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Newly Isolated Rhizobacteria as Plant Growth Promoters For Maize Plant (Zea mays) under Water Stress

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Plant growth promoting rhizobacteria (PGPR) offers an attractive way to replace chemical fertilizers, pesticides, and supplements. In the current study, nine rhizobacterial strains isolated from the rhizoshere of clover (*Trifolium*) and wheat (*Triticum*) plants were tested for multiple plant growth promoting activities (NH_3, IAA production, catalase and oxidase activity). Later, they were genetically idendified by 16S RNA sequence analyses as *Bacillus subtilis*, *Bacillus cereus, Pseudomonas mendocina*, *Pseudomonas putida, Kocuria marina*, and *Kocuria rhizophila.* The rhizobacterial strains were then applied individually, for the evaluation of maize seedling growth promotion activity. Finally, the interaction between the rhizobacterial strains in addition to a previously identified rhizobacterium *Bacillus licheniformis*, was investigated by applying the Plackett-Burman experimental design under different levels of water stress. The percentages of germination, carbohydrate, and protein production were measured as physiological responses indicating the effect of the bacteria on plant health.

Key words: Plant growth promoting rhizobacteria, maize plant, Plackett-Burman, water stress.

Biofertilizers, recently refer to the use of soil microorganisms to increase the availability and uptake of mineral nutrients for plants [1-3]. Biofertilizers are also available for increasing crop nutrient uptake of nitrogen from nitrogen fixing bacteria associated with roots (*Azospirillum*), iron uptake from siderophore producing bacteria (*Pseudomonas*), sulphur uptake from sulfur–oxidizing bacteria (*Thiobacillus*) and phosphorus uptake from phosphate mineral solubilizing bacteria (*Bacillus* and *Pseudomonas*) [1].

Plant growth promoting rhizobacteria (PGPR) are a group of bacteria that actively colonize plant roots and increase plant growth and yield [2, 4]. Inoculation of crop plants with certain strains of PGPR at an early stage of development improves biomass production through direct effects on root and shoots growth. Inoculation of ornamentals, forest trees, vegetables, and agricultural crops with PGPR may result in multiple effects on early-season plant growth, as seen in the enhancement of seedling germination, stand health, plant vigor, plant height, and shoot weight, nutrient content of shoot tissues, early bloom, chlorophyll content, and increased nodulation in legumes [1]. In last few decades a large array of bacteria including species of *Pseudomonas*, *Azospirillum*, *Azotobacter, Klebsiella, Enterobacter, Alcaligens, Arthobacter, Burkholderia*, *Bacillus* and *Serratia* have been reported as rhizobacteria that enhance plant growth[5].

PGPR are now commonly used as inoculants for improving the growth and yield of agricultural crops and offers an attractive way to replace chemical fertilizers, pesticides, and supplements [3,4,6]. Utilization of PGPR in order to increase the productivity may be a viable alternative to organic fertilizers which also helps in reducing the pollution and preserving the environment in the spirit of an ecological agriculture [7]. In addition, PGPR have great

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adaptation to harsh environments including drought stress, salt stress, high temperatures, dryness or heavy rainfalls in tropical countries, and contaminated environments, indicating that they could contribute to ameliorate plant crops in areas with poor agricultural potential [1,8,9].

Maize is a major principal summer field crop in Egypt. It is planted on approximately 728,000 hectares of land. Each year, 6.1 million tonnes of maize are produced domestically. Moreover, 4.1 million tones of yellow maize are imported annually, valued at $US 1.3billion [10]. Maize kernels are technically a fruit 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 [11]. Maize is a major source of starch, cooking oil (Corn oil) and of maize gluten. Maize starch can be hydrolyzed and enzymatically treated to produce syrups, particularly high fructose corn syrup, 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 [11].

MATERIALS AND METHODS

Bacterial strains

Nine bacterial strains were isolated from soil samples collected from the rhizosphere of different agricultural plants (clover and wheat), growing in Nubaria, Egypt. *Bacillus licheniformis* HQ883968 isolated from agricultural soil, was kindly supplied by the Microbiology Lab, Scientific City for Research and Technology Applications, Alexandria, Egypt. Bacteria were maintained as pure cultures in Trypticase soy broth (TSB) and 30% glycerol, stored at 4ºC with periodic transfer to fresh media and stocked for further use.

Maize plants

Seeds of maize plants were obtained as a pure variety from the Agricultural Research Center, Ministry of Agriculture, Giza321, Egypt. The seeds used were selected for uniformity of size, shape, viability.

Isolation of plant growth promoting rhizobacteria

Intact root system of clover and wheat was dugout and the rhizospheric soil samples were carefully taken in plastic bags and stored at 4°C. The plant growth promoting rhizobacteria (PGPR) were isolated from the rhizosperic soil samples by serial dilution plate technique. Appropriate dilution was spread on tripticase soy agar (TSA) [12] and incubated at 28± 2ºC for 24-48h.Colonies showing different morphotypes were picked, purified and identified .

Identification of bacterial isolates

Rhizobacterial isolates were examined for colony and cell and morphology and Gram stain. Further identification was carried by 16S rDNA.

Phylogenetic analysis

DNA was isolated from the selected isolates according to **Sambrook *et al* [13].** The 16S rDNA was amplified by polymerase chain reaction (PCR) using primers designed to amplify 1500 bp fragment of the 16S rDNA region. The forward primer was 5'AGAGTTTGATCMTGGCTCAG3' and the reverse primer was 5'TACGGYTACCTTGTTACGACTT3'. The PCR mixture consists of 30 picomoles of each primer, 10ng of chromosomal DNA, 200 µM dNTPs and 2.5 units of Taq polymerase in 50 µl of polymerase buffer. The PCR was carried out for 30 cycles in 94°C for 1 min, 55°C for 1 min and 72 °C for 2 min. After completion, a fraction of the PCR mixture was examined using agarose gel electrophoresis [14] and the remnant was purified using QIAquick PCR purification reagents (Qiagen).

DNA sequences were obtained using an ABI PRISM 377 DNA Sequencer and ABI PRISM BigDye Terminator Cycle Sequencing (Perkin Elmer). The PCR product was sequenced using the same PCR primers. Blast program was used to asses the DNA similarities and multiple sequence alignment and molecular phylogeny were performed using BioEdit software [15]. The phylogenetic tree was displayed using the TREEVIEW program [16].

In vitro screening for multiple plant growth promoting activities.

Bacterial isolates were tested for ammonia production after growth in peptone water using Nessler's reagent. Indole production was tested by Kovac's reagent after cell growth in tryptone broth. Catalase test was performed by the addition of 3-4 drops of hydrogen peroxide (H_2O_2) to a 48 h old bacterial colony grown on trypticase soya agar

medium. Oxidase production was estimated by oxidase reagent **(**tetramethyl-p-phenylenediaamine dihydrochloride solution) **[5,17].**

Evaluation of maize seedling growth promotion with PGPRs (Pot experiment)

Seeds were surface sterilized with 0.1% $HgCl_2$ for 3 min and washed with distilled water for 4-5 times. The seeds were soaked for 6 h in 48 h old bacterial broth cultures containing at least 10^8cells/ml. 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 1 kg capacity. All treatments (bacterial inoculation and soil condition) were arranged as randomized design with 3 replicates for each treatment. After 15 days, percant of seed germination, root and shoot lengths were recorded. The Vigor Index (VI) was calculated according to **Ashkan and Jalal [18]** as follows:

VI (%) = (mean root length + mean shoot length) × germination %.

Analytical methods

Maize plants were analyzed for the following parameters. Total proteins were extracted according to Rausch [19] and estimated as described by Hartree [20]. Total soluble sugars were determined as described by Dubois *et al* [21].

Interaction of rhizobacterial strains and water stress

Plackett-Burman design is a rapid screening multifactor to find the most significant independent factor [22,23]. Plackett-Burman design was applied to evaluate the influence of 11 independent variables (ten rhizobacterial strains and water stress), and their interactions, on germination percentage of maize and other different responces. Plackett-Burman design was applied to pick organisms that influence germination percentage and physiological responces significantly, whereas, insignificant ones were eliminated in order to obtain smaller, more manageable set of factors (or organisms). Plackett-Burman is a technique devoted to the screening of controlled experimental factors and the measurement of their responses, according to one or more selected criteria.

Each variable was investigated at two levels, +ve sign for presence and –ve for absence, in case of bacterial isolates, while the same signs refer to 60% and 30% water content, respectively in case of water stress (Table 5). All trials were conducted in triplicate and the averages of all measured physiological activities were treated as the responses. The main effect of each variable was determined with the following equation:

$$E_{xi} = (\Sigma M_{i+} - \Sigma M_{i-}) / N$$

Where E_{xi} is the variable main effect, M_{i+} and M_{i-} are the measured physiological activity, where the independent variable (xi) was present in high and low concentrations, respectively, and N is the number of trials divided by 2. A main effect figure with a positive sign indicates that the presence of the variable (bacteria) or its high concentration (water stress) is nearer to optimum and a negative sign indicates that absence of the variable or its low concentration is nearer to optimum. Using Microsoft Excel, statistical *t*-values for equal unpaired samples were calculated for determination of variable significance.

Data analysis of the result of plackett-burman design

Excel (Microsoft Office, 2010) was used for the experiment design and all statistical analyses. The variables with confidence levels above 95% were considered as influencing different measured physiological responses significantly. The application of statistical design was carried out to evaluate the relative importance of various constituents within a complex culture medium and selecting of levels of the variables, which have significant influences on the reponses.

RESULTS AND DISCUSSION

Plant growth promoting rhizobacteria (PGPR) have gained world wide importance and acceptance for agricultural benefits, due to the emerging demand for diminishing of synthetic chemical products, and to focalize environmental protection[1]. The rhizosphere supports the development and acivity of a huge and diversified microbial community capable to promote plant growth. Among the latter, plant growth-promoting rhizobacteria (PGPR) colonize roots, and enhance plant growth by direct and indirect mechanisms [24]. The objective of the present work was to isolate and identify rhizobacterial strains, and applying an experimental design to evaluate their interaction together with water stress on growth of maize plant. Maize plant was chosen as one of the most important economic crops in Egypt, where the nine

isolates, in addition to *B. licheniformis* were evaluated for their effect on plant growth, yield, under water deficiency stress in pots conditions for 14 days to ascertain the involvement of possible PGPR traits in enhancing maize growth.

Isolation and identification of rhizobacterial strains

The count of isolated aerobic heterotrophic bacteria ranged from $50x10^9$ to $60x10^9$ CFU/g soil. Based on morphological differences and frequently appearance, nine isolates were selected. They were preliminary described phenotypically and further identified genetically. Data in Table 1 summarize the phenotypic characteristics of selected isolates. The majority (7 isolates) were rods, five of them formed endospores. Only two were Gram negative rods.

Molecular identification of bacteria

Based on 16S rRNA sequences, five isolates were assigned to Genus *Bacillus*, Family

Table 1. Morphological characteristics of bacterial isolates

Isolate	Cultural characteristics	Cell shape	Gram reaction
Tr1	Cream	Spore former rods	Gram positive
Tr2	White rough	Non spore former rods	Gram negative
Tr3	Lemon-yellow smooth	Cocci	Gram positive
Tr4	White	Spore former rods	Gram positive
Tr5	Lemon-yellow smooth	Cocci	Gram positive
Wh1	White	Spore former rods	Gram positive
Wh2	Blue-green rough	Non spore former rods	Gram negative
Wh3	White	Spore former rods	Gram positive
Wh4	White	Spore former rods	Gram positive

Table 2. Closest strains to bacterial isolates, similarity precent and accession numbers

Isolate code	Closest strain	Percent of identity	Accession number
Tr1	*Bacillus subtilis*	100%	KP878694
Tr2	*Pseudomonas mendocina*	99%	KP939245
Tr3	*Kocuria marina*	98%	KP878695
Tr4	*Bacillus cereus*	99%	KP939246
Tr5	*Kocuria rhizophila*	100%	KP939247
Wh1	*Bacillus subtilis*	100%	KP898878
Wh2	*Pseudomonas putida*	100%	KP898879
Wh3	*Bacillus cereus*	97%	KP898880
Wh4	*Bacillus cereus*	99%	KR007963

Table 3. Multiple PGPR activities investigated in tested rhizobacterial isolates.

Isolate	NH_3 production	IAA production	Catalase activity	Oxidase activity
B. subtilis Tr1	++	+	+	-
P.mendocina Tr2	++	++	_	+
K. marina Tr3	++	+	+	-
B.cereus Tr4	++	+	+	-
K.rhizophila Tr5	++	-	+	-
B.subtilis Wh1	++	+	+	-
P.putida Wh2	++	++	+	+
B.cereus Wh3	++	+	+	-
B.cereus Wh4	+	+	+	-
B.licheniformis HQ883968	+	+	+	-

Table 4. Effect of bacterial inoculations on seed germination and vigour index of maize plant Giza321, 15 days after germination in vivo conditions

Isolates	Average length of seedling (cm)		Germination	Vigour
	Root	Shoot	percentage	Index
B. subtilis Tr1	18.7	10.4	100%	2910
P.mendocina Tr2	18.8	9.5	100%	2830
K. marina Tr3	27	6.8	80%	2704
B.cereus Tr4	27	9.7	100%	3670
K.rhizophila Tr5	17.6	8.0	100%	2560
B.subtilis Wh1	28.4	8.8	100%	3720
P.putida Wh2	19.5	10.12	80%	2962
B.cereus Wh3	19.4	8.4	100%	2780
B.cereus Wh4	23	7.9	70%	2163
B.licheniformis	22	8.9	100%	3090
Control (Water)	16.2	7.6	80%	1904

Bacillaceae, Phyllum Firmicutes. Cells were typical Gram positive with endospore formation. Their 16Sr DNA sequences Tr1 and Wh1 showed 100% similarity to *Bacillus subtilis*. They were isolated from clover and wheat rhizosphere. On the other hand, the three isolates (Tr4,Wh3 and Wh4) were isolated from clover and wheat rhizosphere.They showed similarity percentages of 99, 97, and 99%, respectively to *Bacillus cereus*. Phyllum Actinobacteria was represented by two Gram positive non sporingTr3 and Tr5 isolated from clover rhizospere.Their sequences were 98% and 100% similar to sequences of *Kocuria marina and K.rhizophila*, respectively. The 16S rDNA sequences of the two Gram negative isolates were found to belong to genus *Pseudomonas*, subphylum Gammaproteobacteria. Isolate Tr2 was recovered from the rhizospere of clover, with 99% sequence similarity to *Pseudomonas mendocina*. Isolate Wh2 isolated from rhizospere of wheat was 100% similar to *P.putida.* Isolates code, closest strains, similarity percent and accession numbers of isolated bacteria are shown in Table 2. A tree (Fig 1) was constructed illustrating the relationship among isolated bacteria.

Rhizobacteria members found in this work are well documented to compete aggressively for sites in the rhizosphere and prevent proliferation of phytopathogens by niche exclusion, production of antibiotics and siderophores, or inducing systemic resistance; by stimulating plant growth by facilitating either uptake of nutrients from soil; or by producing certain plant growth promoting substances [1].

Bacillus strains and Actinomycetes are the most important groups of PGPR among Gram positive bacteria. Different *Bacillus* species were reported to be effective biocontrol agents in green house or field trials [25]. Mechanisms involved in *Bacillus* and Actinomycetes, eliciting plant growth promotion include auxin production, increased uptake availability of phosphorus, biocontrol abilities, and induction of systemic resistance [1,26]. In the opposite of *Pseudomonas* and other nonspore-forming bacteria, *Bacillus* spp. are able to form endospores that allow them to survive for extended periods under unfavorable environmental conditions. This trait is relevant in their relative durable viability when stored for a relatively long period (shelf-life) [1,26].

Other researchers demonstrated that plant promotion relies on the ability of the Actinomycetes to solubilize phosphate or to produce phytohormones[27-29], showing the great interest of actinomycetes solubilizing phosphate in soils deficient in available soluble phosphorus (P).

It is likely that more interest was addressed to the antibiotic production by actinomycetes or their biopesticide capacities since almost all works were initially interested in these topics. Thus, most studied PGPR actinomycetes possess antibacterial or antifungal activity as they were initially screened for works aiming to suppress a plant disease[30]. Also some species of *Pseudomonas* were recorded as highly aggressive colonizers of the rhizosphere of various crop plants

Table 5. Plackett-Burman experimental design for evaluation of 11 variables for germination percentage, production of carbohydrates and total proteins, by maize plant Giza 321, 15 days after germination in vivo conditions

Trial	Water stress	*B. subtilis* Tr1	*P. mendocina* Tr2	*K. marina* Tr3	*B. cereus* Tr4	*K. rhizophila* Tr5	*B. subtilis* Wh1	*P. putida* Wh2	*B. licheniformis*	*B. cereus* Wh3	*B. cereus* Wh4	Germination percentage %	Carbohydrate mg/g dwt		Total proteins mg/g dwt	
													Root	Shoot	Root	Shoot
1	+	+	+	+	+	+	+	+	+	+	+	86.6	0.999	1.234	0.279	0.856
2	-	+	-	+	+	+	-	-	-	+	-	80	0.632	0.759	0.241	0.773
3	-	-	+	-	+	+	+	-	-	-	+	93.3	0.617	1.082	0.237	0.619
4	+	-	-	+	-	+	+	+	-	-	-	73.3	0.582	0.700	0.243	0.399
5	-	+	-	-	+	-	+	+	+	-	-	80	0.634	0.732	0.447	0.669
6	-	-	+	-	-	+	-	+	+	+	-	86.6	0.700	0.925	0.322	0.371
7	-	-	-	+	-	-	+	-	+	+	+	66.66	0.588	0.678	0.149	0.237
8	+	-	-	-	+	-	-	+	-	+	+	80	0.613	0.855	0.364	0.649
9	+	+	-	-	-	+	-	-	+	-	+	80	0.476	0.757	0.361	0.425
10	+	+	+	-	-	-	+	-	-	+	-	93.3	0.623	0.838	0.394	0.529
11	-	+	+	+	-	-	-	+	-	-	+	86.6	0.794	1.043	0.408	0.704
12	+	-	+	+	+	-	-	-	+	-	-	93.3	0.687	1.238	0.355	0.618
Control												73.3	0.481	0.668	0.190	0.285

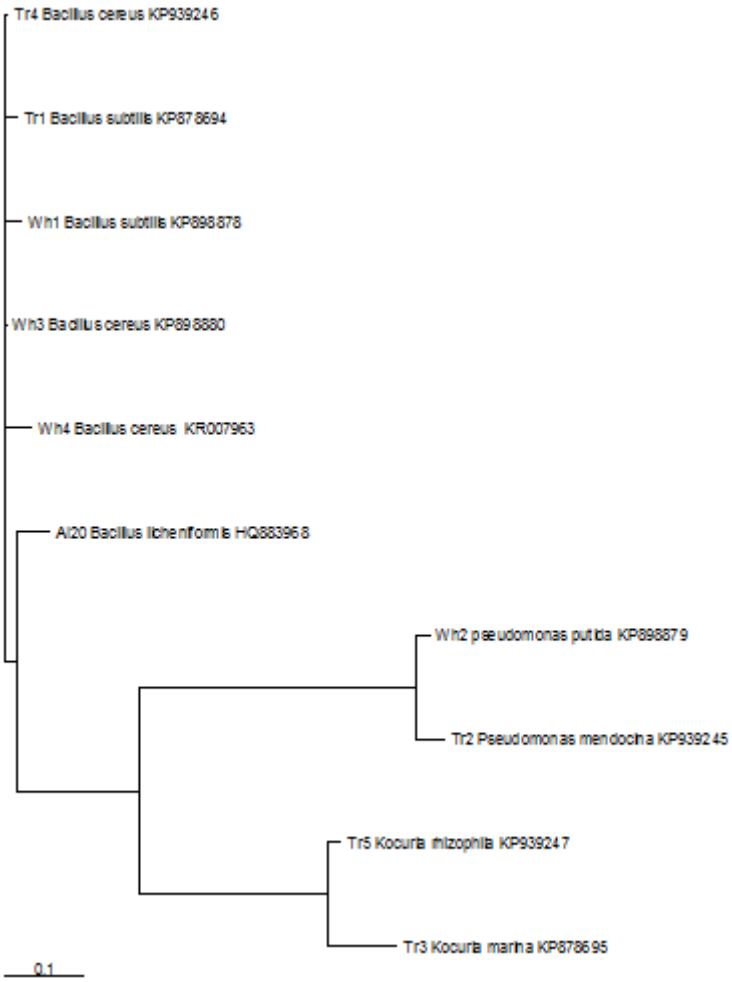

Fig. 1. 16S rDNA-based dendogram showing the phylogenetic position of isolated bacteria among representatives of related bacterial species. The tree was constructed by Bioedit method

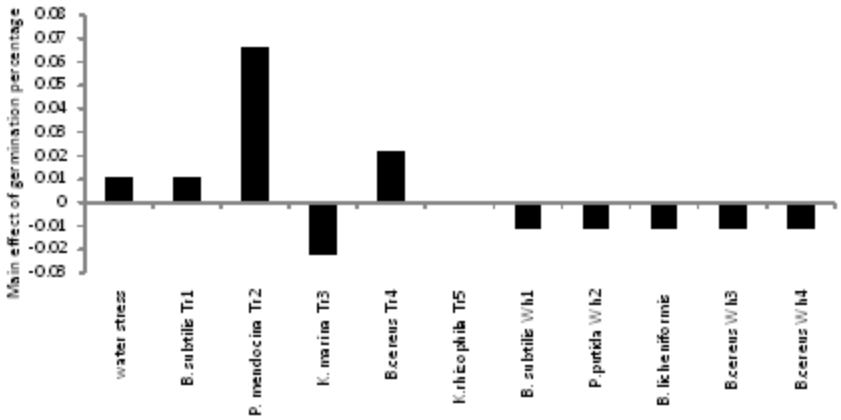

Fig. 2. Main effect of germination percentage for maize Giza 321, after inoculation by different bacterial treatments accompanied by water deficiency stress

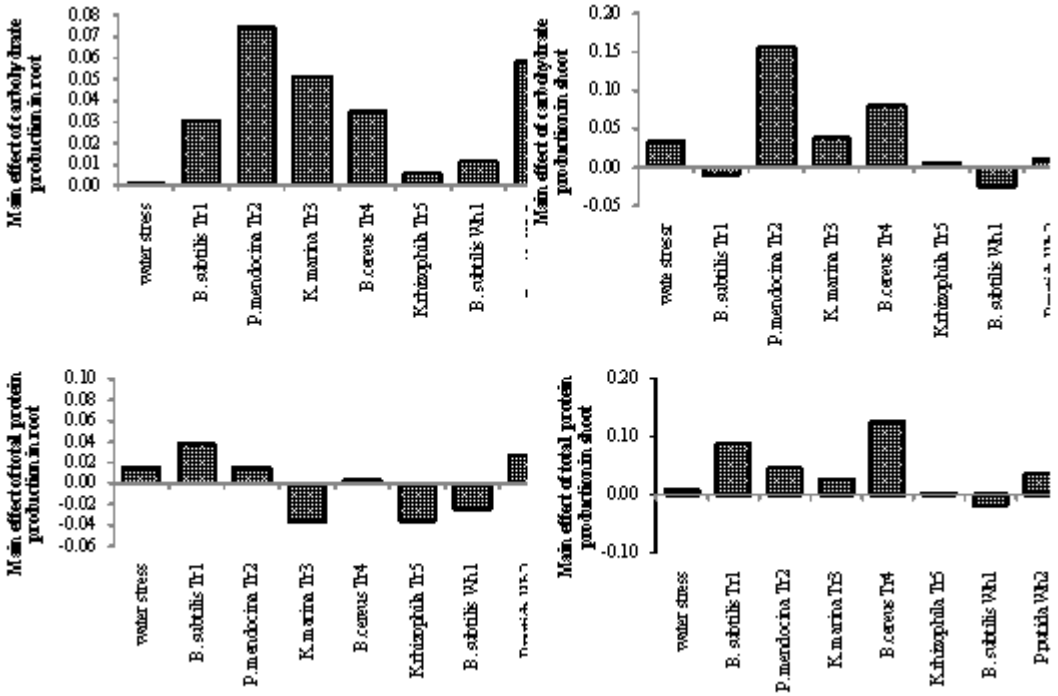

Fig. 3. Main effect for carbohydrates and total protein production, in root and shoot systems of maize Giza 321, after inoculation by different bacterial treatments accompanied by water deficiency stress

and has a broad spectrum antagonistic activity against plant pathogens like nematodes [31].

Screening for multiple plant growth promoting activities

The nine rhizobacterial isolates, in addition to *B.licheniformis* HQ883968 were then screened for their plant growth promoting activities as ammonia production, indole acetic acid (IAA) production, catalase and oxidase activities. All the tested isolates were positive for ammonia production which indirectly influence the plant growth. The same results were recorded for IAA production, except *K.rhizophila* Tr5 as shown in Table 3.

The ability of bacteria to produce indole acetic acid IAA in the rhizosphere depends on the availability of precursors and uptake of microbial IAA by plant. IAA is known to stimulate both rapid increases in cell elongation and long-term responses in plants [32]. Growth promotion may be attributed to other mechanisms such as production of plant growth promoting hormones in the rhizosphere and other PGP activities [1].

It has been assumed that inoculation with bacteria like *Bacillus, Pseudomonas, Rhizobium*, and *Azotobacter* may enhance the plant growth as a result of their ability to fix nitrogen[1]. Bacterial isolates in the present study were able to produce catalase. Bacterial strains showing catalase activity must be highly resistant to environmental, mechanical, and chemical stress[1]. Some of the above-tested isolates could exhibit more than two or three PGP traits, which may promote plant growth directly or indirectly or synergistically. Similar to our findings of multiple PGP activities among PGPR have been reported by some other workers while such findings on indigenous isolates of India are less commonly explored [33].

In vivo screening of isolates for multiple plant growth promoting activities

All rhizobacterial strains were screened individually for their ability to enhance growth promotion. Results revealed that the two strains *B. cereus* Tr4, and *B. subtilis* Wh1, showed pronounced effect in improving plant growth, as observed by almost the double increase in vigour index compared to control. Other *Bacillus* species showed relatively high germination percent (Table 4). *Bacillus* species have been reported as plant promoting bacteria in a wide range of plants [26, 34].

The two *Kocuria* species showed good vigour index. There is a well-documented history for species belonging to *Kocuria* genus as IAA producers [35]. *K. varians, K. rhizophila, K. flava*, and other unnamed species of *Kocuria* have been reported to produce large amount of indole acetic acid (IAA) [1, 36]. *Pseudomonas* spp. are well known as growth promoting rhizobacteria [1]. In the present work, *P.mendocina* Tr2 caused 100% seed germination on maize plant, with vigour index of 2830, whereas, *P.putida* Wh2 caused only 80% germination with a vigour index of 2962.

Plackett-Burman experimental design for evaluation of 11 variables on different reponses in maize plant Giza321

The Plackett-Burman experimental design, a fractional factorial design[22], was used in this research to reflect the relative importance of various rhizobacterial isolates in addition to water stress on growth of maize plant Giza321. Eleven independent variables were screened in twelve combinations organized according to the Plackett-Burman design matrix described in Table 5. For each variable, a high (+) and low (-) level was tested.

In general, the combination of all bacterial strains, in presence of 60% water level, had the most pronounced effect on germination percentage, carbohydrate and total protein content (Trial 1), in comparison to control (Only water with no bacterial addition).

Regarding the germination percentage of maize plant Giza 321, one could recognize that *P.mendocina* Tr2 was the most positively significant rhizobacterium, followed by *K. marina* Tr3 and *B. cereus* Tr4 as shown in Figure 2. Again, the isolated rhizobacterium *P.mendocina* Tr2, proved to have the highest significant effect on carbohydrate production in both shoot and root systems of maize plant, followed by the same previously mentioned strains. However, in case of carbohydrate produced specifically by the root, the rhizobacterium *P.putida* Wh2, showed a very observable significant effect, which makes it comes directly in the second place after *P.mendocina* Tr2 (Figure 3).

The calculated main effect for total proteins produced by root system of maize plant Giza 321, proved that the two isolated rhizobacteria, *B.subtilus* Tr1 and *P.putida* Wh2, had a positive significant effect. On the contrary, the isolates *K. marina* Tr3, *K.rhizoplila* Tr5, *B. subtilis* Wh1, and *B. cereus*Wh3, had a negative effect on total proteins production. On the other hand, in case of shoot system, total proteins were only positively affected by the presence of *B.subtilus* Tr1 and *B.cereus* Tr4 (Figure 3).

Water stress, as abiotic factor, was found to have neither positive nor negative significance on all the previously measured physiological responses. This null effect could be referred to the action of the investigated rhizobacteria, repairing any consequence damages that might take place to the plant.

Several environmental stimuli, such as low nutrient or water availability, can reduce or halt cell division or elongation, leading to an arrest of primary-root growth accompanied by a stimulation of lateral-root emergence [37]. Drought stress is one of the most adverse factors for plant growth and productivity [38]. Drought affects different aspect of plant growth, through a series of morphological, physiological and metabolic changes [39] and reduces the yield of plant. These PGPR *e.g., Rhizobium, Azospirillum*, *Pseudomonas*, *Flavobacterium*, *Arthrobacter* and *Bacillus* utilize osmoregulation, oligotrophic, endogenous metabolism, resistance to starvation, and efficient metabolic processes to adapt under dry and saline environments [40]. These bacteria, with a physiological adaptation and genetic potential for increased tolerance to drought, increasing salt concentration, and high temperatures, could improve plant production in degraded sites. Rajasekar and Elango [41] observed that a combination of PGPR strains *Azospirillum*, *Azotobacter*, *Pseudomona*s and *Bacillus* significantly increased plant height, root length, and alkaloid content in *Withania somnifera* compared to the uninoculated control.

Ordookhani *et al* [42] found an increase in root dry weight, N, P and potassium (K) content and essential oils in *Ocimum basilicum* inoculated with PGPR *Pseudomonas putida* and *Azotobacter chroococcum*.

Accumulation of reactive oxygen species (ROS) is one of the biochemical changes and occurred when plants are exposed to drought stress condition [43]. ROS include super oxide (O_2), hydrogen peroxide (H_2O_2), hydroxyl (OH) [44]. These ROS are cytotoxic for cells [45] and in high density,

hurt cells lipids, proteins and nucleic acids and finally stop the natural metabolism of plant. Chloroplast and mitochondria of plant cell are the major intracellular generator of reactive oxygen species .When plants suffering from drought stress, they show a series of physiological, morphological and biochemical reactions to resist against the stress condition.

The greater induction of stress related enzymes in plants may be the mechanism through which these PGPRs help plants to tolerate the consequences of drought stress and maintenance of plant homeostasis under severe drought. It is also suggested that PGPR, which produce phytohormones, can prevent the deleterious effects of stresses from the environment [1].

Carbohydrates are compounds produced during photosynthesis. In plants, they have two main purposes. First, they provide building blocks for plant structural components, such as cellulose (important in building cell walls). Secondly, carbohydrates are molecules that deliver energy for plant growth.

Proteins serve as important components of the major signaling and biochemical pathways. Protein variation is an essential part of plant response to environmental stress as well as for adaptation to environmental conditions [46]. Abiotic stress with a dehydration component (draught, salt and freezing) involves as a common feature, increased numbers of inactive proteins-denatured, aggregated or oxidetively damaged [47]. Some authors reported that rhizobacteria enhanced protein concentration in plants [48] probably due to stimulation of protein biosynthesis processes in plants, providing in this way plant seeds with higher nutritional value.

CONCLUSION

From the current study, one could conclude that newly isolated rhizobacterial strains belonging to the genera, *Bacillus*, *Pseudomonas*, and *Kocuria*, could have a great potential on acting as biofertilizer and consequently enhancing maize plant growth, inspite of being isolated from other different plants. In addition, such strains acted in this way while the plant was under harsh abiotic condition like water stress. Such strategies will be useful in reducing the chemical loads into plant production and a move towards chemical free herbals.

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The authors declare that there is no conflict of interests.

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