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Enhancement of growth behaviors in *zea maize* seedling using PGPB as safety fertilizers

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Abstract: The need for beneficial microbes as plant growth promoters is extensively growing and widening in agricultural applications. The current study was designed to test the effect of several isolated plant growth promoting bacteria on the growth of maize seedlings under laboratory conditions. The seeds were treated with *Bacillus amyloliquefaciens* MG214652 and *Enterobacter cloacea* MT012825 and left for five days at 25°C. Growth of maize as well as germination parameters showed a significant positive response to bacterization, particularly *Enterobacter* MT012825. Interestingly, flavonoids, proline, and phenols as well as antioxidant enzymes showed the same attitude in response to bacterial treatments. The output of this experiment emphasizes the responsive attitude to *Z. maize* seedlings in response to bacterial treatments to various degrees.

Keywords: growth, flavonoids, proline, phenols, *Zea maize.*

1.Introduction

In the sake of sustainable agriculture, the use of plant growth promoting microorganisms is considered a potential low-cost and plausible solution rather than plant genetic engineering. Additionally, the adaptability of the applied organism(s) should be considered for effective application and subsequently plant productivity**[1]**. Plant growth promoting microorganisms particularly bacteria (PGPB) started to gain attention since last century**[2]**. In that regard, various bacterial strains including *Rhizobium*, *Pseudomonas*, *Bacillus* and *Enterobacter* have shown their ability to improve crop plants growth and productivity**[3]**. The role of these bacteria is not limited to growth promotion as some of them has been employed as bio-control agents**[4]**

The activity of PGPB in this interaction is still to be discovered. Recently it was found that PGPB are sensed and further reprogram gene expression patterns in plants to help plants overcome both biotic and abiotic stresses via activation of induced systematic resistance**[5]**. Directly PGPB could mobilize nutrients such as phosphorus, produce auxins and siderophores to mobilize iron and commit ACC-deaminase activity**[6]**. Additionally, PGPB could antagonize phyto-pathogens in the rhizosphere region**[7]**. These capabilities and more are sufficient for plants to sustain life under biotic and abiotic stress.

Effectively, the application of PGPB would not completely replace but reduce to a significant extent the use of different agrochemicals including chemical fertilizers, pesticides, and growth substances. This is the smart solution that other attitudes failed to approach. In that regard, two isolated PGPB isolated and characterized previously in our lab have been applied for maize seedlings to verify their effect on growth parameters as well as metabolism under controlled laboratory conditions.

2. Materials and methods

2.1.The used Seed material and bacterial isolates:

The used *Z. maize* seeds (Hybrid Sc10) were obtained from the Agricultural Research Center, Ministry of Agriculture, Egypt. *Bacillus* MG214652 was donated by Mona Agha, Botany Department, Faculty of Science, Mansoura University**[8]**. *Enterobacter* MT012825 was donated by Sherouk Tarek,

Botany Department, Faculty of Science, Mansoura University.

2.1.Germination experiment

A group of homogenous seeds were selected and divided into three groups (20 seeds for each group), the seeds were then surface sterilized by soaking in 30% sodium hypochlorite for 15 mins, and then in order to get rid of any chemical remains, they were washed three times with distilled sterilized water under aseptic conditions.

The two strains (Figure 1) of plant growth promoting bacteria were used separately and upon that the treatments were as follow:

- Water
- *Bacillus* MG214652.
- *Enterobacter* MT012825.

Water-treated maize served as a control for this experiment. A single colony of each bacterial strain was allowed to grow in Luria-Bertani (L.B) media and incubated on rotating shaker at 37°C and 150 rpm until the optical density of the bacteria at 600 nm reached about (0.5). The bacterial cultures were then centrifuged, and the pellet was resuspended in 200ml sterilized distilled water. The sterilized seeds were then soaked in each treatment for one hour, the seeds were then sown on sterile wound dressing in small, sterilized boxes and sprayed with 30ml distilled sterilized water, the boxes were then incubated in growth chamber settled at 25 °C for five days. The seeds were checked daily and watered evenly when necessary. After the indicated incubation period, the growth parameters were assessed and calculated using the following equations**[9]**:

$$MGT = \frac{\sum F \times X}{\sum F}$$

Where F is the number of seeds newly germinated at the time of X, and X is the number of days from sowing.

Germination rate = (a/1) +(b-a/2) +(c-b/3) +.....+(n-n-1/N)

Where a, b, c, …, n are numbers of germinated seeds after 1, 2, 3, …, N days from the start of imbibition.

Vigor index I = Germination% × Seedling length (cm)

Vigor index II = Germination% × Seedling weight (g)

Evaluations of Mean Daily Germination (MDG), Pick Value (PV) and Germination Value (GV) were calculated by the following equations:

MDG = Germination% / total experiment days

PV = Maximum germinated seed number at one day / day number

GV= PV × MDG.

2.1.Estimation of total phenols

For total phenolic content, methanolic extract was prepared by extracting 2 g powdered dry tissue in 20 ml of 50% methanol for a week at 37°C, followed by filtration. 1ml of the methanolic extract was mixed with 1ml of (1:10) diluted Folin-Ciocalteu reagent. After 3 mins, 1ml of a saturated sodium carbonate solution was added, the mixture was mixed well and completed to 10ml by distilled water, then kept in the dark for 90mins, after which the absorbance was measured at 725nm**[10]**.

2.1.Estimation of total flavonoids

The reaction mixture was prepared by mixing 1ml methanolic extract, prepared as mentioned previously, with 4 ml distilled water and 0.3ml of 5% $NaNO_2$, after 3mins 0.3ml of 10% $AlCl_3$ was added. The mixture was allowed to stand for 6mins before adding 2ml NaOH (1M), the reaction mixture volume was completed to 10 ml using distilled water, mixed well, and the optical density was measured at 500nm**[11]**.

2.1.Estimation of proline

Plant water extract was prepared by incubating 0.1 gm dry tissue powder in 10ml water at 90°C for 1hr, then centrifuged ,and the pellet was extracted twice, the combined supernatant was raised up to 10ml. Proline was estimated by mixing 1ml plant water extract with 1ml glacial acetic acid and 1ml ninhydrin reagent, the mixture was then incubated in boiling water bath for an hour, after cooling, the absorbance was measured at 510nm**[12]**.

2.1.Estimation of antioxidant enzymes

2.6.1.Enzyme extraction:

Leaves (0.2 g) were grounded into liquid nitrogen and homogenized at 4°C in 5ml potassium phosphate extraction buffer. The

homogenate was then centrifuged at 4°C at 10,000 rpm for 15 min. The supernatant was obtained, fractionated into 1ml aliquots, and kept in -20^0 till use**[13]**.

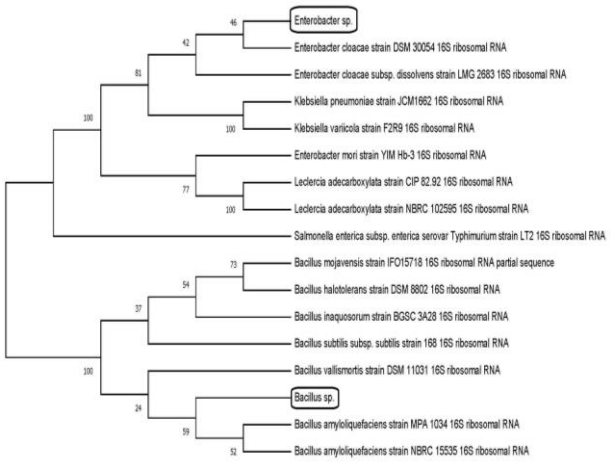

Figure (1): Phylogenetic analysis of the used strains in this experiment. 16s rRNA analysis along with phylogenetic analysis with relevant strains show that *Enterobacter* and *Bacillus* are closely relevant to *Enterobacter cloacea* and *Bacillus amyloliquefaciens* respectively.

2.6.2. Detection of peroxidase (POD) activity:

The assay mixture contains: 125 µM of phosphate buffer, pH 6.8, 50 µM of pyrogallol, 50 µM of H_20_2, and the enzyme extract. And the increase in absorbance was measured at 420 nm**[14]**.

2.6.3. Detection of polyphenol oxidase (PPO) activity:

The PPO will be assayed using the same mixture as peroxidase but without the addition of H_20_2. The increase in absorbance was recorded at 420 nm**[15]**.

2.6.4. Detection of catalase (CAT) activity:

Catalase will be assayed in reaction mixture containing potassium phosphate buffer, pH 7.0, 11 mM H_20_2, and enzyme extract. Activity was determined by monitoring the change in absorbance at 240 nm**[16]**.**Results**

3.1. The used bacterial strains greatly affect the apparent growth parameters of *Z. maize* seedlings as shown in Table 1. The measurements of root length showed a significant increase in response to bacterization with *Enterobacter* MT012825 as well as *Bacillus* MG214652 compared to the control. Shoot length increased in case of *E.* MT012825, while there was a significant

decrease in case of *B.* MG214652 treatment compared to that of the control, as shown in

figure (2). Looking for root and shoot fresh weights, the seedlings showed a significant

increase in case of treatment *E.* MT012825 while there was almost no change in case of *B.* MG214652 treatment. As for the dry root and shoot weights, the increase was significant with

E. MT012825, but no or a very slight increase in the case of *B.* MG214652. The treatment

E. MT012825 also showed a significant increase considering seedling length, seedling fresh and dry weights.

Table 1: Effect of bacterial treatments on growth parameters of *Z. maize* plants.

Treatment	Rootlengh (cm)	Shoot length(cm)	Root fresh weight (g)	Shoot fresh weight (g)	Rootdry weight (g)	Shootdryweight(g)	Seedling length(cm)	Seedling freshweight (g)	Seedling dry weight(g)
Water	10.43^c ±0.38	6.73^b ±0.1	0.10^b ±0.01	0.20^b ±0.01	0.02^b ±0	0.02^b ±0	17.7^c ±0.24	0.30^b ±0.01	0.04^b ±0
***B.*MG214652**	13.50^b ±0.29	6.10^c ±0.06	0.12^a ±0.01	0.20^b ±0	0.02^b ±0	0.02^b ±0	19.60^b ±0.32	0.31^b ±0.01	0.04^b ±0
***E.*MT012825**	18.33^a ±0.17	8.07^a ±0.12	0.14^a ±0	0.23^a ±0.02	0.03^a ±0	0.03^a ±0	26.40^a ±0.26	0.37^a ±0.02	0.07^a ±0
LSD	1.02	0.39	0.02	0.04	0.00	0.00	0.96	0.05	0.01

The results are recorded as Mean of triplicates ± Standard Error (S.E). Different superscript letters refer to significant variation; with the least significant difference (LSD) at *P = 0.05* using COSTAT software.

Considering the germination parameters; mean germination time (MGT), germination percentage (GP), mean daily germination (MDG), germination rate (GR), pick value (PV) and germination value (GV) the bacterial treatments showed

a non-significant increase compared to the control, while in case of vigor index I and vigor index II the treatments *E.* MT012825 and *B.* MG214652 showed a significant increase respectively, compared to the water control as shown in Table 2.

Table 2: Effect of bacterial treatments on the germination parameters; mean germination time (MGT), germination percentage (GP), mean daily germination (MDG), germination rate (GR), pick value (PV), germination value (GV), vigor index I (VI1), vigor index II (VI2) of *Z. maize* seedlings.

Treatment	MGT	GP	MDG	GR	VI1	VI2	PV	GV
Water	2.43^{a} ±0.03	96.6^{b}±1.67	19.33^{b} ±0.33	8.33^{c}± 0.14	1660^{c} ±47.91	3.57^{c} ±0.12	5.83^{b} ±0.17	112.83^{b}± 4.51
***B.*MG214652**	2.38^{b} ±0.03	96.6^{b} ±1.67	19.33^{b} ±0.33	8.56^{b}± 0.24	1894.83^{b} ±4819	4.09^{b} ±0.16	5.67^{c} ±0.44	109.33^{c}± 7.20
***E.* MT012825**	2.38^{b} ±0.03	100^{a} ±2.89	20^{a} ±0.58	8.75^{a}± 0.29	2640.50^{a} ±89.24	6.59^{a} ±0.10	6.33^{a} ±0.44	126.83^{a}± 10.1
LSD	0.11	7.45	1.49	0.81	224.10	0.45	1.29	27.47

The results are recorded as Mean of triplicates ± Standard Error (S.E). Different superscript letters refer to significant variation; with the least significant difference (LSD) at *P = 0.05* using COSTAT software.

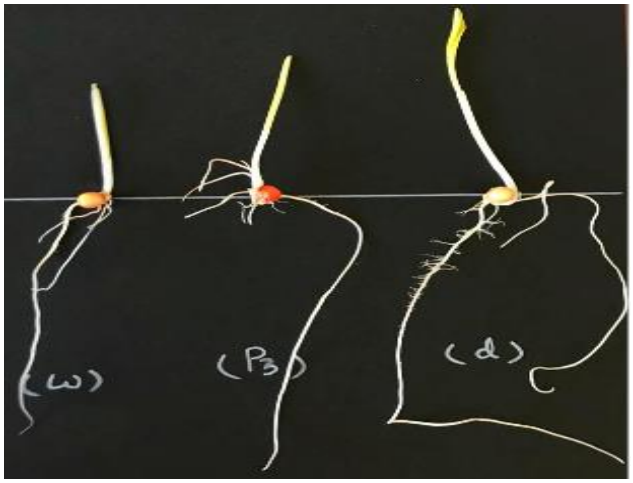

Figure (2): *Zea maize* seedlings bacterized with two candidates of PGPB compared to the control; (W) the water control; (P3) *Bacillus.*MG214652; (d) *Entero.*MT012825**.**

3.2. As shown on Table 3, the difference among the treatments in root and shoot water percentage was not significant. For proline, phenolic and flavonoid content the treatments showed non-significant difference in case of proline and phenolic content, while the amount of flavonoids was significantly decreased with the bacterial treatments compared to the control. The antioxidant enzymes: catalase, peroxidase, poly phenol oxidase showed differences in their activity with the different treatments. The catalase activity found to be significantly increased in case of *B.*MG214652 and *E.*MT012825 treatment respectively, while the increase in the activity of poly phenol oxidase and peroxidase enzymes was minor compared to the control.

Table 3: effect of bacterial treatments on root and shoot water percentage (w%), proline, total phenolic and flavonoid content, and antioxidant enzymes of *Z. maize*.

Treatment	Root water %	Shoot water %	Proline (µmolesg^{1}d.wt)	Phenols (µg g^{1}d. wt.)	Flavonoids (µg g^{-1}d. wt.)	CAT (U mg^{-1} protein)	PPO (U mg^{-1} protein)	POD (Umg^{-1} protein)
Water	81.63^{b} ±2.49	90.43^{a} ±0.62	2.35^{b}±0.08	11.46^{a} ±0.30	0.52^{a} ±0.06	35.99^{c} ±0.21	1.34^{b} ±0.02	6.38^{a} ±0.12
*B.*MG214652	82.82^{a} ±2.02	88.39^{b} ±0.26	2.50^{a}±0.01	10.68^{c} ±0.51	0.29^{b} ±0.05	111.71^{a} ±0.66	1.70^{a} ±0.09	6.68^{a} ±0.12
*E.*MT012825	76.17^{c} ±0.92	85.39^{c} ±0.88	2.14^{c}±0.05	11.42^{b} ±0.52	0.35^{ab} ±0.06	61.09^{b} ±0.01	1.51^{ab} ±0.5	2.47^{b} ±0.13
LSD	6.68	2.21	0.19	1.58	0.20	2.04	0.22	0.42

The results are recorded as Mean of triplicates ± Standard Error (S.E). Different superscript letters refer to significant variation; with the least significant difference (LSD) at *P = 0.05* using COSTAT software.

4.Discussion

Plant growth promoting bacteria (PGPB) have proven themselves in agricultural applications not only as a biofertilizers but also as biocontrol agents against phytopathogens. Moreover, its effectiveness in abiotic stress conditions of drought and salinity and its ability

to give plants mechanisms to confront these conditions made it an ideal solution to many agricultural problems.

Both strains used in this study; *Enterobacter cloacae* (MT012825) and *Bacillus amyloliquefaciens* (MG214652), have shown a significant ability to support soybean growth under salt stress**[17]**. That might be attributed to its assessed promoting criteria such as the production of IAA, siderophores, phosphate solubilization and others, criteria those might be behind their ability to promote Z. maize seedling root, shoot length, water content and total growth. Several articles support the capability of *Enterobacter* as a growth promoter**[2, 18, 19]**. *Bacillus amyloliquefaciens* not only regarded as growth promotor but also it is considered as a potential bio-control agent against various phyto-pathogens**[20-22]**. The enhancement in germination parameters observed for *Enterobacter* treated seedlings was also recorded for sunflower and soybean seedlings in previous studies**[17, 23]**. *Bacillus amyloliquefaciens* showed the same effect on *Vicia faba* germination in a greenhouse experiment**[24]**. This effect might be attributed to their ability to stimulate plant growth via IAA, siderophores and ammonia production in addition to their ability to solubilize phosphate. Phytohormonal level alters plant metabolic pathways toward nutritional needs and immunity.

In this study there was no clear effect of the used PGPB upon phenols or flavonoid quantities although other studies reported the significant effect of PGPB upon these secondary metabolites**[25, 26]**. Catalase and polyphenol oxidase activity were affected due to bacterization, a result that has been obtained when mung bean was treated with *Pseudomonas***[27]**. PGPB not only affect the nutritional status of plant, but also it affects plant defense pathways against both biotic and abiotic factors via a poorly understood mechanism**[28]**.

Collectively the results represented in this study needs further experiments to fully elucidate the molecular mechanism behind the effect of PGPB on *Z. maize* immunity and growth response.

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