Journal of
Plant Pathology & Microbiology
ISSN: 2157-7471

Abd El-Ghany et al., J Plant Pathol Microb 2015, 6:9
DOI: 10.4172/2157-7471.1000305

Research Article Open Access

Maize (*Zea Mays* L.) Growth and Metabolic Dynamics with Plant Growth-Promoting Rhizobacteria under Salt Stress

Abd El-Ghany TM*, Masrahi YS, Mohamed A, Al Abboud, Alawlaqi MM and Nadeem I Elhussieny

Biology Department, Faculty of Science, Jazan University, 114, KSA

Abstract

Maize (*Zea mays* L.) biomass and its allied attributes were assessed under salinity stress and three plant growth-promoting rhizobacteria (*Pseudomonas fluorescens*, *Pseudomonas putida* and *Azotobacter vinelandii*) treatments. The three PGPRs inocula exhibited a different pattern of shoot growth under both normal and saline stress conditions. Plant biomass, carbohydrates, protein and chlorophyll content were reduced by saline stress, however application of PGPRs treatments improved them either in comparison to control samples or to untreated samples under saline stress. Lipids and antioxidant enzymes (catalase and peroxidase) increased as a response for saline stress as an indication of oxidative stress. Plant growth-promoting rhizobacteria treatment restored them to semi-normal levels. Sodium/ potassium balance was observed to be disturbed by saline stress through higher levels of Na^+ and lower levels of K^+, but treating samples balance was clearly restored close to normal conditions especially in the root system.

Keywords: *Zea mays* L.; Plant growth-promoting rhizobacteria; Salt stress

Introduction

Biofertilizers diminish the need for expensive chemical fertilizers in crop farming systems because of they are an inexpensive source of nitrogen that increase crop yields. Thus the extensive use of biofertilizers would provide economic benefits to farmers, improve the socio-economic condition of the people and preserve natural resources. Biofertilizers are ecofriendly inputs and are less damaging to the environment when compared to chemical fertilizers [1,2]. Beneficial rhizobacteria, often referred to as plant growth-promoting rhizobacteria (PGPR), affect plant growth either directly or indirectly through various mechanisms of action [3-6]. Although, the mechanisms by which PGPRs promote plant growth are not fully understood, some mechanisms include gibberellic acid and/or cytokinins production, nitrogen fixation, and solubilization of mineral phosphate and other nutrients [7].

Maize (*Zea mays* L.) is the third most important cereal after wheat and rice all over the world [8]. Subramaniyan *et al.* [9] stated, "the application of biofertilizers improved the total carbohydrate, protein, amino nitrogen and chlorophyll content of of *Zea mays*". Five growth-promoting strains (*Azotobacter* sp. Lx191, *Pseudomonas* sp. Jm92, *Bacillus* sp. LM4-3, *Bacillus* sp.LH12-3, and *Azospirillum* sp. LHS11) were previously isolated from rhizosphere of wheat, maize, oat in arid fields,. These strains were proofed to stimulate these plant growth under controlled conditions via *in vitro* and pot experiments [10,11].

Soil salinity decreases plant growth, reduces photosynthetic activity and results in nutrient imbalance in plants.It was reported that PGPR significantly increased shoot/root fresh weight, shoot/root dry weight, chlorophyll a, b and cartenoid contents of maize under salt stress. PGPR can induce plant tolerance to salinity by producing various hormones and enhancing the availability of nutrients from the soil matrix [12]. Hasnain and Sabri [13] reported that inoculation with *Pseudomonas* sp. stimulated plant growth by reduction of toxic ion uptake and production of stress-specific proteins in plant. PGPR strains can also produce exopolysaccharides (EPSs) to bind cations including sodium, thus help alleviating salt stress in plants grown under saline environment [14]. The rhizosphere is the soil portion found around the root and under the influence of the root. It is the site with complex interaction between the root and associated microorganisms [15]. The rhizosphere harbors a multitude of microorganisms that are affected by both abiotic and biotic stresses. Among these are the dominant rhizobacteria that prefer living in close vicinity to the root or on its surface and play a crucial role in soil health and plant growth [16,17]. It has been noted by many workers that *Pseudomonas*, *Bacillus*, *Arthrobacter*, *Azospirillum*, *Klebsiella*, and *Enterobacter*, isolated from the rhizosphere of various crops, showed synergistic effects on plant growth [18]. Weller [19] reported that PGPR belong to several genera, e.g. *Agrobacterium, Alcaligenes, Arthrobacter, Actinoplanes*, Azotobacter, *Bacillus*, *Pseudomonas* sp., *Rhizobium*, *Bradyrhizobium*, *Erwinia*, *Enterobacter*, *Amorpho sporangium*, *Cellulomonas*, *Flavobacterium*, *Streptomyces* and *Xanthomonas* .These groups of bacteria are important as they are involved in various soil biochemical processes such as fixation of atmospheric nitrogen, solubilization of minerals such as phosphorus, production of siderophores that solubilize and sequester iron and/or production of plant growth regulators [20]. Nitrogen-fixing bacteria are widely distributed in nature where they reduce atmospheric nitrogen in soil or in association with plant [21]. They have been found in a wide variety of terrestrial and aquatic habitats in both temperate and tropical regions of the world [22]. Biofertilizer contains living microorganisms and promotes growth by increasing the availability of primary nutrients (nitrogen and phosphorus) to the host plant [23-26]. Among the free-living nitrogen-fixing bacteria those belonging to genus Azotobacter play a remarkable role, being broadly dispersed in

***Corresponding author:** Abd El-Ghany TM, Biology Department, faculty of Science, Jazan University, KSA, E-mail: tabdelghany@yahoo.com

Received September 16, 2015; **Accepted** September 28, 2015; **Published** September 30, 2015

Citation: Abd El-Ghany TM, Masrahi YS, Mohamed A, Abboud A, Alawlaqi MM, et al. (2015) Maize (*Zea Mays* L.) Growth and Metabolic Dynamics with Plant Growth-Promoting Rhizobacteria under Salt Stresses. J Plant Pathol Microb 6: 305. doi:10.4172/2157-7471.1000305

different environments, such as soil, water and sediments [27]. Several authors have shown the beneficial effects of *Azotobacter chroococcum* on vegetative growth and yields of maize [27-29], as well as the positive effect of inoculation with this bacterium on wheat [30]. *Bacillus* group was the most dominant strain found in the three types of biofertilizer products. The other bacteria were *Azospirillum*, *Corynebacterium*, *Pseudomonas* and *Proteus mirabilis*. These bacteria have the potential to fix atmospheric nitrogen, able to produce IAA with the supplemented tryptophan, and showed some phosphorus solubilizing activity [15,31]. The aim of this work was to assess the effect of PGPR on the growth of *Z. mays*. Also it was carried out to elucidate the role of PGPR on growth and ion uptake of maize under salt stress condition.

Materials and Methods

Description of the study area

The present study focused on the area in Jazan. The study area is situated in Jazan, Kingdom of Saudi Arabia (Lat. 16°53′21″ N , Long. 42°33′03″ E and 19 m Elevation above sea level) with the significant features of evergreen forests and also it was a less explored ecosystem for the investigation of biofertilizers population.

Bacterial strains used

Plant growth-promoting rhizobacteria were isolated from maize rhizosphere growing in Jazan area. Selection of isolates was performed on the basis of the PGPR traits. Selected isolates identified according standard microbiological methods as described in Bergys Manual of Systematic Microbiology [32]. The physiological and biochemical characters, included: starch hydrolysis, gelatin liquefaction, indole production, nitrate reduction, urease activity, citrate utilization, production of oxidase, catalase, methyl red, voges proskauer, tryptophane deaminase, gelatinase, lysine decarboxylase, arginine dihydrolase, ß-galactosidase and fermentation oxidation of the following carbon sources (D-glucose, D-mannitol, inositol, D-sorbitol, rhamnose, D-sucrose, D-melibiose maltose, fructose, inulin and L-arabinose) were used for identification of bacterial isolates. Bacterial isolates were grown on yeast manitol agar (YMA) supplemented with different concentrations of NaCl for salt tolerance test. Identification of highly NaCl tolerance was done using biochemical analysis. Three isolates including *Pseudomonas fluorescens, Azotobacter vinelandii* and *Pseudomonas putida* were used in this study.

Inoculums preparation and *Zea mays* L. growth experimental design

Fresh cultures of selected isolates were inoculated in pikovisky media and shaken at 37°C for two days in an orbital shaker at 100 rpm. After two days bacterial cultures were centrifuged at 3000 rpm for 15 mins. Maize (*Z. mays* L.) grains were surface sterilised with 0.5% (v/v) NaOCl for 10 min and were subsequently washed with sterilized deionised water. The sterilized grains were soaked in distilled water in case of un-inoculated control. The rest of sterilized seeds were soaked in broth cultures of isolates form 4-5 hr prior to sowing at two different concentrations 10^4 (low concentration) and 10^8 (high concentration) bacterial cells. Grains were germinated in plastic pots (15 cm diameter) with 2 kg sterilized soil. After sowing, seedlings were reduced to three per pot. The pots were treated as the following: 1) Untreated control, 2) 10^4/ml bacterial cell suspension, 3)10^8/ml bacterial cell suspension, 4) 35 mM Na Cl solution, 5) 70 mM Na Cl solution, 6) 35 mM Na Cl +10^4/ml bacterial cell mixture, 7) 35 mM Na Cl +10^8/ml bacterial cell mixture, 8) 70 mM Na Cl +10^4/ml bacterial cell mixture and 9) 70 mM Na Cl +10^8/ml bacterial cell.

Growth parameters including dry weight, height, leaves length and width of Maize plants were recorded.

Na^+ and K^+ content analysis

Oven - dried samples of *Zea mays* roots and shoots were powdered for estimation of Na^+ and K^+ by flame photometric method [33].

Quantitative determination of chlorophyll

Chlorophylls content was determined with using the following equations:

Chlorophyll a (mg)/tissue (g) = 11.63 (A 665) –2.39 (A 649)

Chlorophyll b (mg)/tissue (g) = 2.11 (A 649) –5.18 (A 665)

Where A denotes the reading of the optical density.

Antioxidant enzymes catalase and peroxidase of healthy and infected plant were determined according to Kar and Mishra [34].

Estimation of Total protein content

Total protein was estimated calorimetrically [35] by recording absorbance at 595 nm. Bovine serum albumin was used as standard. Protein content in plant samples was recorded as mg of protein per g of sample.

Estimation of Total carbohydrate content

Plant extract was taken in 25 ml test tubes and 6 ml anthrone reagent (150 mg of anthrone in 72 % H_2SO_4) was added, and then heated in boiling water bath for 10 min. The test tubes were ice cooled for 10 min and incubated for 20 min at 25°C. Optical density (OD) was read at 625 nm on a spectrophotometer. The carbohydrate content was calculated from the standard curve using glucose with the same method which mentioned above [36].

Estimation of total lipid content

Total lipid was estimated using Vanillin reagent (6.1 g of vanillin was dissolved in water and diluted to 1 liter). The OD was read on a spectrophotometer at 540 nm. The lipid content was calculated from the standard curve using standard solution of cholesterol with the same method which mentioned above.

Results

Plant biomass growth

Maize biomass in terms of plant height, stem diameter, leaf surface area, and plant dry weight was investigated in relation to saline stress (0, 35, and 70 mM) and PGPRs (*Azotobacter vinelandii, Pseudomonas fluorescens* and *Pseudomonas putida*) treatments. Plant height (Table 1) was reduced from 103.33 cm to 91 cm by saline stress in untreated samples. PGPRs treatments improved plant height in both stressed and normal conditions. *A. vinelandii* at both their concentrations significantly increased plant height from 103.33 to 144.33 cm under normal conditions. Under saline stress *A. vinelandii* increased plant height at 35 mM by 33.77% and 13.55% at 70 mM saline stress. *P. fluorescens* was inferior to *A. vinelandii* as it was able to increase plant height in untreated samples to 131 cm in normal conditions while it was 29.17% more than untreated sample at 35 mM saline stress and no significant increase at 70 mM. *P. putida* did not improved plant height significantly both in normal and stressed conditions.

PGPR(Cell ml^{-1})		Saline stress (mM)		
		0	35	70
Control		103.33 ± 1.45cdef	101.67 ± 9.28def	91.00 ± 3.79f
A. vinelandii	10^4	132.33 ± 7.84ab	114.33 ± 5.33bcde	102.67 ± 7.06cdef
	10^8	144.33 ± 4.37a	136.00 ± 6.51ab	103.33 ± 11.67cdef
P. fluorescens	10^4	125.00 ± 2.89abc	100.67 ± 8.35def	89.33 ± 7.31f
	10^8	131.00 ± 4.58ab	131.33 ± 10.27ab	92.33 ± 8.88ef
P. putida	10^4	119.33 ± 1.86bcd	105.67 ± 7.45cdef	85.33 ± 2.03f
	10^8	119.33 ± 3.84bcd	115.67 ± 10.84bcd	98.67 ± 2.96def

Means followed by the same letter are not significantly different.

Table 1: Mean comparison and standard error of maize plant height (cm) under saline stress and PGPRs treatment.

PGPR(Cell ml^{-1})		Saline stress (mM)		
		0	35	70
Control		2.93 ± 0.12efg	2.80 ± 0.06fg	2.17 ± 0.19g
A. vinelandii	10^4	4.07 ± 0.22abcde	3.43 ± 0.23cdef	2.63 ± 0.19fg
	10^8	4.67 ± 0.38ab	3.57 ± 0.23bcdef	3.23 ± 0.46defg
P. fluorescens	10^4	4.27 ± 0.15abcd	3.60 ± 0.15bcdef	3.07 ± 0.35defg
	10^8	4.80 ± 0.06a	4.03 ± 0.09abcde	3.43 ± 0.09cdef
P. putida	10^4	4.60 ± 0.21abc	3.80 ± 0.44abcdef	2.77 ± 0.19fg
	10^8	4.87 ± 0.09a	3.17 ± 0.67defg	3.37 ± 1.07def

Means followed by the same letter are not significantly different.

Table 2: Mean comparison and standard error of maize stem diameter (cm) under saline stress and PGPRs treatment.

PGPR(Cell ml^{-1})		Saline stress (mM)		
		0	35	70
Control		231.25 ± 16.16bcdef	77.00 ± 3.82j	81.85 ± 17.82j
A. vinelandii	10^4	258.68 ± 14.81abcde	157.70 ± 22.05fghij	128.78 ± 24.20hij
	10^8	293.08 ± 25.93abc	179.88 ± 50.12efghi	134.30 ± 26.04hij
P. fluorescens	10^4	243.88 ± 2.99abcde	190.30 ± 37.53defghi	125.05 ± 18.71ij
	10^8	312.70 ± 1.76a	268.75 ± 33.11abcd	141.60 ± 17.33ghij
P. putida	10^4	209.13 ± 35.58defgh	157.73 ± 21.21fghij	126.83 ± 22.48ij
	10^8	298.35 ± 11.89ab	217.35 ± 27.07cdefg	152.38 ± 18.59fghij

Means followed by the same letter are not significantly different.

Table 3: Mean comparison and standard error of maize leaf surface area (cm^2) under saline stress and PGPRs treatment.

Stem diameter was not significantly affected by saline stress (Table 2), however it was clearly influenced by PGPRs treatment. The three tested PGPRs (at $10^4$and 10^8 cell ml^{-1}) improve stem diameters by 38 – 66% under no saline stress. *P. fluorescens* was the best PGPR treatment that enhanced stem diameter from 2.8 to 4.03 cm at 35 mM saline stress and 10^8 cell ml^{-1} ; while stem diameter was 3.43 cm at 70 mM saline and 10^8 cell ml^{-1} against 2.17 cm at 70 mM saline untreated samples. *A. vinelandii* treatment improve plant stems diameter by 27.5% and 48.85 % at 35 and 70 mM saline stress respectively. *P. putida* both concentrations improve plant stems diameter to 3.8 at low saline stress and 3.37 at the higher one.

Leaf surface area of maize plant was significantly reduced by 66.7% due to saline stress (Table 3). All tested PGPRs treatment increased plant leaf surface area to 298.35 cm^2 under no saline stress. *A. vinelandii* and *P. putida* treatment limited leaf surface area reduction by saline stress from 66.7% to range between 31.8 and 6% at 35 mM stress and between 44.3 and 34.1% at 70 mM. *P. fluorescens* treatment was superior it was capable of increasing leaf surface area to 268.75 cm^2 that is 16% more than control leaf surface area

Plant dry weight (Table 4) was reduced from 6.4 g to 3.4 g by saline stress in untreated samples. PGPRs treatment improve plant dry weight in both stressed and normal conditions. *A. vinelandii* both concentrations significantly increased plant dry weight from 6.4 g to 11.2 g under normal conditions. Under saline stress *A. vinelandii* increased plant dry weight at 35 mM to 12.8 g. *P. fluorescens* was able to increase plant dry weight under no saline stress to 13.1 g while it was 11.9 g at 35 mM saline stress and 9.9 g at 70 mM. *P. putida* improved plant height significantly both in normal and stressed conditions.

Biochemical contents

Maize plant carbohydrate content (Table 5) was investigated during the current study. However, the differences in carbohydrates content among treatments was limited; it showed high degree of significance. Carbohydrate content was 79.2 mg/g in the control plant. PGPRs treatments at no saline stress increased carbohydrates content of the plant up to 82.2 mg/g. Although saline stress reduced plant carbohydrate content in all treated and untreated samples as compared to control sample; it is obvious that except of *P. putida*, the other treatments improve plant carbohydrate content as compared to untreated samples (Table 6).

Maize proteins were 26.17 mg/g in control sample. PGPRs treatments significantly improve plant protein content up to 33.17 mg/g under no saline stress. Saline stress reduced maize protein content to 24.17 and 20.17 mg/g at 35 and 70 mM saline stress, respectively. *A. vinelandii* and *P. fluorescens* treatment improve palnt protein content by 7.5% as compared to untreated sample at 35 mM. PGPRs treatment showed no considerable differences in the plant protein content when compared to untreated samples at 70 mM. Unlike other investigated

PGPR(Cell ml^{-1})		Saline stress (mM)		
		0	35	70
Control		6.40 ± 0.29f	3.40 ± 0.23h	4.00 ± 0.29h
A. vinelandii	10^4	10.00 ± 0.23d	11.10 ± 0.29c	5.40 ± 0.29g
	10^8	11.20 ± 0.29c	12.80 ± 0.29b	6.80 ± 0.35ef
P. fluorescens	10^4	9.30 ± 0.17d	11.93 ± 0.20c	5.07 ± 0.26g
	10^8	13.10 ± 0.58b	11.30 ± 0.23c	9.90 ± 0.06d
P. putida	10^4	7.30 ± 0.17ef	14.00 ± 0.29a	6.20 ± 0.12f
	10^8	13.00 ± 0.29b	14.20 ± 0.23a	6.80 ± 0.23ef

Means followed by the same letter are not significantly different.

Table 4: Mean comparison and standard error of maize dry weight (g) under saline stress and PGPRs treatment.

PGPR(Cell ml^{-1})		Saline stress (mM)		
		0	35	70
Control		79.20 ± 0.13f	69.20 ± 0.13j	66.20 ± 0.13m
A. vinelandii	10^4	81.00 ± 0.13c	69.95 ± 0.13i	67.18 ± 0.13l
	10^8	81.69 ± 0.13b	70.98 ± 0.13h	67.89 ± 0.13k
P. fluorescens	10^4	80.20 ± 0.13e	70.20 ± 0.13i	69.89 ± 0.13i
	10^8	82.20 ± 0.13a	71.98 ± 0.13g	69.94 ± 0.13i
P. putida	10^4	80.00 ± 0.13e	67.95 ± 0.13k	65.28 ± 0.12n
	10^8	80.62 ± 0.12d	67.58 ± 0.13k	62.80 ± 0.13o

Means followed by the same letter are not significantly different.

Table 5: Mean comparison and standard error of maize carbohydrates (mg/g) under saline stress and PGPRs treatment.

PGPR(Cell ml^{-1})		Saline stress (mM)		
		0	35	70
Control		26.17 ± 0.02e	24.17 ± 0.01h	20.17 ± 0.18l
A. vinelandii	10^4	28.18 ± 0.01d	25.18 ± 0.06g	20.12 ± 0.18l
	10^8	32.13 ± 0.06b	26.00 ± 0.02e	20.93 ± 0.01k
P. fluorescens	10^4	30.17 ± 0.01c	25.17 ± 0.06g	21.10 ± 0.18k
	10^8	33.17 ± 0.06a	25.69 ± 0.02f	21.99 ± 0.01j
P. putida	10^4	26.19 ± 0.01e	22.12 ± 0.06j	18.94 ± 0.01m
	10^8	28.11 ± 0.07d	23.33 ± 0.32i	20.12 ± 0.12l

Means followed by the same letter are not significantly different.

Table 6: Mean comparison and standard error of maize protein (mg/g) under saline stress and PGPRs treatment.

PGPR(Cell ml^{-1})		Saline stress (mM)		
		0	35	70
Control		18.65 ± 0.13h	19.14 ± 0.14g	19.65 ± 0.13f
A. vinelandii	10^4	18.02 ± 0.06i	20.41 ± 0.06de	23.05 ± 0.02b
	10^8	20.56 ± 0.12d	21.15 ± 0.01c	23.95 ± 0.06a
P. fluorescens	10^4	19.65 ± 0.12f	18.65 ± 0.05h	18.15 ± 0.05i
	10^8	19.85 ± 0.14f	18.45 ± 0.12h	19.65 ± 0.12f
P. putida	10^4	17.00 ± 0.06j	19.21 ± 0.07g	21.02 ± 0.07c
	10^8	18.54 ± 0.12h	20.15 ± 0.02e	20.90 ± 0.12c

Means followed by the same letter are not significantly different.

Table 7: Mean comparison and standard error of maize lipid content (mg/g) under saline stress and PGPRs treatment.

primary metabolites, lipid content was significantly proportion to saline stress. Lipid content (Table 7) of maize was also improve by PGPRs treatments. *A. vinelandii* treatment raised lipid content in maiz plant from 18.65 to 20.56 mg/g at no saline stress, and to 23.95 mg/g at 70 mM saline stress. *P. fluorescens* treatment did not increase maize lipid content significantly as compared to untreated samples at no saline stress. *P. putida* improved lipid content in maize plant at 35 and 70 mM saline stress.

Chlorophyll content of maize plant was investigated in the current study. Under no saline stress *A. vinelandii* and *P. Fluorescens* significantly improve chlorophyll a content (Table 8) to 6.99 and 7.99 mg/g, respectively. Saline stress reduced plant chlorophyll a content in both PGPRs treated and untreated samples. *A. vinelandii* and *P. fluorescens* treatments significantly limited chlorophyll a content reduction from 46.86% to 31.59 and 27.99% at 35 mM saline stress, respectively. Chlorophyll b content (Table 9) was reduced due to saline stress from 3.21 to 2.1 mg/g. PGPRs treatments either did not influence or reduced chlorophyll b content under no saline stress, 35 mM and 70 mM saline stress.

Antioxidant enzymes as indicators for oxidative stress exerted on the plant were investigated. Saline stress increased catalase concentration (Figure 1) in untreated and PGPRs treated samples. PGPRs treatments reduced the amount of catalase in plants under saline stress when compared to untreated stressed plants. Salinity stress

PGPR(Cell ml^{-1})		Saline stress (mM)		
		0	35	70
Control		6.68 ± 0.05c	3.55 ± 0.09i	3.50 ± 0.01i
A. vinelandii	10^4	6.77 ± 0.06c	4.31 ± 0.05f	3.62 ± 0.14hi
	10^8	6.99 ± 0.01b	4.57 ± 0.06e	3.65 ± 0.13hi
P. fluorescens	10^4	7.91 ± 0.03a	4.81 ± 0.03d	3.78 ± 0.07h
	10^8	7.99 ± 0.02a	4.98 ± 0.03d	3.99 ± 0.05g
P. putida	10^4	6.68 ± 0.06c	3.65 ± 0.02hi	3.50 ± 0.02i
	10^8	6.66 ± 0.04c	3.66 ± 0.06hi	3.62 ± 0.07hi

Means followed by the same letter are not significantly different.

Table 8: Mean comparison and standard error of maize chlorophyll a (mg/g) under saline stress and PGPRs treatment.

PGPR(Cell ml^{-1})		Saline stress (mM)		
		0	35	70
Control		3.21 ± 0.01b	2.15 ± 0.12efg	2.10 ± 0.13fg
A. vinelandii	10^4	3.25 ± 0.12ab	2.14 ± 0.06efg	1.91 ± 0.06g
	10^8	3.32 ± 0.04ab	2.10 ± 0.12fg	1.91 ± 0.13fg
P. fluorescens	10^4	3.45 ± 0.12ab	2.45 ± 0.08de	2.12 ± 0.07fg
	10^8	3.52 ± 0.04a	2.52 ± 0.12d	2.01 ± 0.12fg
P. putida	10^4	2.19 ± 0.12efg	2.45 ± 0.08de	2.18 ± 0.07efg
	10^8	2.89 ± 0.04c	2.50 ± 0.12d	2.23 ± 0.12def

Means followed by the same letter are not significantly different.

Table 9: Mean comparison and standard error of maize chlorophyll b (mg/g) under saline stress and PGPRs treatment.

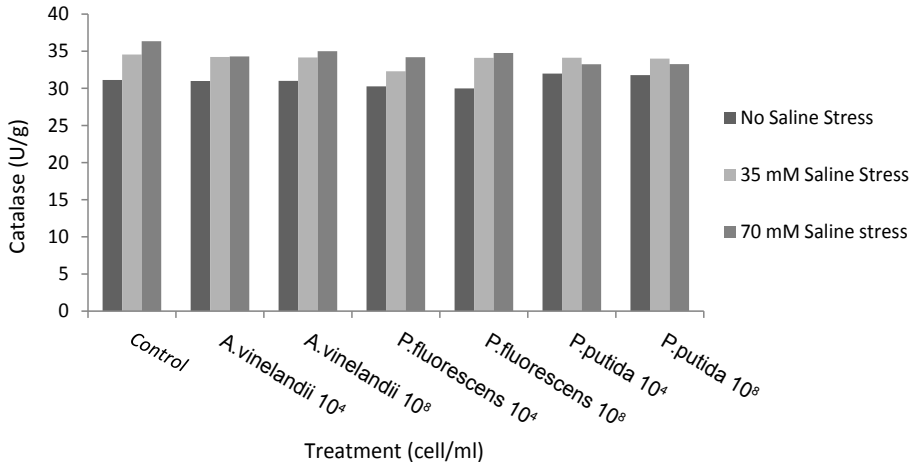

Figure 1: Effect of saline stress and PGPRs treatments on catalase production (U/g) by maize plant.

increased peroxidase concentration (Figure 2) in untreated samples. PGPRs treated samples showed irregular response to saline stress as peroxidase concentration increased at 35 mM saline stress over the untreated samples concentrations while it was reduced at 70 mM to minimum when compared to samples under no saline stress.

Sodium potassium flux

Root system sodium potassium balance (Table 10) was investigated in this study. It was found that Sodium potassium balance did not influence by PGPRs treatments under normal conditions. Salinity stress disturbed the Na^+ K^+ balance as Na^+ jumped up to 8.14 at 35 mM and 9.25 mg/g at 70 mM while K^+ withdrawn to be 4.43 and 4.22 at 35 and 70 nM saline stress, respectively. *A. vinelandii* treatment restored Na^+ K^+ balance at 35 and 70 mM saline stress close to normal balance. *P. fluorescens* treatment reduced Na content of plant roots when compared to untreated samples at both 35 and 70 mM saline stress; while the K^+ content slightly increased. *P. putida* was disabled to restore the balance as Na^+ remained high and K^+ low. Sodium and potassium contents in shoot system (Table 11) were influenced by saline stress as Na^+ content increased up to 3.95 mg/g while the K^+ content reduced to 23.85mg/g. PGPRs treatments slightly reduced Na content of the plant shoot system and increased its K^+ content. Under 35 mM saline stress also, Na^+ was slightly reduced by PGPRs treatments while the K^+ content increased especially by *A. vinelandii* and *P. fluorescens*. The same pattern observed at 70 mM saline stress but both Na^+ reductio and K^+ improvement were limited.

Discussion

The effect of three PGPRs (*Pseudomonas fluorescens*, *Pseudomonas putida* and *Azotobacter vinelandii*) application on the Maize (*Zea mays* L.) growth and its allied attributes was assessed under saline stress in the current study. The results showed that the application of PGPRs significantly increased the shoot and root growth as compared to untreated plants. Parida and Das [37] reported that, the negative effects

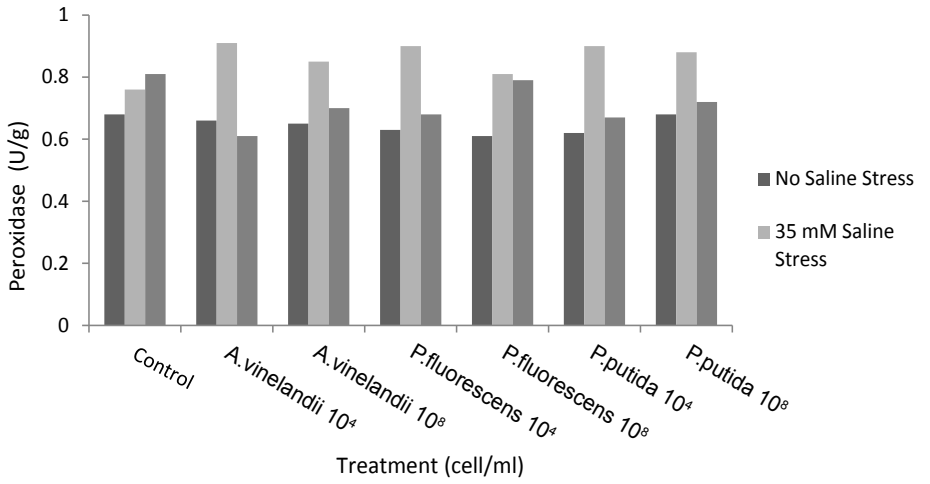

Figure 2: Effect of saline stress and PGPRs treatments on peroxidase production (U/g) by maize plant.

PGPR(cell ml^{-1})		0		35		70	
		Na^+	K^+	Na^+	K^+	Na^+	K^+
Control		5.78 ± 0.032h	6.51 ± 0.01e	8.14 ± 0.027bc	4.43 ± 0.03l	9.25 ± 0.088a	4.22 ± 0.01m
A. vinelandii	10^4	5.62 ± 0.024h	7.07 ± 0.04b	6.37 ± 0.032f	6.31 ± 0.05f	7.24 ± 0.038e	5.13 ± 0.01g
	10^8	5.38 ± 0.029i	8.07 ± 0.10a	6.11 ± 0.009g	6.22 ± 0.05f	7.19 ± 0.003e	5.20 ± 0.03g
P. fluorescens	10^4	5.63 ± 0.022h	6.59 ± 0.02e	6.32 ± 0.052f	4.75 ± 0.03i	7.21 ± 0.006e	4.54 ± 0.02l
	10^8	5.39 ± 0.030i	6.96 ± 0.01c	6.38 ± 0.262f	4.91 ± 0.01h	7.21 ± 0.022e	4.44 ± 0.01l
P. putida	10^4	5.72 ± 0.007h	6.53 ± 0.01e	8.04 ± 0.031c	4.60 ± 0.02jk	8.28 ± 0.044bc	4.43 ± 0.04l
	10^8	5.67 ± 0.022h	6.75 ± 0.03d	7.77 ± 0.075d	4.66 ± 0.02ij	7.83 ± 0.092d	4.44 ± 0.03l

Na^+ means followed by the same letter are not significantly different; K^+ means followed by the same letter are not significantly different.

Table 10: Mean comparison and standard error of maize root system Na^+ and K^+ content (mg/g) under saline stress and PGPRs treatment.

PGPR(cell ml^{-1})		0		35		70	
		Na^+	K^+	Na^+	K^+	Na^+	K^+
Control		2.95 ± 0.03gh	25.28 ± 0.04g	3.51 ± 0.03c	23.76 ± 0.03i	3.95 ± 0.08a	23.85 ± 0.08i
A. vinelandii	10^4	2.29 ± 0.03j	27.54 ± 0.27d	3.15 ± 0.03ef	28.57 ± 0.01c	3.83 ± 0.03b	26.23 ± 0.12ef
	10^8	2.15 ± 0.01k	31.91 ± 0.08a	3.12 ± 0.01f	29.25 ± 0.04b	3.32 ± 0.01d	26.54 ± 0.03e
P. fluorescens	10^4	2.31 ± 0.03j	27.90 ± 0.03d	3.15 ± 0.03ef	28.47 ± 0.07c	3.81 ± 0.05b	26.16 ± 0.08ef
	10^8	2.23 ± 0.09jk	32.17 ± 0.41a	3.11 ± 0.01f	29.31 ± 0.05b	3.33 ± 0.02d	26.42 ± 0.07ef
P. putida	10^4	2.84 ± 0.03h	26.05 ± 0.07f	3.24 ± 0.04de	24.42 ± 0.05h	3.54 ± 0.06c	23.94 ± 0.07i
	10^8	2.57 ± 0.01i	27.56 ± 0.06d	2.98 ± 0.05gh	26.28 ± 0.03ef	3.56 ± 0.02c	24.38 ± 0.04h

Na^+ means followed by the same letter are not significantly different; K^+ means followed by the same letter are not significantly different.

Table 11: Mean comparison and standard error of maize shoot system Na^+ and K^+ content (mg/g) under saline stress and PGPRs treatment.

of salinity stress on plant-growth include a reduction in growth rate and biomass, shorter stature, smaller leaves, osmotic effects, nutritional deficiency and mineral disorders. Therefore, according to Bacilio et al. [38] the use of PGPR to promote plant-growth in saline conditions is an important technology. The three PGPRs inocula exhibited a different pattern of shoot growth under both normal and salinity stressed conditions. Plant height, dry weight, stems diameter,and leaf surface area were clearly improved by PGPRs treatments in normal conditions. In fact, field trials have demonstrated that, inoculation with Azotobacter has beneficial effects on plant yields, due to the increase of fixed nitrogen content in agricultural soil [28,39-43], and to the microbial secretion of stimulating hormones, like gibberellins, auxins and cytokinins [44,45]. Our results showed that *P. fluorescens* was the best inoculum for *Zea mays* growth enhancing. These results confirm previous findings where the enhancing effect of *Zea mays* inoculation with *P. fluorescens* on dry weight and yield of maize was reported [3,46], increase in plant height, root weight and total biomass were observed in response to inoculation.

Reduction in plant growth parameters under salt stress condition were recorded. Salinity is one of serious environmental problems that cause reduction in plant growth and yield productivity in irrigated

areas of arid and semi-arid regions of the world [37]. The obtained adverse effects of salt stress on the *Zea mays* L. growth was alleviated by the PGPRs inoculation and decreased with concentration of inoculants. From the current study the effect of *P. fluorescens* was more pronounced than that of other two PGPRs. Our results confirm previous findings that inoculated plants grew better and had higher biomass compared to non-inoculated plants under salt stress conditions [38,47-49]. Jagnow [50] found that wheat and maize inoculated with Azotobacter increases both the plant biomass of the above ground (26- 50%) and the yield (19-30%). Recently, Zafar-ul-Hye *et al.* [51] found that maize productivity increased under salt stress inoculated with *P. syringae* and *P. fluorescens.*

Plants inoculated with PGPRs showed higher protein and carbohydrate content compared to control plants. Thus inoculation with *P. fluorescens* induced *Zea myes* L soluble protein and Carbohydrate yields (33.17 and 82.20 mg/gm respectively) compared with control (26.17 and 79.20 mg/gm respectively). On the other hand, under salt stress and without *P. fluorescens* treatment plant contents of protein and carbohydrate were decreased. The inoculation with *P. fluorescens* induce synthsis of protein and carbohydrate. Similar effects where also showed by the *A. vinelandii* and *P. putida.* Usually the increase of protein yield is related to higher nitrogen fixation activities, this knowledge was confirmed with many authors [52-54]. On the other hand, lipid content was observed to increase under salinity stress and reduced when PGPRs treatments were applied. The results indicate that *P. fluorescens* increased chlorophyll a and b (7.99 and 3.52 mg respectively) of *Zea mays* L. compared with the control (6.68 and 3.21 mg respectively) while *A. vinelandii* and *P. putida* were less effective on chlorophyll contents. Our results showed the co-inoculation of stress *Zea mays* L. markedly stimulated chlorophyll a and b content as compared to plants cultivated under salt stress without inoculation especially at low concentration 35 mM Na Cl. The effect of salinity on the synthesis of chlorophyll depended on the specific concentration of NaCl. Nevertheless, the inoculation with PGPRs of current study enhanced the content of chlorophyll revealing a positive effect on growth and plant development. A similar trend has also been observed in other researchers [12,55,56]. In the present investigation, the responses of *Zea mays*L. plant to high level of salinity were reflected by increased of catalase and peroxidase activities. Mittler [57] stated that antioxidant enzyme activities are usually affected by salinity and used as indicators of oxidative stress in plants. To protect against oxidative stress, plant cells produce both antioxidant enzymes such as peroxidase and catalase, and non-enzymatic antioxidants such as ascorbate, glutathione and tocopherol [58]. The results showed that the exogenous application of PGPRs decreased catalase and peroxidase activities of cultivated *Zea mays* under salt stress. A similar trend has also been observed in other researchers [59, 60].

Salinity causes an imbalance in the ion flux inside plants. The present results showed that during salinity stress, the plants had higher Na^+ and lower K^+ contents, compared with control plant in root and shoot system (Tables 10 and 11). This is also according to the results [61], salinity increases the uptake of Na^+ or decreases the uptake K^+ which lead to nutritional imbalances. Accumulation of excess Na^+ may cause metabolic disturbance in processes where low Na^+ and high K^+ are required for optimum plant function [62]. Increased K^+ concentration under salinity conditions may help to decrease Na^+ uptake and this can indirectly maintain the growth of the plant [63]. Based on the results obtained, applying PGPRs treatment significantly increased the K^+ content of maize under salt stress conditions. The higher K^+ uptake may demonstrate the role of K^+ in salt tolerance. This is also according to the results of [64] where PGPR strains from *Azotobacter sp.* increased the maize plant growth and potassium and phosphorus intake under different levels of salinity stress. Recently, Sang-Mo et al. [65] found that the PGPR-applied plants had reduced sodium ion concentration; while the potassium was abundantly present as compared to control under stress of *Cucumis sativus* cultivation. K^+ play a key role in plant water stress tolerance and has been found to be the cationic solute responsible for stomata movements in response to changes in bulk leaf water status [66]. There are several reports of lower Na^+ concentrations in plants inoculated with PGPR under salinity conditions [63,67,68].

Conclusion

P. fluorescens, A. vinelandii and *P. putida* had significant impact on maize growth, suggesting that can be applied as biofertilizers for improved maize production under salinity stress. Further greenhouse studies should provide more definitive information about the movement and uptake of macro-elements (Na^+ and K^+) to plants with the impacts of PGPR–based inoculants.

Acknowledgement

Part of this study was conducted as part of the Project No. 30-G5-1434: "Modern integrated management of halothermophilic biofertilizers for enhancing agricultural soil fertility in Jazan", which is supported and funded by the Deanship of Scientific Research, Jazan University, KSA.

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