production of various plant growth promoting substances and could play a role as bio-fertilizers (Karlidag *et al*., 2013). Bacterial bio-fertilizers are proposed to designate the biological products which contain microorganisms providing direct and indirect enhancement in yield of crops.

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Vessey (2003) defines a bio-fertilizer as a substance that contains living microorganisms and when applied to seeds, plant surfaces, or soil colonizes the rhizosphere or the interior of the plant. This promotes growth by increasing the supply or availability of primary nutrients for the host plant. Rhizobacteria, associated with rhizosphere, can fix nitrogen, solubilize phosphorus and be used as an inoculum in non-leguminous species such as maize, rice, wheat, and sugar cane (Dobereiner, 1997). Bio-fertilizers have been used as an alternative to mineral fertilizers to increase the yield and plant growth in sustainable agriculture (Canbolat *et al*., 2006).

In addition, PGPR enhance plant adaptation to harsh environments including drought or heavy rainfalls in tropical countries, salt stress, high temperatures and contaminated environments, indicating that they can contribute to ameliorate such stresses on crops of areas with poor agricultural potential (Arzanesh *et al*., 2011; Saharan and Nehra, 2011; Shrivastava and Kumar, 2015).

Drought caused a great reduction in productivity and yield of crops, although some crops have characteristics of reducing water loss rates, and maintenance of higher physiological activity (Zhu *et al*., 2012). Inoculation of plants with native beneficial microorganisms may increase drought tolerance of plants growing in arid or semi-arid areas (Marulanda *et al*., 2007). The PGPR containing deaminase are present in various soils and offer a promise as a bacterial inoculum for improvement of plant growth, particularly under unfavorable environmental conditions such as flooding or drought, heavy metals, phyto-pathogens and high salt (Kohler, 2008).

Maize is a major summer field crop in Egypt. It is planted on approximately 728,000 hectares of land. Each year, 6.1 million tons of maize grains are produced domestically. Moreover, 4.1 million tons of yellow maize grains are imported annually, valued at $US 1.3billion (Ezezika and Daar, 2012). Maize kernels are technically fruits but are used in cooking as a vegetable or starch. Sugar-rich varieties called sweet corn are usually grown for fresh consumption, while field-corn varieties are used for animal feed and as chemical feedstocks (Krenz *et al*., 1999). Maize is also a major source of, cooking oil (Corn oil) and of maize gluten. Maize starch can be hydrolyzed and enzymatically treated to produce syrups, particularly high fructose corn syrup, used as a sweetener; and also fermented and distilled to produce grain alcohol. The corn steep liquor, a plentiful watery byproduct of maize wet milling process, is widely used in the biochemical industry and research as a culture medium to grow many kinds of microorganisms (Krenz *et al*., 1999).

This study aims to evaluate the effect of some rhizosphere bacteria isolated from local agricultural soil to be used as bio-fertilizers on the growth and productivity of *Zea mays* plants and concomitant changes in some metabolic and biochemical processes. The study also intended to investigate the best bacterial combinations for further utilization as bio-fertilizers in *Zea mays.*

Materials and Methods

Materials

Grains of maize (*Zea mays* cv. Giza321) were obtained as a pure lot from the Agricultural Research Center, Ministry of Agriculture Egypt. The grains used were selected for uniformity of size, shape and viability. The used bacteria were isolated from the rhizosphere in the cultivated fields by *Trifolium alexandrina* L. and *Trifolium aestevum* L. at Nubaria area in Egypt. The isolated bacteria were purified and identified as in Mostafa (2015).

Methods

Six combinations of the purified bacteria were used as inoculants for maize grains. The combinations of bacteria are shown in Table 1.

TABLE 1. Bacterial combinations under investigation of *Zea maize*. Bacteria were collected from the rhizosphere of *Trifolium alexandrina* L. and *Trifolium aestevum* L. cultivated at Nubaria, Egypt.

Combination	No. of strains	Bacterial combinations
1	5	1, 3, 4, 5, 9
2	5	2,4,5,6,10
3	5	1,4,6,7,8
4	5	2,5,7.8.9
5	5	3.6.8.9.10
6	5	1.2.3.7.10
Control	0	

1= *Bacillus subtilis, 2= P. pseudoalcaligenes, 3= Kocuria marina, 4= Bacillus cereus, 5=Kocuria rhizophila, 6= Bacillus subtilis sp. qingdao, 7= Pseudomonas putida, 8= Bacillus licheniformis, 9= Bacillus thuringiensis, 10= Bacillus subtilis sp. spizizenii*

Grains of Zea mays were surface sterilized with 0.1% $HgCl_2$ for 3 min and washed with distilled water for 4-5 times. The grains were soaked for 6 h in 48 h old bacterial broth cultures containing at least 108cells/ml of the chosen bacterial combinations recorded in Table 1. The used soil was sterilized by autoclaving twice for 20 min at 120ºC with a 24 h interval. Soil was placed in plastic pots (14 cm diameter x 14 cm height) of 1kg capacity. Equal number of the treated grains was cultivated in each pot. A control treatment was prepared by cultivating pots by non-treated grains with bacteria. All treatments were arranged as randomized block design with 3 replicates for each treatment.

Measurement of the root depth, shoot height and shoot and root weights.

After fourteen days post sowing, *Zea mays* plant samples were harvested and the root depth and shoot height were measured according to Abdel-Nasser (2002). The control and the plants treated by bacterial combinations were washed by distilled water and laid flat on a moist black cloth with a millimeter scale fixed alongside of the plant and the whole set was photographed. The photos were then projected on a rigid screen and the length of roots and height of the shoots were measured from the image using a measuring wheel (curve

meter). The exact length of the organ in millimeter was then determined by reference to the image of the millimeter scale. The root and shoot samples were then weighed as fresh and dried to constant weight at 60º C in an electric oven. Water content percentage in both organs was calculated from the fresh and dry weights.

Photosynthetic pigments

Photosynthetic pigments, (chlorophyll a, chlorophyll b and carotenoids) were extracted and determined from expanded young leaves according to Inskeep and Bloom (1985). The absorbance was measured at 665, 650 and 450nm. Formulas used for determination of photosynthetic pigments was carried out according to the following equations:

Chl a = 10.3* abs. 665 - abs. 650
Chl b = 19.7* abs. 650 - abs. 665
Carotenoids = 4.2* abs. 450 - (0.0264 * chl a + 0.426 * chl b).
The pigment content was calculated as mg/g. F.wt.

Estimation of total antioxidant capacity

Total antioxidant capacity was determined according to Madhujith and Shahidi (2009).

Analysis of carbohydrates, protein and proline

Samples of maize roots and leaves from the control and the treated plants were used for analysis. Total proteins were extracted according to Rausch (1981) and estimated as described by Hartree (1972). Total soluble sugars were determined as described by Dubois *et al*., (1956). Analysis of proline was carried out according to the method described by Bates *et al*. (1973).

Protein banding pattern

Protein extraction

Samples of 500 mg of maize fresh shoot and root were extracted and kept in the refrigerator for 24 hr with 200 µl of 0.5M Tris-HCl buffer, at pH 6.5. Then, the extract was centrifuged at 3000 rpm for 30 minutes at 40 C. The residue was discarded and the supernatant was used for application on the gel.

Gel electrophoresis

Dissociating polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to Laemmli (1970) as modified by Hames and Rickwood (1990).

Electrophoretic separation of denatured proteins

Electrophoresis was carried out using II-Cell (BIO-RAD) at 75v through stacking gell followed by 125v until the bromophenol blue dye reached the bottom of the gel. The gel was directly placed in the Coomassie brilliant blue staining solution overnight. The gels were destained several times for twelve hours in the destaining solution. After destaining, the gel was photographed,

dried and kept for comparison using lab.110 Software nonlinear dynamics, Newcastle UK, to analyze banding pattern, molecular mass and band percentage.

Statistical analysis

The obtained data were statistically analyzed using standard deviation and student t- test analysis was carried out by SPSS statistical package. All of the statistical methods were carried out according to the method described by Bishop (1983).

Results

Length, fresh and dry weight of Zea mays seedlings:

The average length of *Zea mays* seedling (Table 2) was affected by the used bacterial combinations where the seedlings shoot length was decreased by all bacterial combinations with the maximum decrease by combination 5, as compared with the control. On the opposite, seedlings root length was increased by three of the bacterial combinations (2, 4 & 6) with maximum increase by combination 6. The other three bacterial combinations reduced the root length with a marked effect by combination 5 in comparison with the control. This was reflected on the total plant length that was increased by bacterial combinations 2,4 and 6 the last one led to the maximum plant length while other three combinations especially combination 5 reduced the plant total length in comparison with the control. The root/shoot length ratio was 1.96 in the control plants and this ratio was increased by 5 bacterial combinations (combinations.2, 3, 4, 5, and 6) with a maximum increase by combination 4 whereas combination 1 led to a reduction of the root/shoot length ratio, as compared with the control.

The shoot fresh weight (Table 2) was increased by the used six bacterial combinations with the maximum increase at combination 6 as compared with the control. For the root fresh weight there were remarkable increases by five of the six bacterial combinations (combinations.1, 2, 4, 5, 6) and the maximum increase (40%) over the control resulted by combination 6. On the other hand, one bacterial combination (combination 3) caused a slight reduction of the root fresh weight, as compared with the control.

The shoot dry weight was increased by three bacterial combinations (combinations.1, 3, 6) and the last one (combination 6) led to the maximum increase by about 57%, compared to the control. The other three combinations (2, 4, and 5) reduced the shoot dry weight with a remarkable reduction by combination 5, compared to the control. The root dry weight was increased by four bacterial combinations (combination.1, 4, 5 and 6), with the maximum increase by combination 6. The other two bacterial combinations (2, 3) showed root dry weights comparable with that of the control. All of the bacterial combinations except combination 3 increased the root to shoot dry weight ratio, especially by combination 5. All of the bacterial combinations also increased the total fresh weight of the plant while only combinations 1, 3, 4 and 6 increased the total dry weight of seedling. In this respect, combination 6 led to a marked increase.

TABLE 2. The effect of bacterial combinations applied pre-planting to Zea mays grains on the average length of seedling, fresh weight, dry weight and water percentage of seedlings after 14 days from sowing. Each result is a mean of three replicates ± SD or SE.

Combination	Average length of seedling (cm)				Fresh Weight (g)				Dry Weight (g)				Water content%	
	Root	Shoot	Total length (cm)	Root/ shoot	Root	Shoot	Total weight (g)	Root/ shoot	Root	Shoot	Total weight (g)	Root/ shoot	Root	Shoot
Combination.1	16.59	8.76	25.35	1.89	2.56	5.76	8.32	0.44	0.12	0.39	0.51	0.31	2033.3	1376.9
Combination.2	21.1	8.6	29.7	2.45	2.51	4.13	6.64	0.61	0.1	0.28	0.38	0.36	2410	1375
Combination.3	19.04	8.21	27.25	2.32	2.08	7.6	9.68	0.27	0.1	0.55	0.65	0.18	1980	1281.8
Combination.4	21.77	8.56	30.33	2.54	2.63	5.3	7.93	0.50	0.13	0.35	0.48	0.37	1923.1	1414.3
Combination.5	14.88	6.48	21.36	2.3	2.6	6.64	9.24	0.39	0.12	0.26	0.38	0.46	2066.7	2453.8
Combination.6	24	9.71	33.71	2.47	3.6	8.28	11.88	0.43	0.16	0.58	0.74	0.28	2150	1327.6
Control	19.09	9.72	28.81	1.96	2.15	4.08	6.23	0.53	0.1	0.37	0.47	0.27	2050	1002.7
	Statistical analysis													
Standard deviation	3.11	1.09	3.94	0.25	0.50	1.63	1.93	0.11	0.02	0.12	0.13	0.09	158.78	458.67
t-test	16.57	20.78	18.84	23.65	13.78	9.71	11.75	11.02	14.31	8.47	10.14	9.47	34.79	8.43
P<	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

Photosynthetic pigments

The results of photosynthetic pigments of maize plants are represented in Table 3. The content of Chl.a was increased by five bacterial combinations (.1, 2,3,5 and 6) with remarkable increases due to combinations 5 and 6 which doubled the Chl.a content in comparison with the control. Only the bacterial combination 4 caused a slight reduction of Chl.a content. The content of Chl.b was also increased by four bacterial combinations (2, 3, 5and 6) and the maximum increase was due to combination 6. On the other hand, bacterial combinations (1, 4) caused reductions in the Chl.b content. However, the calculated Chl, a/b ratio was increased by five bacterial combinations (1, 2, 4, 5 and 6) with maximum increase by combination 1 but reversibly, the bacterial combination 3 led to a slight reduction of the Chl. a/b ratio, as compared with the control.

Carotenoid contents were reduced remarkably by all bacterial combinations, as compared with the control (Table 3). The greatest reduction was obtained in treatment with combination 1. This reduction by all bacterial combinations to carotenoids content increased the ratio of Chl. (a+b) / Carotenoids. Where maximum value was obtained in response to combination 2.

Photosynthetic pigment efficiency (the amount of dry weight in gram per the amount of total pigments (mg/g F.wt.) under the effects of different bacterial combinations (Table 3) showed remarkable increases by four bacterial combinations (1, 3, 4 and 6). The highest enhancement was induced by the bacterial combination 1. In the contrary, combinations 2 and 5 reduced the photosynthetic pigment efficiency.

TABLE 3. The content of chlorophylls a, b and carotenoids (mg/g F.wt.) of the leaves of 14- day- old seelings of of *Zea mays* due to six bacterial combinations applied pre-planting to the grains. Each result is a mean of three replicates ± SD or SE.

Combinations	Photosynthetic pigments					Total pigment efficiency (g/mg)
	Chl.a	Chl.b	Ratio Chl, a/b	Carotenoids	Chl. (a + b)/ Carotenoids	
Combination.1	4.50	2.35	1.91	1.10	6.23	64.151
Combination.2	7.55	6.91	1.09	2.09	6.92	22.961
Combination.3	5.69	7.74	0.74	3.43	3.92	38.553
Combination.4	3.53	3.32	1.06	1.70	4.02	56.140
Combination.5	8.51	6.11	1.39	3.06	4.78	21.493
Combination.6	6.81	7.95	0.86	2.57	5.74	42.701
Control	3.89	5.05	0.77	4.63	1.93	34.635
Statistical analysis						
Standard deviation	1.91	2.16	0.41	1.17	1.68	15.88
t-test	8.003	6.882	7.113	5.963	7.522	6.676
P<	0.001	0.001	0.0001	0.001	0.0001	0.0011

Carbohydrate /protein ratio

The carbohydrate content in maize was increased by all bacterial combinations especially combinations 2 and 6, where the values were nearly doubled (Fig. 1). The least increase was due to combination 5. The protein content in the plant increased remarkably by all the used bacterial combinations except combination 5 which caused a slight decrease. Bacterial combinations 1, 3 and 6 doubled the protein content, as compared to control. These variations in carbohydrate and protein contents in response to application of the bacterial combinations led to significant increases in protein to carbohydrate ratios due to all bacterial combinations, especially 1 and 3. Other bacterial combinations led to a decrease in protein to carbohydrate ratios which resulted from decreased protein contents.

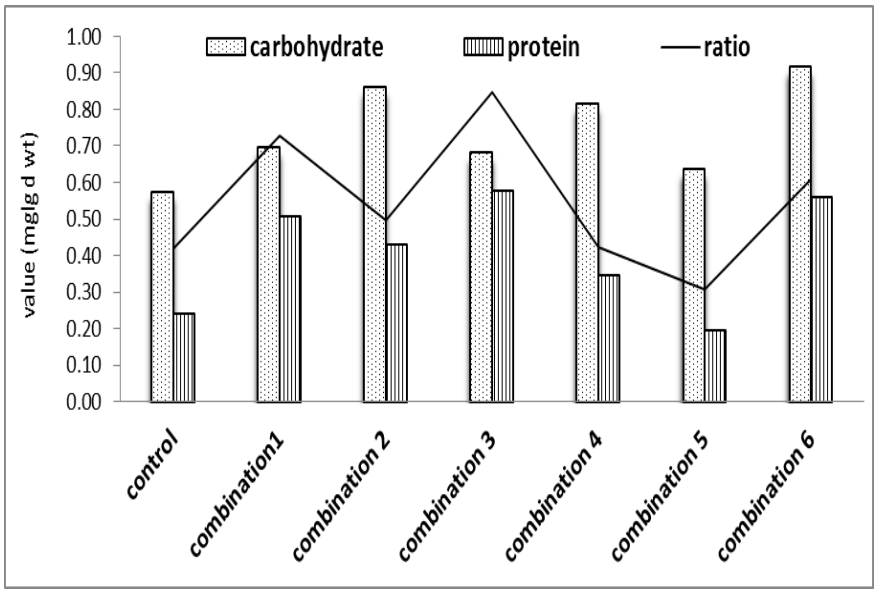

Fig. 1. The content (mg/g D. wt) of carbohydrate and protein and their ratio in maize seedlings (14- day- old) as a result of pre-sowing treatments of grains with bacterial combinations. Each value is a mean of three replicates.

Proline

Proline content in maize shoot (Table 4) was increased by five of the bacterial combinations (.1, 3, 4, 5, and 6) under study, with a maximum increase due to combination 6. On the other hand, only one bacterial combination (2) caused a slight reduction of the shoot proline content, as compared with the control. The proline in maize root was increased by three bacterial combinations (1, 5 and 6) and the maximum increase was recorded with combination 6 which led to 23% increase in proline content. The other three combinations (2, 3 and 4) caused reduction of the root proline particularly by combination 2.

Antioxidant capacity

The antioxidant capacity in the shoot showed an increase by three bacterial combinations (4, 5 and 6) with maximum increase by combination 4. The other three bacterial combinations (1, 2 and 3) led to reduction in the shoot antioxidant capacity, especially combination 1. The root antioxidant capacity was induced by all the bacterial combinations under investigation, compared with the control. The highest induction was due to bacterial combination 3.

TABLE 4. The content of proline (µg g^{-1}f.wt.) and total antioxidant capacity (m M/L) in shoots and roots of maize seedlings (14- day- old) as a result of pre-sowing treatments of grains with six bacterial combinations. Each value is the mean of three replicates.

Combinations	Proline µg g^{-1}f.wt.		Total antioxidant capacity mM/L	
	Root	Shoot	Root	Shoot
Combination.1	5.54	7.67	2.32	2.41
Combination.2	4.27	5.83	2.39	2.46
Combination.3	4.73	7.50	2.47	2.53
Combination.4	5.08	6.98	2.40	2.62
Combination.5	5.88	10.10	2.17	2.58
Combination.6	6.75	12.06	2.43	2.61
Control	5.48	6.92	2.11	2.56
Standard deviation	0.80	2.16	0.13	0.07
t-test	17.674	9.982	45.036	85.895
P<	0.0001	0.0001	0.0001	0.0001

Protein profile of maize

SDS-PAGE analysis of concentrated roots and shoots Tris-HCl extracts was assayed for protein pattern by vertical slab gel electrophoresis. The obtained results are presented in Figures (2 and 3) and Tables 5, 6, 7 and 8. The electrophoresis pattern of proteins for the seedlings shoots and roots revealed the presence of several new protein bands, due to treatments with the different bacterial combinations. Protein bands belonged to different molecular weights that ranged from 112 to 8 kDa in the root and from 109 to 7 kDa in the shoot under the different bacterial combinations.

Concerning root system under the treatment of different bacterial combinations, SDS-PAGE protein bands revealed a sum of eighteen bands that appeared on the gel. With reference to the marker including 18 protein bands ranging from 112 to 8 kDa, most of the bands in the lanes corresponding to the different treatments, appeared in the region between 41 and 10 KDa.

The number of bands obtained for root control were 4 bands and the bacterial combination number 2 exhibited the maximum number of bands (ten bands) followed by combination 5 (eight bands) while combination number 5 showed the lowest number of bands (three bands). However, two combinations showed specific protein bands, the protein profile of combination number 3 showed the occurrence of a specific protein band with a molecular weight of 96 kDa.

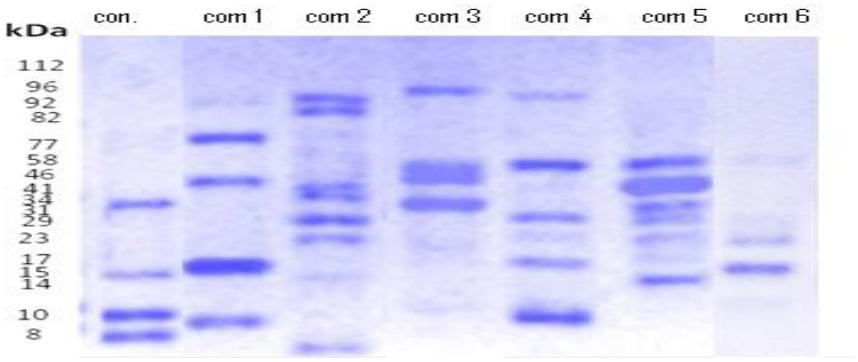

Fig. 2. SDS-PAGE analysis of root proteins of 14-day-old seedlings of maize in response to pre-sowing treatments of the grains with six bacterial combinations. M- Molecular mass markers indicated in kDa. Lane1 represents control and lanes 1 to 6 represent protein under the effect of different bacterial combinations

TABLE 5. Banding patterns of soluble proteins in the roots of 14-day-old seedlings of *maize*, in response to pre-sowing treatments of the grains with six bacterial combinations.

Band No.	Molecular weight (kDa)	Control	Bacterial combinations					
			1	2	3	4	5	6
1	112	-	-	-	-	-	-	-
2	96	-	-	-	+	+	-	-
3	92	-	-	+	-	-	-	-
4	82	-	+	+	-	-	-	-
5	77	-	-	+	-	-	-	-
6	58	-	+	+	+	-	-	-
7	52	-	-	-	-	-	+	-
8	46	-	-	-	+	+	-	-
9	41	-	+	+	-	-	+	-
10	34	-	-	+	-	-	-	-
11	31	+	-	-	+	-	+	+
12	29	-	-	+	+	+	+	-
13	23	-	-	+	-	+	+	-
14	17	-	-	-	-	+	+	-
15	15	+	+	+	-	-	+	+
16	14	-	-	-	-	-	-	+
17	10	+	+	-	+	+	-	-
18	8	+	-	+	-	-	+	-

+ = Band present - = Band absent

TABLE 6. Soluble protein profile bands pattern of root system of the studied Maize cultured for 14 days in response to different bacterial combinations showing sum, unchanged, disappeared and newly formed bands

Characters	Control	Bacterial combinations					
		1	2	3	4	5	6
Sum of bands	4	5	10	6	6	8	3
Unchanged bands	--	2	2	2	1	3	2
Disappeared bands	--	2	2	2	3	1	2
Newly formed bands	--	3	8	4	3	7	1

Comparing the resulting bands from different combinations to control (Table 6), it could be concluded that the number of unchanged bands ranged from one to two bands. Consequently, the number of disappeared bands ranged from a maximum of three bands with the bacterial combination number 4 to a minimum of one band at combination 5. The newly formed bands were 8 bands (maximum) with combination number 2 and one band (minimum) by combination number 6.

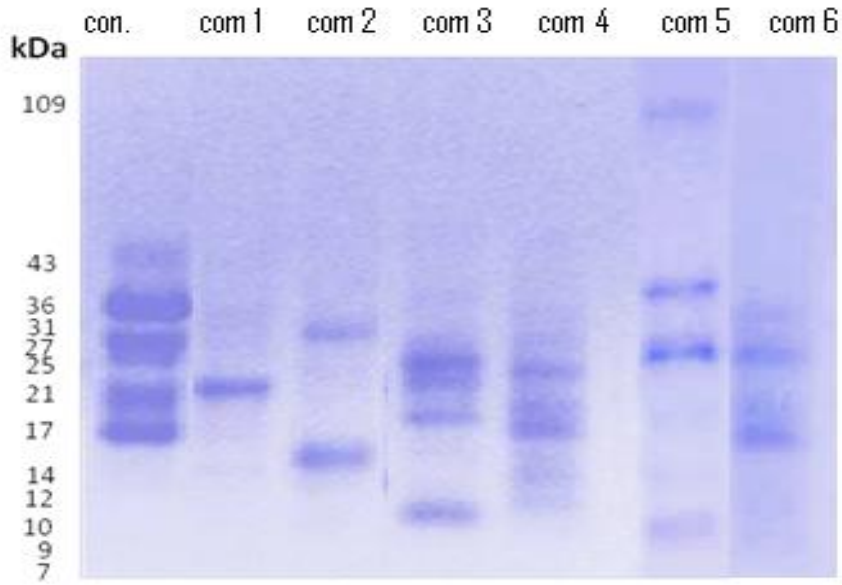

Fig. 3 . SDS-PAGE analysis of shoot proteins of 14-day old seedlings of m*aize*. M-Molecular mass markers are indicated in kDa. Lane1 represents control and lanes 1 to 6 represent protein of the plant under the different bacterial combinations

TABLE 7. Soluble protein profile bands pattern of shoot system of the studied maize cultured for 14 days in response to different bacterial combinations

Band No.	Molecular weight (kDa)	Control	Bacterial combinations					
			1	2	3	4	5	6
1	109	-	-	-	-	-	+	-
2	43	+	-	-	-	-	+	-
3	36	+	-	-	-	-	-	-
4	31	-	-	+	+	+	+	+
5	27	+	-	-	-	+	-	+
6	25	-	-	-	+	-	-	-
7	21	+	+	-	+	+	-	+
8	17	+	-	-	+	+	-	+
9	14	-	-	-	-	+	-	-
10	12	-	-	+	-	-	-	-
11	10	-	-	-	-	+	-	-
12	9	-	-	-	+	-	+	-
13	7	-	-	-	-	-	-	-

(+) = Band present (-) = Band absent

TABLE 8. Soluble protein profile bands pattern of shoot system of the studied maize cultured for 14 days in response to different bacterial combinations showing sum, unchanged, disappeared and newly formed bands

Character	Control	Bacterial combination					
		1	2	3	4	5	6
Sum of bands	5	1	2	5	6	4	4
Unchanged bands	--	1	0	2	3	1	2
Disappeared bands	--	4	5	3	2	4	3
Newly formed bands	--	0	2	3	3	3	1

The results obtained concerning shoot system under treatment of different combinations and SDS-PAGE protein bands are recorded in Figure 3 and Tables 7 and 8. These results revealed that the sum of the bands that appeared on the gel plate and further confirmed by scanning using the band peak were therteen bands. However, most of the bands appeared in the region between 31 and 10 kDa. The number of bands obtained for the control was five bands. Bacterial combination number 4 exhibited the maximum number of bands (6 bands) followed by combination number 3 (five bands) while combination number 1 showed the least number of bands (only one band). However, five combinations (2- 6) showed specific protein bands, where the protein profile of combination number 2 showed the presence of two newly and one specific protein band with a molecular weight of 12 kDa. Combination 3, 4 and 5 showed three newly protein bands with one specific protein band at 25, 14 and 9 kDa, respectively. The bacterial combination number 6 also exhibited a new protein band at 31 kDa, so resembling the other combinations except combination 1. The disappearance of protein bands by bacterial combinations was remarkable with a maximum with combination 2 where five bands became absent, compared with the control.

Discussion

Rhizosphere, the layer of soil influenced by plant root (Saharan and Nehra, 2011), is known to play a pivotal role in plant growth and development (Hrynkiewicz and Baum, 2012). Rhizobacteria aggressively multiply and colonize on the roots at all stages of plant growth. Plant Growth Promoting Rhizobacteria (PGPR) are free living soil microorganisms that exert beneficial effects on plants. The well-known genera of PGPR are *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Klebsiella*, and *Pseudomonas.* The means by which PGPR enhance the nutrient status of host plants can be categorized into five areas: (1) biological nitrogen fixation, (2) increasing the availability of nutrients in the rhizosphere, (3) inducing root surface area, (4) enhancing other beneficial symbiosis of the host, and (5) combination of modes of action (Apastambh *et al*., 2016**)**. There are several PGPR inoculants currently commercialized that seem to promote growth. The use of PGPR inoculants as biofertilizers and/or antagonists of phytopathogens provide a promising alternative to chemical fertilizers and pesticides. (Hrynkiewicz and Baum 2012).

In the present work, PGPR application at six bacterial combinations caused a decrease of maize (*Zea mays*) seedling shoot length, with a maximum decrease by combination number 5, as compared with the control. On the other hand, three bacterial combinations (2, 4 & 6) enhanced the root length of seedlings, especially combination 6, while the other three combinations (1, 3 and 5) reduced the root length with a marked effect by combination 5. However, the total plant length was increased by bacterial combinations 2, 4 and 6 but decreased by the other three combinations, especially combination 5. The root to shoot length ratio of the control was increased by five bacterial combinations (2, 3, 4, 5, and 6) with a maximum increase by combination 4. The six bacterial combinations generally increased the shoot and root fresh weights, with maximum increase by combination 6. On the other hand, three or four bacterial combinations increased shoot and root dry weights, maximum increase by also combination 6. In this respect, bacterial inoculations improved the growth parameters of strawberry plants (Karlidag *et al*., 2013). Liu *et al*., (2013) stated that. *Bacillus subtilis* inoculation increased the shoot dry weight of well-watered *Platycladus orientalis* (oriental thuja). Our data mentioned above concluded that combination 6 was the best bacterial combination followed by combination 4 in inducing the extension growth and weights of the shoots and roots of maize seedlings. This increase in the metabolic activity of the treated plants resulted in the induced root surface area that is one of objective effect of PGPR (Apastambh *et al*., 2016). PGPR induced efficient translocation of metabolites into roots

Chlorophyll status is a key index for evaluating plant photosynthetic efficiency and response to environmental stress (Zhu *et al*., 2012). Improving soil conditions by PGPR improved the accumulation rates of chlorophylls (Fork, 1973), and impaired ultra structural differentiation (Duysen, and Freemani,

1974). Most of the bacterial combinations experimented in the present work enhanced the levels of chlorophylls a & b, with a remarkable value for the former, especially by combination 6. Carotenoids content was markedly reduced by all bacterial combinations which led to increased Chl. (a+b) / Carotenoids ratios. These changes in the photosynthetic pigments content were reflected as increases in photosynthetic pigment efficiency by four bacterial combinations (1, 3, 4 and 6), whereas combinations 2 and 5 markedly reduced the photosynthetic efficiency.

Maize carbohydrate content increased in response to treatments with all bacterial combinations, especially combinations 2 and 6 where the carbohydrate values were nearly doubled. Protein content was also remarkably increased by all of the used bacterial combinations, especially combinations 1, 3 and 6 where the protein content was approximately doubled. Some authors reported that rhizobacteria enhanced protein concentration in plants (Sannazzaro *et al.*, 2006) probably due to stimulation of nitrogen fixation in addition to enhancing protein biosynthesis processes in plants, thus in this way providing plant seeds with higher nutritional value.

The increases were higher in protein than in carbohydrate, which increased their ratio in response to all bacterial combinations, especially combinations 1 and 3. Ullah *et al.* (2013) stated that proteins serve as important components of the major signaling and biochemical pathways which were activated by the presence of PGPR. Protein variation is also an essential part of plant response to environmental stress as well as for adaptation to environmental conditions. Thus, the increased protein to carbohydrate ratios obtained in the present work by bacterial combinations would appreciably increase the nutritive value of maize plants. Meanwhile protein biosynthesis produced a number of growth enhancing effects (Skimmer *et al*., 2009).

Except one combination (2), all bacterial combinations led to increased proline content in maize shoot with a maximum increase by combination 6, while only three bacterial combinations increased proline in root, with maximum increase with also combination 6. According to the results of Moslemi *et al* (2011), bacteria could increase proline that acts as reactive oxygen species (ROS) scavenger. Meloni *et al*. (2001) suggested that proline also serves: as an important source of nitrogen in plant metabolism, as a readily available source of energy, and as a reducing agent. In our work, the obtained increase in proline was reflected on the antioxidant capacity in shoots which was increased by three bacterial combinations (.4, 5 and 6). The root antioxidant capacity was induced by all the bacterial combinations, compared to the control, and the greatest induction was due to bacterial combination number 3. Accumulation of ROS is one of the biochemical changes occurring when plants are exposed to stressful environmental conditions (Pan *et al*., 2006). ROS include super oxide (O^{-}_{2}), hydrogen peroxide (H_2O_2), hydroxyl ($^{\bullet}OH$) radical (Lai *et al*., 2007). These ROS are cytotoxic (Badawi *et al*., 2004) and at high concentrations negatively affect

proteins and cell nucleic acids which finally seriously affected the natural metabolism of plant.

The PGPR mediated plant response to environmental changes has been reported in many studies and numerous genes induced by various stress conditions have recently been identified using molecular approaches (Dardanelli *et al*., 2008). In the present study, the obtained results from SDS-PAGE analysis of the protein banding patters in roots and shoots revealed the presence of several new bands, which differed in migration position and band's intensity due to the different bacterial combinations. The molecular weights of the protein bands ranged from 112 to 8 kDa in root system and from 109 to 7 kDa in shoot under the influence of different bacterial combinations. A significant role of the used bacterial combinations was represented in the induction of new protein bands of low molecular weight and disappearance of others that might reflect the improvement of the plant growth criteria and protein accumulation under the effect of the applied bacterial combinations (Dobereiner, 1997).

The number of protein bands for roots of the control were four bands and the bacterial combinations showed increased number with a maximum value (10 bands) in treatment with the bacterial combination number 2 followed by combination 5 (8 bands), while the lowest number of bands (three bands) was shown in treatment with combination number 5. Specific protein bands appeared in the profile of combination number 3 with a molecular weight of 96 kDa. The number of unchanged bands ranged from one to two bands. Consequently, the number of the disappeared bands ranged from a maximum of three bands at combination number 4 to a minimum of one band by combination number 5. The newly formed bands were eight bands (maximum) with combination number 2 and one band (minimum) with combination number 6.

In shoots, a greater number of bands were shown representing five bands in the control and therteen bands with treatments. In most lanes of bacterial combination, the protein bands appeared for low molecular weight protein in the region between 31 and 10 kDa. Combination number 4 exhibited the maximum number of bands (six bands) followed by combination number 3 (five bands) while combination number 1 showed the least number of bands only one band. However, five combinations (2- 6) showed a specific protein bands. Thus, the protein profile of the combination number 2 showed the presence of two new bands with a specific one having a molecular weight of 12 kDa. Combinations number 3, 4 and 5 showed three newly protein bands and each had one specific protein band at 25, 14 and 9 kDa, respectively as environmental response (Radwan *et al*.,2013). Combination number 6 had one new protein band at 31 kDa. The disappearance of protein bands by bacterial combinations was remarkable with a maximum with combination 2 (five bands). In this connection, Vaseva *et al*. (2012) revealed that plant responses to soil environment changes involved, as a common feature, increased numbers of inactive denatured proteins, aggregated or oxidatively damaged

In conclusion, the study of rhizosphere, is an important goal in the present time due to the prevailing environmental changes. In this context, soil microorganisms play a pivotal role in plant growth and development. The used bacterial combinations in this study were promising in improving the growth criteria and metabolism of maize seedlings. The applied treatments also induced changes in the protein banding pattern of shoots and roots represented in the occurrence of new protein bands and repression of others. Thus, the applied bacterial combinations may be assumed to exert effects on the gene expression.

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(*Received* 1 / 8 / 2016;
accepted 6 / 2 / 2016)

فعالية البكتيريا الجذرية المعزوله من الحقول على النمو وتعزيز القدرة المضادة للأكسدة في نبات الذرة .

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كان هدف هذه الدراسة هو استخدام البكتيريا الجذرية (PGPR) بديلاً عن المخصبات الكيميائية لتعزيز نمو وانتاجية نبات الذرة. في هذه الدراسة عزلت سلالات البكتيريا الجذرية من المحيط الجذري لنباتي البرسيم والقمح واخبترت لتعزيز نمو وأنشطة النبات في التربة مثل الأمونيا، نشاط إندول حمض الخليك ونشاط انزيمي الكتاليز والأوكسيديز. وفي وقت لاحق تم تعريف ٦ سلالات من هذه البكتيريا المعزوله *Pseudomonas mendocina Bacillus subtilis Bacillus Pseudomonas putida, Kocuria marina, K. rhizophilla, cereus*, استخدمت المجموعات الستة من البكتيريا الجذرية لدراسة تطور نمو ونشاط نبات الذرة. أدت تركيزات التركيبات المختلفة المستخدمة إلى نسبة انبات ١٠٠%. قدرت قيمة المواد السكرية في صورة الكربوهيدرات الذائبة والمواد النيتروجينية في صورة بروتين وقيمت فعاليتها في كل التركيبات على نوعية انتاج نبات الذرة وعلى نضارة النبات. كان تأثير كل التركيبات البكتيريا هو زيادة الوزن الطازج للمجموع الخضري بينما كان تأثير كل من التركيبات (١، ٣، ٤، ٦) هو زيادة الوزن الطازج للمجموع الجذري ووضحت النتائج أن التركيبة رقم ٦ كانت أحسن التركيبات البكتيرية تلتها التركيبة رقم ٤ في زيادة النمو في الطول والوزن لكل من المجموع الخضري والجذري لنبات الذرة. وكذلك التركبتين (٦، ٤) شجعت زيادة انتقال المواد الأيضية إلى الجذر. كل تركيب من هذه التركيبات يحتوى على سلالتين من *Bacillus* spp وسلالتين من *Pseudomanas spp* وسلالة واحدة من *Kocuria spp*. أظهرت النتائج ان كفاءة أصباغ البناء الضوئي قد زادت زيادة محلوظة في وجود مجموعات (١، ٣، ٤، ٦) من البكتيريا الجذرية.

كما وجد أن الزيادة في نسبة كمية البروتين إلى المواد الكربوهيدراتية كانت ملحوظة في وجود هذه التركيبات البكتيرية. ولقد زاد محتوى البروتين مع استخدام التركيبات البكتيرية (١، ٣، ٤، ٥، ٦) وكانت أعلى زيادة في وجود التركيبه رقم (٦). وبالنسبة لمضادات الأكسدة في المجموع الخضري فقد زادت في وجود ثلاثة تركيبات بينما في الجذر كانت كل التركيبات محفزة لزيادة قدرة مضادات الأكسدة. ونتيجة لاستخدام التركيبات البكتيرية فلقد وجد أن هناك تغيرات واضحة في كل البيتيدات التي لم تتغير وعدد البيتيدات التي اختفت وكذلك التي تكونت مجدداً كاستجابة لاستخدام المجموعات البكتيرية.