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Response of Three Soybean Genotypes to Inoculation with *Bradyrhizobium* and Some Rhizobacteria under Calcareous Soil Condition

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ABSTRACT

A field trail was conducted during the two successive summer seasons of 2011 and 2012, at Nubaria Agricultural Research Station, to study the effect of inoculation with *Bradyrhizobium japonicum* either solely or in combination with some rhizobacteria *i.e. Bacillus polymyxa* and *Bacillus megaterium* as Plant Growth Promoting Rhizobacteria (PGPR) on nodulation, growth and productivity of three soybean genotypes i.e. Hybrid 30, Giza 21 and Giza 22. Results of combined analysis could be summarized as follows: The highest values of number and dry weight of nodules/plant, shoot dry weight/plant at 60 DAS and plant height were obtained from Hybrid 30. The maximum values of pods number/plant and 100 seed weight were recorded by Hybrid 30. While, seed and straw yields were obtained by Giza 22. The maximum values of phosphorus and nitrogen contents of shoot at 60 DAS were recorded by Hybrid 30. Crude protein percentage of seeds and straw recorded the highest values in Giza 22, while, the minimum values were obtained by Giza 21. *Bradyrhizobium* in combination with each of the tested rhizobacteria exhibited significant increases in nodulation status, shoot dry weight/plant, branches number/plant, plant height, pods number/plant, 100 seed weight, seed and straw yields, as well as phosphorus content of shoot and crud protein content of seeds and straw compared with plants inoculated with *Bradyrhizobium* alone. On the other hand, nitrogen content of shoot recorded the maximum value with inoculated soybean seeds by *Bradyrhizobium* plus *B. mega*. compared with other inoculation treatments. Total microbial counts in the rhizosphere of soybean were markedly affected by biofertilization types: *Brady*. + *B. poly*. + *B. mega*. > *Brady*. + *B. mega* > *Brady*. + *B. poly*. > *Brady*. Total microbial counts and CO_2 evaluation were higher in the second season than in the first one and the high densities of both were obtained by using the triple biofertilization treatment on Hybrid 30 seeds. The interaction between soybean genotypes and inoculation recorded the highest values for number and dry weight of nodules/plant, shoot dry weight/plant, plant height, pods number/plant, 100 seed weight and phosphorus content of shoot when Hybrid 30 seeds inoculated with *Bradyrhizobium* in combination with *B. polymyxa* and *B. megaterium*. While, the maximum values of branches number /plant, straw and seed yields as well as crude protein contents of seeds and straw were obtained when Giza 22 seeds received inoculation with *Bradyrhizobium* plus *B. polymyxa* and *B. megaterium*. The highest value of nitrogen content of shoot were recorded when Hybrid 30 seeds inoculated with *Bradyrhizobium* in combination with *B. megaterium*.

Key words: Soybean Genotypes, inoculation, *Bradyrhizobium japonicum*, Rhizobacteria, Calcareous Soil

Introduction

Soybean [*Glycine max* (L.) Merrill] is one of the most important annual pulse crops in the world. The cultivated form is used in human food and livestock feeds (Harry and Kwon, 1987). Soybean seeds are one of the main sources of protein and oil in world. The seed contains approximately 40% protein and 21% edible oil, which used in making margarine, salad oils, cooking oil. Moreover, soybean products become more important because of low costs, nutritionally balanced and beverages for human consumption.

Biological nitrogen fixation is considered the second important biological process after photosynthesis and it can contribute by about 40-48 million tons of N $year^{-1}$ via agricultural crops. This process accomplishes in nature by few genera of prokaryotic organisms via their ownership genetic information to convert the di-nitrogen molecules to ammonia under mild temperature and normal atmospheric pressure. These nitrogen fixing organisms can fix atmospheric nitrogen through different systems, including free living, associative and symbiotic. Biological nitrogen fixation of *Rhizobium*-legume symbiosis considered the more efficient and important for agricultural environment, particularly sustainable agricultural systems (Jensen and Hauggaard, 2003).

Over the last few years, plant growth promoting rhizobacteria (PGPR) is known as beneficial soil rhizospher to promote plant growth. *Bacillus polymyxa*, which is now called *Paenibacillus polymyxa,* is a Gram-positive bacterium capable of fixing nitrogen. It is found in soil, plant roots, and marine sediments (Lal and

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Silvia, 2009). It has range of reported properties, including nitrogen fixation (Coelho *et al.,* 2003; Cakmakcl *et al.,* 2006), P-solubilisation (De Freitas *et al.,* 2007), cytokinin production (Timmusk *et al.,* 2009), and increased root and shoot growth (Sudha *et al.,* 2009). *P. polymyxa* is used as a soil inoculant in agriculture and horticulture. Biofilms of *P. polymyxa* growing on plant roots have been shown to produce exopolysaccharides which protect the plants from pathogens (Yegorenkova, *et al*., 2013). The interaction between these bacterial species and plant root also cause the root hairs to undergo physical changes. Some strains of *B. polymyxa* produce polymyxin antibiotic compounds (Shaheen *et al.,* 2011). Also, *Bacillus megaterium* is a biofertilizers bacteria based on a selected strain of naturally-occurring beneficial eubacteria, it produces organic such as lactic acid, gluconic acid, citric acid, succinic acid, propionic acid and enzymes that help solubilize the fixed phosphorus into exchangeable form. These organic acids through their hydroxyl and carboxyl groups chelate the cations (mainly Calcium) bound to phosphate converting them into the soluble forms. It produce cytokinine (López, *et al.*, 2007)

Logically, potentials for improving plant yield by combining these plant growth promoting organisms with rhizobia have also been a subject of several investigators (Verma *et al.,* 2010). In this respect, Bai *et al.,* (2003) and Badawi *et al.,* (2011) found that co-inoculation of legumes with rhizobia and PGPR (such as *Pseudomonas, Bacillus* and *Serratia* strains) led to positive effects on nodulation, growth and yield of legume crops.

Therefore, study the response of soybean genotypes to inoculation with *Bradyrhizobium* in conjugation with some rhizobacteria is very important aspect to optimize soybean genotype for high production In this respect, Fardoas and Ali (2001) concluded that inoculation soybean seeds of Giza 111 with *Bradyrhizobium japonicum* significantly increased number and dry weight of nodules at 60 and 90 days after sowing, plant height, number of branches and pods/plant, pod, seed and straw weight/plant, 100- seed weight, seed and straw yields/fad. Abd El-Hafez and Abo El-Soud (2007) reported that, soybean cultivar Giza 111 superior Crawford on number, dry weight of nodules, shoot dry weight, plant height, branches number and pods number/plant, seed weight, 100-seed weight, seed and straw yields/fad.

This work was conducted to study the response of three soybean genotypes *i.e.* Hybrid-30, Giza-21 and Giza-22 to inoculation with *Bradyrhizobium japonicum* either solely or in combination with some rhizobacteria i.e. *Bacillus polymyxa* and *Bacillus megaterium* for nodulation, growth and yield and its compontes under calcareous soil conditions.

Material and Methods

A field trail was conducted during the two successive summer growing seasons, 2011 and 2012, at Nubaria Agricultural Research Station, El-Behira governorate, Egypt; to study the effect of inoculation with *Bradyrhizobium japonicum* either solely or in combination with some rhizobacteria *i.e. Bacillus polymyxa* and *Bacillus megaterium* as Plant Growth Promoting Rhizobacteria (PGPR) on nodulation, growth and productivity of three soybean genotypes *i.e.* Hybrid 30, Giza 21 and Giza 22. The seeds of such soybean genotypes were kindly provided by Leguminous Crop Research Department, Field Crop Research Insititute, ARC, Giza, Egypt.

Microorganisms used:

Bradyrhizobium japonicum (USDA 3456) was the most suitable for producing biological nitrogen fixation but *Bacillus megaterium* and *Bacillus polymyxa* strains were active in solubilizing phosphate being 1.4 and 1.79 mg/ 100ml, respectively. These strains were supplied by Biofertilizer Production Unit, Soils, Water and Environment Research Institute, Agric. Res. Center (ARC), Giza, Egypt.

Inoculants preparation:

Bradyrhizobium was cultured in yeast mannitol broth medium (Vincent, 1970), *B. polymyxa* and *B. megaterium* were grown in Kings medium B (Atlas, 1995). All bacterial cultures were incubated at 28°C for three days on a rotary shaker until early log phase was developed of 10^9 cfu/ml culture. Vermiculite supplemented with 10% Irish peat was packed into polyethylene bags (300g carrier per bag), then sealed and sterilized with gamma irradiation (5.0×10^6 rads). Cultures were injected into the carrier to satisfy 60 % of the maximum water holding capacity.

The experiment was laid out in a split plot design with four replicates. The main plots were occupied by soybean genotypes, while sub-plots contained inoculation treatments. Each sub-plot was 10.5 m^2 (3×3.5 m) and included 5 ridges, 3.5 m long and 60 cm apart. Representative soil samples were collected from the top 30 cm layer of the experimental site, air-dried and sieved through 2 mm screen. The main physical and chemical properties of the experimental site in both growing seasons were analyzed according to Page *et al.,* (1982). Total microbial counts in the soil samples determined before sowing according to Bunt and Rovira (1955). Also, CO_2 evolution (µg/g dry soil/ hr.) was determined according to Pramer and Schmidt (1964). The data of the soil analyses were recorded in Table (1). At 60 DAS, total microbial counts and CO_2 evaluation were determined for each sub-plot and tabulated in Table (5).

All sub-plots were received the recommended dose of single superphosphate (15.5 % P_2O_5) and potassium sulphate (48% K_2O) at rate of 200 kg/fad and 50 kg/fad, respectively and incorporated into soil before sowing.

Table 1: Mechanical and chemical analyses of the experimental sit in both investigated seasons

Property	Season 2011	Season 2012
Mechanical analysis:		
Sand %	51.4	52.1
Silt %	24.9	24.5
Clay %	23.7	23.4
Texture grade	Sandy clay loam	Sandy clay loam
$CaCO_3$ (%)	18.10	19.10
S.P (%)	28.40	29.20
pH (soil paste)	8.40	8.50
EC (dS m^{-1}, at 25ºC)	0.98	1.05
Soluble cations (meq/L) :		
Ca^{++}	2.40	2.45
Mg^{++}	1.40	1.53
Na^+	3.30	3.69
K^+	2.60	2.78
Soluble anions (meq/L):		
$CO^=_3$	0.00	0.00
HCO^-_3	3.50	3.66
Cl^-	3.10	3.41
$SO^=_4$	3.10	3.38
Total-N (%)	0.016	0.018
Total soluble- N (mg kg^{-1})	12.50	13.20
Available-P (mg kg^{-1})	4.10	3.95
Available-K (mg kg^{-1})	115.00	126.00
Organic matter (%)	0.21	0.18
DTPA-extractable (mg kg^{-1})		
Fe	0.68	0.61
Mn	0.54	0.48
Zn	0.41	0.39
Cu	0.09	0.11
Total microbial counts X 10^6		
(CFU / g. dry soil)	82.0	90.0
CO_2 evolution (µg/g dry soil/ hr.)	18.5	22.6

The treatments are as follows:

I-Main plots (soybean genotypes)

A- Hybrid-30

B- Giza 21

C- Giza 22

II-Sub-plots (inoculation)

1- Inoculated with *Bradyrhizobium* (*Br.*) (Control)

2- Inoculated with *Bradyrhizobium* (*Br.*) + *Bacillus polymyxa* (*B. poly.*).

3- Inoculated with *Bradyrhizobium* (*Br.*) + *Bacillus megaterium* (*B. mega.*).

4- Inoculated with *Bradyrhizobium* (*Br.*) + *B. poly.* + *B. mega.*

Soybean seeds were sown on 5/4/2011 and 10/4/2012, in hills 10 cm apart and treated with different inoculants at the rate of 300 g per 35 kg seeds, prior to sowing using Arabic gum solution (16%) as an adhesive agent. Soybean seedlings were thinned out to two plants /hill at 21 days after sowing. 10 kg N/fad was added in the form of ammonium sulphate (20.5% N) as a starter dose.

Cultural practices were practiced according to the methods being adopted for growing soybean plant in the locality. At 60 days after sowing (DAS), sample of five plants were random taken from the outer rides of the four replications to determine the following traits:

1- Nodules number/plant

2- Nodules dry weight (mg/plant)

3- Shoot dry weight (g/plant)
4- Nitrogen content of shoot (mg/plant)
5- Phosphorus content of shoot (mg/plant)

Shoot P and N contents were determined according to page *et al.* (1982).

At harvest time of 29/8/2011 and 1/9/2012 in the first and second seasons, respectively, ten guarded plants were randomly taken from central ridges in each sub-plot to determine:

1-Plant height (cm)
2-Branches number/plant
3-Pod number/plant
4-100 seed weight (g)

Seed and straw yields/fad were calculated from a central area (4.8 m^2) in each sub-plot to avoid the border effect. Mature seeds and straw samples from the two growing seasons were subjected to chemical analysis to determine crude protein percentage according to A.O.A.C. (1990).

Data of the two seasons were combined and statistically analyzed according to Steel and Torrie (1980). The discussion of the results was carried out on the basis of combined analysis of the two seasons.

Results and Discussion

1-Soybean genotypes:

Regarding the growth behavior of soybean genotypes, data of Table (2) recorded a significant difference in number and dry weight of nodules/plant, shoot dry weight/plant at 60 DAS and plant height. The maximum values of number, dry weight of nodules/plant and shoot dry weight/plant was obtained from Hybrid 30 followed by Giza 22. However the lowest values were gained from Giza 21. With respect to plant height, results revealed that Hybrid 30 had the tallest plants, while the shortest plants were obtained from Giza 22. However, Giza 21 had an intermediate values. These results are in harmony with those reported by Bakheit (1990) who concluded that the differences between the long stem and the short stem cultivars could be attributed to the genetic marks up to of cultivars. It can be noticed that branches number/plant recorded insignificant effect. Giza 21 and Giza 22 recorded the same figure for the two genotypes (5.9 branches/plant).

Table 2: Number, dry weight of nodules/plant, shoot dry weight, branches number and plant height of three soybean genotypes as affected by inoculation with *Bradyrhizobium* and some *Rhizobacteria* (PGPR) in 2011 and 2012 summer seasons.

Genotypes	Inoculation	Number of nodules / plant (60 DAS)			Nodules dry weight (mg / plant) (60 DAS)			Shoot dry weight (g/plant) (60 DAS)			Branches number / plant			Plant height (cm)		
		2011	2012	Comb.	2011	2012	Comb.	2011	2012	Comb.	2011	2012	Comb.	2011	2012	Comb.
Hybrid-30	Bradyrhizobia	26.3	31.0	28.7	120.0	130.0	125.0	13.30	15.63	14.47	4.7	5.3	5.0	95.0	97.3	96.2
	Brady.+B. polymyxa	29.3	34.0	31.7	142.7	147.3	145.0	14.50	14.83	14.67	4.7	5.3	5.0	95.7	97.0	96.4
	Brady.+B. megaterium	34.0	41.0	37.5	148.7	152.3	150.5	15.53	18.20	16.87	5.7	5.7	5.7	96.7	96.0	96.4
	Brady.+B. polymyxa + B.megaterium	38.3	46.7	42.5	161.0	168.0	164.5	17.70	19.07	18.39	6.3	6.3	6.3	97.6	98.0	97.8
Mean		32.0	38.2	35.1	143.1	149.4	146.3	15.26	16.93	16.10	5.4	5.7	5.5	96.3	97.1	96.7
Giza 21	Bradyrhizobia	18.7	24.3	21.5	108.3	111.7	110.0	12.27	13.27	12.77	5.0	5.3	5.2	81.5	82.5	82.0
	Brady. + B. polymyxa	22.0	28.3	25.2	126.0	130.3	128.2	12.70	13.70	13.20	5.7	6.0	5.9	83.6	84.6	84.1
	Brady.+B. megaterium	24.7	30.7	27.7	128.0	132.7	130.4	14.47	15.13	14.80	5.7	6.7	6.2	88.8	90.2	89.5
	Brady. + B. polymyxa + B.megaterium	25.0	31.7	28.4	130.7	136.0	133.4	16.63	17.63	17.13	6.0	6.3	6.2	90.6	91.6	91.1
Mean		22.6	28.8	25.7	123.3	127.7	125.5	14.02	14.93	14.48	5.6	6.1	5.9	86.1	87.2	86.7
Giza 22	Bradyrhizobia	21.7	28.7	25.2	114.0	126.0	120.0	13.43	14.43	13.93	5.0	5.7	5.4	67.9	68.9	68.4
	Brady. + B. polymyxa	26.0	32.7	29.4	130.7	137.3	134.0	12.63	12.97	12.80	5.3	5.7	5.5	75.8	78.1	77.0
	Brady.+B. megaterium	27.3	33.3	30.3	135.7	141.7	138.7	15.27	17.07	16.17	6.0	6.3	6.2	79.2	80.5	79.9
	Brady. + B. polymyxa + B.megaterium	30.7	37.3	34.0	141.3	144.7	143.0	16.37	17.33	16.85	6.3	6.7	6.5	85.4	85.4	85.4
Mean		26.4	33.0	29.7	130.4	115.6	133.9	14.43	15.45	14.94	5.7	6.1	5.9	77.1	78.2	77.7
General mean of inoculation	Bradyrhizobia	22.2	28.0	25.1	114.1	122.6	118.3	13.00	14.44	13.72	4.9	5.4	5.2	81.5	82.9	82.2
	Brady. + B. polymyxa	25.8	31.7	28.8	133.1	138.3	135.7	13.28	13.83	13.56	5.2	5.7	5.5	85.0	86.6	85.8
	Brady.+B. megaterium	28.7	35.0	31.8	137.5	142.2	139.9	15.09	16.80	15.95	5.8	6.2	6.0	88.2	88.9	88.6
	Brady. + B. polymyxa + B.megaterium	31.3	38.6	35.0	144.3	149.6	147.0	16.90	18.01	17.46	6.2	6.4	6.3	91.2	91.7	91.4
LSD 5%	Genotypes	5.2	4.2	2.8	9.2	7.4	5.0	1.06	1.26	0.80	N.S.	N.S.	N.S.	2.9	2.5	1.6
	Inoculation	3.3	3.6	2.3	5.8	4.3	3.4	0.97	1.21	1.15	0.7	0.6	0.4	2.5	2.2	1.4
	Interaction	5.7	6.2	4.1	10.1	10.9	6.3	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	4.3	3.9	2.5

DAS = days after sowing

Concerning the yield and its components of soybean genotypes, results in Table (3) showed significant differences among genotypes in pods number/ plant, 100 seed weight, seed and straw yields. The highest value of pods number/plant and 100 seed weight were obtained from Hybrid 30. On the contrary Giza 21 scored the lowest values of such characters, whereas Giza 22 scored intermediate values.

Giza 22 recorded the maximum values of straw and seed yields (1060.1 and 1010.3 kg/fad, respectively) followed by Hybrid 30, with insignificant differences between the both genotypes. Whereas, the lowest values of such traits were obtained by Giza 21 (892.0 and 855.2 kg/fad, respectively). These results are in agreement with those reported by Mohamed and Morsi (2005).

Results in Table (4) showed significant differences among soybean genotypes in phosphorus and nitrogen contents of shoot at 60 DAS and crud protein percentage of seeds and straw. The maximum values of phosphorus and nitrogen contents of shoot were recorded by Hybrid 30 followed by Giza 22, while, the lowest values of such traits were gained by Giza 21.

As for the difference between genotypes with respect to crude protein percentage of seeds and straw, it could be observed that Giza 22 had the maximum values of such traits followed by Hybrid 30 and Giza 21, respectively. Such differences between genotypes might be attributed to the growth habit of each genotype, which is governed by genetically factors and/or environmental condition. These results are in agreement with those reported by El-Karamity (1998) and Abd El-Hafez (1999).

Effect of inoculation:

Results of *bradyrhizobia* inoculation combined with plant growth promoting rhizobacteria (PGPR) are presented in Table (2). The number and dry weight of nodules developed on soybean roots samples collected after 60 days from sowing revealed that, inoculated plants with bradyrhizobia alone had the lowest nodulation of 25.1 nodules/plant with dry weight of 118.3 mg/plant. Inoculated soybean with *Bradyrhizobium* combined with either *B. poly.* or *B. mega.* give significant increases in nodules number by 14.7 or 26.7%, respectively and nodules dry weight by 14.7 or 18.3%, respectively over the bradyrhizobia inoculated alone (control). The maximum nodules number (35 nodules/plant) and their dry weight (147 mg/plant), were recorded from plants inoculated with *Bradyrhizobium* combined with *B. polymyxa* and *B. megaterium*. These results are in accordance with those obtained by Abdel-Wahab *et al*., (2006) and Mekhemar *et al*., (2006).

Concerning the shoot dry weight of soybean plant at 60 day-old in Table (2) clearly illustrated that the plants inoculated with *Bradyrhizobium* combined with *B. polymyxa* and *B. megaterium* led to the highest increase in shoot dry weight. *Bradyrhizobium* combined with *B. megaterium* recorded higher values of shoot dry weight than *Bradyrhizobium* combined with *B. polymyxa*. These results due to a high proportion of rhizobacteria are capable to exhibit the plant growth hormone (*i.e*., indole acetic acid), which acts to stimulate root growth and provides it with more branching and larger surface area. Many investigators consider the Indole secretion by PGPRs, as vital mechanism to clarify the plant promotion (Verma *et al.,* 2010 and Badawi *et al.,* 2011).

The effect of *Bradyrhizobium* inoculation associated with rhizobacteria on plant height, pods number and 100 seed weight are given in Tables (2, 3).

Table 3: Pods number, 100 seed weight, as well as seed and straw yields of three soybean genotypes as affected by inoculation with *Bradyrhizobium* and some *Rhizobacteria* (PGPR) in 2011 and 2012 summer seasons.

Genotypes	Inoculation	Pods Number / plant			100 seed weight (g)			Straw yield (kg/fad.)			Seed yield (kg/fad)		
		2011	2012	Comb.	2011	2012	Comb.	2011	2012	Comb.	2011	2012	Comb.
Hybrid-30	Bradyrhizobia	80.67	87.67	84.17	15.70	16.03	15.87	935.3	985.7	960.5	866.0	912.7	889.4
	Brady. + B. polymyxa	85.33	89.33	87.33	16.97	17.63	17.30	972.4	1048.0	1010.2	900.3	970.3	935.3
	Brady. + B. megaterium	87.00	91.33	89.17	17.10	17.77	17.44	1016.3	1052.3	1034.3	907.7	974.4	941.1
	Brady.+ B. polymyxa + B.megaterium	92.67	95.67	94.17	17.63	18.30	17.97	1089.4	1129.0	1109.2	1175.3	1145.3	1160.3
Mean		86.42	91.00	88.71	16.85	17.43	17.15	1003.4	1053.8	1028.6	962.3	1000.7	981.5
Giza 21	Bradyrhizobia	67.67	70.33	69.00	13.77	14.10	13.94	802.8	878.4	840.6	743.3	846.7	795.0
	Brady. + B. polymyxa	73.67	76.33	75.00	14.10	14.43	14.27	848.9	920.9	884.9	786.0	840.0	813.0
	Brady. + B. megaterium	79.33	81.00	80.17	14.17	14.83	14.50	852.5	906.5	879.5	789.3	852.7	821.0
	Brady.+ B. polymyxa + B.megaterium	81.67	84.33	83.00	15.93	16.60	16.27	930.6	995.4	963.0	961.7	1021.7	991.7
Mean		75.59	78.00	76.79	14.49	14.99	14.75	858.7	925.3	892.0	820.1	890.3	855.2
Giza 22	Bradyrhizobia	77.33	80.67	79.00	15.40	15.73	15.57	985.0	1028.5	1006.8	945.3	952.3	948.8
	Brady. + B. polymyxa	78.67	82.00	80.34	16.13	16.33	16.23	1023.1	1062.7	1042.9	924.4	964.0	944.2
	Brady. + B. megaterium	81.33	83.33	82.33	16.73	16.77	16.75	987.5	1052.3	1019.9	947.3	981.0	964.2
	Brady.+ B. polymyxa + B.megaterium	84.33	86.33	85.33	17.07	17.30	17.19	1134.7	1206.7	1170.7	1150.7	1217.3	1184.0
Mean		80.42	83.08	81.75	16.33	16.53	16.44	1032.6	1087.6	1060.1	991.9	1028.7	1010.3
General mean of inoculation	Bradyrhizobia	75.22	79.56	77.39	14.96	15.29	15.13	907.7	964.2	936.0	851.5	903.9	877.7
	Brady. + B. polymyxa	79.22	82.55	80.89	15.73	16.13	15.93	948.1	1010.2	979.3	870.2	924.8	897.5
	Brady. + B. megaterium	82.55	85.22	83.89	16.00	16.46	16.23	952.1	1003.7	977.9	881.4	936.0	908.8
	Brady.+ B. polymyxa + B.megaterium	86.22	88.78	87.50	16.88	17.40	17.14	1051.6	1110.4	1081.0	1095.9	1128.1	1112.0
LSD 5%	Genotypes	3.85	3.40	2.18	0.48	0.52	0.32	110.5	102.2	63.4	89.4	82.6	51.5
	Inoculation	3.14	3.03	1.85	0.41	0.44	0.25	77.7	71.5	44.8	62.8	57.8	37.1
	Interaction	N.S.	N.S.	N.S.	0.71	0.78	0.45	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

DAS = days after sowing

Results revealed that the plant inoculated with *Bradyrhizobium* alone recorded the lowest values of plant height (82.2 cm), branches number per plant (5.2), pods number (77.39 pod/plant) and 100 seed weight (15.13 g/plant). Plants inoculated with *Bradyrhizobium* plus *B. polymyxa* and *B. megaterium* caused a significant increase in plant height, branches number/plant, pods number/plant and 100 seed weight. These increases were 11.2, 21.2, 13.1, and 13.3%, respectively over the *Bradyrhizobium* inoculated alone. The synergistic effect of rhizobacteria on soybean yield may be elucidated by their ability to enhance the nodulation development, nitrogen fixation performance and nutrients availability uptake from soil such as phosphorus and micronutrients, which resulted in various mechanisms mainly the production of substances like hormones, siderophores, phosphate solubilization and improvement nutrient and water uptake by increasing the root proliferation. Such mechanisms have been reported by many investigators (Kloepper, 2003 and Abdel-Wahab *et al*., 2008).

Concerning the seed and straw yields of soybean as affected by *Bradyrhizobium* inoculation combined with rhizobacteria are given in Table (3). Obtained results revealed that *Bradyrhizobium* plus *B. polymyxa* and *B. megaterium* exhibited significant increases of seed and straw yields. These increases were 26.7 and 15.5%, respectively compared with control.

Regarding the effect of inoculation treatments on phosphorus and nitrogen contents of shoot at 60 DAS, crude protein percentage of seeds and straw yields, Table (4) showed significant effect of the inoculation treatments on such characters. The maximum value for phosphorus content of shoot and crud protein percentage of seeds and straw yields were obtained when soybean seeds inoculated with *Bradyrhizobium* plus *B. polymyxa* and *B. megaterium* compare with other inoculation treatments. Whereas, the highest value for nitrogen content of shoot was gained when soybean seeds inoculated with *Bradyrhizobium* plus *B. megaterium* with insignificant differences between such treatment and inoculated soybean seeds pre sowing by *Bradyrhizobium* plus *B.polymyxa* and *B.megaterium*. These results are in line with those obtained by Desoky *et al*. (2011) on peanut plant, who found that the increases in accumulated N of peanut tissues due to the interaction effect between *Bradyrhizobium* and PGPR could be explained through the role of available N supplemented by the inoculating organisms and the production of growth promoting substances.

Table 4: Phosphorus and nitrogen contents of shoot, crude protein percentage of seeds and straw for three soybean genotypes as affected by inoculation with *Bradyrhizobium* and some *Rhizobacteria* (PGPR) in 2011 and 2012 summer seasons.

Genotypes	Inoculation	P-content of shoot (mg/ plant) (60 DAS)			N-content of shoot (mg/plant) (60 DAS)			Crude protein in seeds (%)			Crude protein in straw (%)		
		2011	2012	Comb.	2011	2012	Comb.	2011	2012	Comb.	2011	2012	Comb.
Hybrid-30	Bradyrhizobia	84.26	107.60	95.93	370.73	461.04	415.89	36.54	37.68	37.11	4.68	4.94	4.81
	Brady. + B. polymyxa	122.72	123.31	122.93	410.96	441.74	426.35	38.44	39.20	38.82	5.04	5.14	5.09
	Brady. + B. megaterium	125.28	153.99	139.64	514.22	542.69	528.46	37.62	39.14	38.38	4.93	5.12	5.03
	Brady.+B. polymyxa+B.megaterium	154.71	171.31	163.01	471.90	571.82	521.86	40.20	40.10	40.15	5.14	5.16	5.15
Mean		121.74	139.01	130.38	441.95	504.32	473.14	38.20	39.03	38.62	4.95	5.09	5.02
Giza 21	Bradyrhizobia	83.83	107.47	95.65	344.52	373.16	358.84	36.86	36.92	36.89	4.73	4.84	4.79
	Brady. + B. polymyxa	94.56	111.85	103.21	358.34	388.37	373.35	37.04	37.22	37.13	4.85	4.88	4.87
	Brady. + B. megaterium	113.17	127.52	120.34	458.55	490.62	474.59	36.84	37.40	37.12	4.83	4.90	4.87
	Brady.+B.polymyxa+ B.megaterium	143.38	136.96	140.17	416.25	445.88	431.06	39.44	39.14	39.29	5.05	4.96	5.01
Mean		108.73	120.95	114.84	394.42	424.51	409.46	37.55	37.67	37.61	4.87	4.90	4.89
Giza 22	Bradyrhizobia	97.38	117.88	107.63	388.08	409.29	398.69	37.90	37.22	37.56	4.97	4.88	4.93
	Brady. + B. polymyxa	105.84	110.38	108.12	378.35	383.25	380.80	39.30	38.84	39.07	5.15	5.09	5.12
	Brady. + B. megaterium	143.56	146.14	144.85	448.82	512.57	480.69	39.60	39.38	39.49	5.06	5.16	5.11
	Brady.+B.polymyxa+ B.megaterium	137.94	151.19	144.56	427.16	522.40	474.78	39.98	40.82	40.40	5.24	5.49	5.37
Mean		121.18	131.40	126.29	410.60	456.88	433.74	39.20	39.07	39.13	5.11	5.16	5.13
General mean of inoculation	Bradyrhizobia	89.49	110.98	99.74	367.78	414.50	391.14	37.10	37.27	37.19	4.79	4.89	4.84
	Brady. + B. polymyxa	107.71	115.12	111.42	382.55	404.45	393.50	38.26	38.42	38.34	5.01	5.04	5.03
	Brady. + B. megaterium	127.34	142.55	134.94	473.86	515.29	494.58	38.02	38.64	38.33	4.94	5.06	5.00
	Brady.+B.polymyxa+ B.megaterium	145.34	153.15	149.25	438.44	513.37	475.90	39.87	40.02	39.95	5.14	5.20	5.18
LSD 5%	Genotypes	8.68	7.87	4.97	45.94	50.67	28.98	0.84	0.94	0.54	0.21	0.10	0.08
	Inoculation	7.28	6.60	4.16	38.53	42.50	24.31	0.70	0.78	0.44	0.12	0.10	0.08
	Interaction	12.84	11.64	7.34	67.96	74.95	42.87	1.24	1.40	0.80	N.S.	0.16	0.11

DAS = days after sowing

Regarding the effect of inoculation treatments on total microbial counts (60 DAS), data presented in Table (5) showed that total microbial counts in the rhizosphere of soybean were markedly affected by biofertilization types in this order: *Brady. + B. poly + B. mega > Brady. + B. mega > Brady. + B. poly > Brady*. Total microbial counts were higher in the second season than the first one and the high densities were obtained by using the triple biofertilization treatment on Hybrid 30 seeds which produced 163 and 165 X 10^6CFU (g^{-1} dry soil) for first and second seasons, respectively. It may be due to that mixture of the most active *B. polymyxa* and *B. megaterium* which added as soil application produced growth promoting substances beside the fixed nitrogen which produced by Bradyrhizobium, help to increase in soil microflora densities which activities the soil microbes in the rhizosphere when compared with control.

CO_2 evolution (60 DAS) from soybean plant rhizosphere was periodically detected to throw light on microbial activity in such region as influenced by application of dual biofertilizer inoculants. In all cases, the rhizosphere of control treatment give the lowest CO_2 evolution values. Data presented in Table (5) showed that CO_2 evolved (mg CO_2 $100g^{-1}$ dry soil 24 hr^{-1}) of the rhizosphere of soybean were markedly affected by soybean genotypes in this order: Hybrid 30 > Giza 22 > Giza 21. CO_2 evolution values were much higher in the second season than the first one and the most effective CO_2 evolution obtained by using the triple biofertilization treatment on Hybrid 30 seeds which produced 40.8 and 46.0 (mg CO_2 $100g^{-1}$ dry soil 24 hr^{-1}) for first and second seasons, respectively. It may be due to that mixture of the most active *B. polymyxa* and *B. megaterium* which added as soil application produced growth promoting substances beside the fixed nitrogen, help to increase in soil microflora densities which increased in respiratory and activities of soil microbes so it produced

CO_2 in the rhizosphere when compared with control. Data of CO_2 evolution were almost in harmony with those of total microbial counts (Visser and Dennis, 1992).

Interaction effect:

The interaction between soybean genotypes and inoculation, data of Table (2) recorded a significant effect on number and dry weight of nodules at 60 DAS and plant height. The maximum values of such traits were obtained when Hybrid 30 seeds inoculated with *Bradyrhizobia* plus *B. polymyxa* and *B. megaterium.* It could be noticed that there was insignificant effect on branches number /plant and shoot dry weight. Similar results were reported by Abo El-Soud *et al.* (2003) and Rizk *et al.* (2011).

As for the interaction effect between soybean genotypes and inoculation treatments, data in Table (3) showed that a significant effect was observed on 100 seed weight. Whereas, insignificant effect was recorded on pods number/plant, seed and straw yields. The highest values of pods number/plant and 100 seed weight were obtained when Hybrid -30 seeds inoculated pre sowing by *Bradyrhizobium* in combination with *B. polymyxa* and *B. megaterium*. While, the maximum values of seed and straw yields were gained when Giza 22 seeds inoculated with *Bradyrhizobium* plus *B. polymyxa* and *B. megaterium*. Such results were in harmony with those obtained by Ragab (1998) and El-Sayed (1998) who found that co-inoculation with rhizobia and phosphate dissolving bacteria caused significant increase in seed yield of soybean and lentil.

Regarding the interaction effect on phosphorus and nitrogen contents of shoot at 60 DAS as well as crude protein percentage in seed and straw results of Table (4) showed significant effect on such traits. The highest value of phosphorus content was obtained when Hybrid 30 seeds inoculated with *Bradyrhizobium* in combination with *B. polymyx*a and *B. megaterium*. Whereas, the maximum value for nitrogen content of shoot was gained when Hybrid 30 seeds inoculated with *Bradyrhizobium* plus *B. megaterium*. These results are in harmony with those obtained by Desoky *et al.,* (2011) who found that on peanut, Dual inoculation with *Bradyrhizobium* and PGPR recorded a significant increase on N-content of shoot compared to inoculation with *Bradyrhizobium* alone.

The maximum values of seed and straw crude protein were recorded when Giza 22 seeds inoculated pre sowing by *Bradyrhizobium* in combination with *B. polymyxa* and *B. megaterium*. In these respect Abdel-Wahab *et al.*, (2006) and Mekhemar *et al.*, (2007) reported that co-inoculation of leguminous plants with *Rhizobium* and some *rhizobacteria* significantly increased crude protein of seeds.

Table 5: Effect of Biofertilization treatments on densities of total microbial counts and CO_2 evolution in the rhizosphere of some soybean genotypes at 60 DAS.

Genotypes	Inoculation	Total microbial counts X 10^6 CFU / g. dry soil			CO_2 evolution (µg /g dry soil/ hr.)		
		2011	2012	mean	2011	2012	Mean
Hybrid-30	Bradyrhizobia.	95.0	100.0	97.5	23.8	27.0	25.4
	Brady. + B. polymyxa	122.0	133.0	127.5	30.5	36.6	33.6
	Brady. + B. megaterium	139.0	149.0	144.0	34.8	40.0	37.4
	Brady. + B. poly + B. mega	163.0	165.0	164.0	40.8	46.0	43.4
	Mean	129.8	136.8	133.3	32.5	37.4	34.9
Giza 21	Bradyrhizobia.	95.0	101.0	98.0	23.8	28.0	25.9
	Brady. + B. polymyxa	103.0	115.0	109.0	25.8	30.0	27.9
	Brady. + B. megaterium	120.0	133.0	126.5	30.0	36.0	33.0
	Brady. + B. poly + B. mega	140.0	154.0	147.0	35.0	43.0	39.0
	Mean	114.5	125.8	120.1	28.7	34..3	31.5
Giza 22	Bradyrhizobia.	107.0	117.0	112.0	26.8	32.1	29.5
	Brady. + B. polymyxa	108.0	119.0	113.5	27.0	35.0	31.0
	Brady. + B. megaterium	144.0	155.0	149.5	36.0	40.0	38.0
	Brady. + B. poly + B. mega	148.0	160.0	154.0	37.0	43.0	40.0
	Mean	126.8	137.8	132.3	31.7	37.5	34.6
General mean of inoculation	Bradyrhizobia.	107.0	117.0	112.0	26.8	32.1	29.5
	Brady. + B. polymyxa	108.0	119.0	113.5	27.0	35.0	31.0
	Brady. + B. megaterium	144.0	155.0	149.5	36.0	40.0	38.0
	Brady. + B. poly + B. mega	148.0	160.0	154.0	37.0	43.0	40.0

Conclusion

In the light of present results, it clearly that the maximum straw and seed yields as well as crude protein contents of straw and seeds were obtained when Giza 22 seeds inoculated pre sowing by *Bradyrhizobium* in combination with *Bacillus polymyxa* and *Bacillus megaterium.*

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