Cereal-PGPR interweave in salt-affected environments: towards plant persistence and growth promotion

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Abstract

Among the corner stones for crop productivity in the semi-arid areas of the world, is the use soil and irrigation water with high contents of soluble salts. The growth and physiological responses of two maize (TWC 329 and TWC 353) and two wheat (Egypt 1 and Sakha 93) cultivars to salinity in presence of plant growth promoting (PGP) bacterial and cyanobacterial isolates were assessed in gnotobiotic and pot experiments. The phylogenetic analyses inferred from 16S rRNA gene sequence of 2 superior isolates of either bacteria or cyanobacteria demonstrated that these isolates are closely related to the species; *Bacillus megaterium*, *Lysinibacillus sphaericus*, *Dolichospermum circinale* and *Dolichospermum spiroides.* Salinity decreased cereal shoot and root lengths and dry weights as well as chlorophylls, carotenoids and nutrients (K^+, Ca^{++}, Mg^{++}) contents but increased phenol, proline, catalase and peroxidase activities as well as sodium accumulation. Microbiota inoculation greatly alleviated the detrimental effect of salinity. An striking point was the ability of the introduced inocula to modify the K^+/Na^+ ratios towards the proper values required for better establishment of plants in salt-affected environments. Nitrogen balance measurements indicated N gain of up to 23 mg pot^{-1} in saline soil inoculated with the dual culture of *Lysinibacillus sphaericus* and *Dolichospermum spiroides.* This N level is equivalent to > 4 kg N $acre^{-1}$. Acetylene reducing activities were rather low in saline soil even in presence of onrganic amendments. It could be speculated that PGPR inoculation strategies reverse the deletorious effects of high osmolarity in soil and help in improving crop productivity under salt stress.

Keywords: Salt stress- bacteria- cyanobacteria- maize- wheat- 16S rRNA- N balance- acetylene reducing activity.

Introduction

Agronomists around the globe have to deal with challenges of various biotic and abiotic stresses that diminish crop productivity. Nearly 82 % of potential yield of crops is lost due to abiotic stresses every year and the available productive areas of arable land are decreasing world-wide. There is a number of abiotic stresses common in nature such as salinity, drought, extreme temperatures, moisture, light, pH, mineral deficiencies or toxicities, heavy metals and pollutants that seriously reduce plant yields (Munns *et al*., 2006; Chedly *et al*., 2008).

Of these stresses, salinity deems disastrous because it has many direct and indirect injurious effects; inhibits seed germination, induces physiological dysfunctions and often kills non-halophytes even at low concentrations and limits the agricultural development (Bartels and Sunkar, 2005). More than 80 million hectares are facing this problem which accounts for > 6 % of the world's total area (FAO, 2008). Two major stresses affecting plants under salinity are osmotic and ionic stresses, occurring immediately in the root medium on exposure to salts, they result in inhibition of water uptake, cell expansion and lateral bud development (Munns and Tester, 2008). Ionic stress develops when toxic ions (*e.g.* Na^+) accumulate in cells causing increase in leaf mortality, chlorosis, necrosis and decrease in the activity of cellular metabolism including photosynthesis. As mentioned by Munns (2002), ionic stress results in premature senescence of older leaves and in toxicity symptoms (chlorosis, necrosis) in mature leaves due to high Na^+ and Cl^{-1} which affect plants by disrupting protein synthesis and by interfering with enzyme activity. Excess Na^+ and Cl^{-1} have the potential to affect plant enzymes, resulting in reduced energy production and other physiological processes.

Actually, salinization transforms fertile and productive lands to barren ones, and often leads to loss of habitat and reduction of biodiversity. These salts have accumulated over time due to natural causes particularly in arid and semi-arid zones, or as a result of weathering of parent material or deposition of oceanic salts carried in wind or rain (Munns and Tester, 2008).

It is difficult to quantify the salt tolerance of plants because it varies appreciably with many environmental factors (*e.g*. soil fertility, soil physical conditions, distribution of salt in soil profile, irrigation regime and climate) and plant factors (*e.g.* growth stage, variety and root stock) (Juan *et al*., 2005). In this respect, plants have developed a wide range of mechanisms to sustain productivity in saline environments. These mechanisms are osmotic adjustment, Na^+ and / or Cl^{-1} exculsion, and tissue tolerance of low concentrations of Na^+ and / or Cl^{-1}. Research on various

crops has indicated that salt tolerance depends largely on genera and species and even on cultivars within certain species (Niu *et al*., 2012).

Egypt is an agricultural country, the poor irrigation practices and expanded canal system results in development of soil salinity. Fortunately, the role of salt-tolerant microbiota in plant nutrition and growth promotion makes them ideal candidates for alleviating, to an extent, the deleterious impacts of salinity on plant growth and improve crop yield. Here, Xiang *et al*. (2008) mentioned that the halophilic microorganisms possess a number of mechanisms to adapt to salt-affected areas. They are able to form biofilms containing extracellular polysaccharides (EPS) of high water content. EPS as a boundary between cells and surrounding environment also acts as a protective mechanism against desiccation, salt stress and UV radiation and helps their survival (Chen *el al*., 2008). Beside the well established contribution of plant growth promoting (PGP) bacteria to plant welfare in stressed environments, cyanobacteria are well adapted to a wide array of environmental conditions and can support plant growth directly and / or indirectly. The direct ways encompass the production of phytohormones such as auxin (Prasanna *et al*., 2010), gibberllins (Rodrigues *et al*., 2006) and cytokinins (Hussain and Hasanain, 2009). The indirect promotion of plant growth is due to restricting the deleterious effects of one or more phytopathogenic microorganisms (Kim and Kim, 2008).

As reported by Barriuso *et al*. (2008), the identification and assessment of PGPR potential in stressed environments follows a common scenario based on massive microbial isolation on solid media, followed by pure culture tests aiming to determine their effectiveness. Isolates giving best results are then examined usually under gnotobiotic conditions, and only those promising ones are finally tested in pot experiments with soil and eventually under field conditions.

Keeping all in mined, this study was designed to isolate, identify and screen the potential native PGP halophytes of a number of salted areas for their capabilities to support maize and wheat development in salt-stressed environments. This is to provide fundamental biological understanding and knowledge on some aspects of cereal-microbiota interweaves in these particular habitats.

MATERIALS AND METHODS

Soils

Four soil samples representing various textural classes and salinity levels were collected from 5-15 cm sub-surface layers. Those are non-saline sandy taken from Ismailia Experimental Station fields and three from El-Hussinia Plan representing moderately saline, saline and highly saline clayey ones. The mechanical and chemical properties of soils are present in Table (1). Soils were air-dried, crushed and sieved to pass a 2 mm screen and kept at 4 °C until using for isolation and inoculation studies.

Table 1. Mechanical and chemical properties of the experimental soils

Analyses	Ismailia	El-Hussinia Plan1	El-Hussinia Plan2	El-Hussinia Plan3
Mechanical analysis				
Coarse sand (%)	86.12	5.94	6.40	3.14
Fine sand (%)	5.67	7.34	9.15	8.29
Silt (%)	4.06	28.09	27.23	28.76
Clay (%)	2.13	57.43	56.12	59.81
Textural class	Sandy	Clayey	Clayey	Clayey
Chemical analysis				
pH	7.85	7.93	8.30	8.25
EC (dSm^{-1})	3.10	6.50	15.06	18.57
Cations (meq l^{-1})				
Ca^{++}	12.28	17.65	28.95	12.46
Mg^{++}	4.64	16.90	18.11	21.73
Na^{+}	13.95	30.78	139.53	150.02
K^{+}	2.19	0.73	1.94	0.76
Anions (meq l^{-1})				
SO_4^{--}	22.58	45.90	147.12	44.70
HNO_3^{-}	2.36	1.42	1.42	8.25
Cl^{-}	8.13	18.75	40.03	132.43

Cereals

Seeds of maize (*Zea maize*, cvs. TWC 329 and TWC 353) and wheat (*Triticum aestivum*, cvs. Egypt 1 and Sakha 93) were obtained from Field Crops Institute, Agricultural Research Center, Giza. Prior to planting, seeds were washed in tap water to get rid of any traces of pesticides possibly added during storage.

Isolation and identification of PGPR

To isolate PGP bacteria and cyanobacteria, semi-solid malate N-deficient (Döbereiner and Day, 1976) and BG11 (Stainer *et al.*, 1971) media respectively were used. For bacteria, ten gram saline and moderately saline soils were added to 250 ml capacity glass bottles containing 90 ml sterile distilled water and serial decimal dilutions were prepared. One ml aliquotes from the suitable dilutions were inoculated into the N-deficient agar medium. Plates were incubated @ 30°C for 48 hr. Developed colonies were picked up and subjected to purification by sub-culturing several times on the same medium. Cyanobacteria were isolated (Allen and Stanier, 1968) from the same soil types, as follows: 10 g samples were transferred to 250 ml capacity conical flasks containing 100 ml sterile distilled water, shaked for 1 hr and filtered through Whatman No. 41 filter paper. Enriched cultures were prepared by adding 10 ml supernatent to 50 ml BG11 medium and kept under continuous illumination of 2000 Lux with shaking for 2 weeks @ 25 ± °C. One ml culture was introduced into BG11 agar medium. Two-week old colonies were picked up and streaked on the same medium several times to obtain uni-algal cultures. Those were then exposed for 24 hr to a sterile mixture of pencillin, tetracycline, streptomycin and chloramphenicol of 200.0, 2.0, 0.4 and 20.0 mg l^{-1} respectively (Droop, 1967). Pure isolates were microscopically examined and based on colony and cell morphology, representative isolates of bacteria (8 isolates) and cyanobacteria (8 isolates) were assessed for a number of biochemical reactions. Those encompassed acetylene reducing activity (Hardy *et al.*, 1973) and phosphate solubilzation (Sundara and Sinha, 1963) in addition to indol acetic acid (Brick *et al.*, 1991), hydrogen cyanide (Bakker and Schippers, 1987) and siderophore production (Alexander and Zuberer, 1991). This is beside tolerance to increased NaCl concentrations in selective culture media.

According to biochemical potentials, the superior 2 bacterial isolates were subjected for molecular identification according to Lane (1991), by sequencing of the 16S rRNA gene as

follows: fresh bacterial cultures were cultivated on nutrient agar @ 28 °C for 4 days. A small amount of each bacterial isolate was individually scraped and suspended in 100 µl autoclaved distilled water in 2 ml sterile vials and boiled @ 100 °C for 15 min. The non-living bacterial cells were sent to Sol Gent Company for rRNA gene sequencing. Bacterial DNA was extracted and isolated using Sol Gent purification bead. Prior to sequencing, the ribosomal rRNA gene was amplified using the polymerase chain reaction (PCR) technique in which two universal bacterial primers 27F (forward) and 1492R (reverse) were incorporated into the reaction mixture. Primers used for gene amplification have the following composition: 27F (5'AGAGTTTGATCMTGGCTCAG) and 1492R (5'TACGGYTACCTTGTTACGACTT). The purified PCR products (amplicons) were reconfirmed using a size nucleotide marker (100 base pairs) by electrophoreses on 1 % agarose gel. These bands were eluted and sequenced with the incorporation of dideoxynucleotides (dd NTPs) into the reaction mixture. Each sample was sequenced in the sense and antisense directions using the same primers. Sequences were further analyzed using Basic Local Alignment Search Tool (BLAST) from the National Center of Biotechnology Information (NCBI) website. Phylogenetic analysis of sequences was done with the help of MegAlign (DNA Star) software version 5.05.

Similarly, the molecular identification of the selected 2 pioneeric cyanobacteria was confirmed using the 16S ribosomal DNA (rDNA) amplification and sequencing, which was determined by direct sequencing of PCR-amplified 16S rDNA. Genomic DNA was isolated from the selected cyanobacteria species *via* a phenol-chloroform method (Chomczynski and Sacchi, 1987) on a pellet obtained by centrifugation of 10 ml of algal culture at the late-log phase. DNA amplification from genomic DNA containing a partial 16S ribosomal RNA region was performed by PCR using CYA106F and CYA781R primer as follows: forward: 5'CGG ACG GGT GAG TAA CGC GTG A and reverse: 5'GAC TAC TGG GGT ATC TAA TCC CAT T (Nu¨bel *et al.*, 1997).

The identified isolates were quantitatively estimated for IAA, gibberellin and cytokinin according to Unyayar *et al*. (1996).

Maize seed germination indices

Maize seeds of cultivars TWC 329 and TWC 353 were immersed in 20 ml 1 % sodium hypochlorite in 100 capacity beakers for surface sterilization. They were left in the solution for 5 min. followed by washing under running distilled water. Surface sterilized seeds were placed in

Petri dishes (10 seeds/dish) with a double layer of Whatman No. 1 filter paper moistened with distilled water of different NaCl concentrations (0-500 mM). Along 7 days @ ambient temperature, seed germination was recorded daily. Several germination attributes were calculated to characterize the salt tolerance including germination percentages after 1 and 7 days, germination velociety coefficient (GVC) (Kader and Jutzi, 2004), germination rate index (GRI) (Kader, 2005) and mean germination time (MGT) (Kader, 2005) as follows: GVC (% day^{-1}) = Σ Ni / Σ (Ni Ti) ×100; GRI (% day^{-1}) = Σ Ni / i and MGT (days) = Σ (Ni Ti) / Σ Ni

where: N is the number of seeds germinated on day i and Ti is the number of days from sowing.

Gnotobiotic model experiment

Glass bottles of 9 cm diameter and 30 cm length were filled with 100 ml semi-soild (7 % agar, w/v) Hoagland nutrient solution (Hoagland and Arnon, 1950) supplemented with NaCl in different amounts to secure the EC at either 1.50, 6.75 or 9.50 dSm^{-1} and autoclaved @ 121 °C for 15 min. Seeds of maize cv. TWC 329, characterized by high germination rate in presence of salt, were surface sterilized by immersion in 0.2 % $HgCl_2$ for 3 min. followed by several washings with sterile distilled water to remove any traces of $HgCl_2$. Surface sterilized seeds were allowed to germinate under aseptic conditions on moistened Whatman No.1 filter paper for 7 days. Developed seedlings of similar lengths were aseptically transferred to the glass bottles containing salted semi-solid Hoagland nutrient solution where root system was gently inserted downward. The PGP bioformulations were added as 10 ml freshly prepared cultures per tube. The introduced inocula were *Bacillus megaterium*, *Lysinibacillus sphaericus*, *Dolichospermum spiroides* and *Dolichospermum circinale* as mono- or dual-cultures beside a quadriple preparation of all. Bottles were cotton plugged and kept @ ambient temperature. After 2 weeks, seedings were taken and measured for shoot and root lengths as well as fresh and dry weights (oven dry @ 70 °C to constant weight). Leaf area was determined using Leaf Area Measurement Software. Plant shoots were chemically analyzed as well for the photosynthetic pigments chlorophyll a, chlorophyll b and carotenoids (Metzner *et al*., 1965) as well as catalase (Goth, 1991) and peroxidase (Chance and Maehly, 1955) activities.

Cereal-PGP microbe panorama in pot experiments

Two pot experiments were executed to expound how far the tested PGP microorganisms can alleviate the deleterious effects of salinity on cereal plant development.

Maize-microbiota interweave

Plastic pots of 25 cm diameter and 25 cm depth were filled with non-saline sandy soil (EC, 3.10 dSm^{-1}) @ the rate of 5 kg pot^{-1}. All pots were supplemented with PK fertilization regimes equivalent to the recommended field levels of 200 and 50 kg $acre^{-1}$ superphosphate (P_2O_2, 15.5 %) and potassium sulphate (K_2O, 50 %) respectively. Nitrogen in the form of urea (N, 46.5 %) was incorporated into soil at either recommended dose of 120 kg $acre^{-1}$ or its half. Seeds of maize cvs. TWC 329 and TWC 353 were sown as 7 seeds pot^{-1}. For inoculated treatments, seeds were soaked in dense preparations of either *Bacillus megaterium, Lysinibacillus sphaericus, Dolichospermum circinale* or *Dolichospermum spiroides* single cultures for 30 min. Beside uninoculated non-saline water-irrigated pots, all inoculated potting soils were irrigated with NaCl-salted water of 6.50 dSm^{-1}. Six treatments were allocated as indicated in Table (2). Pots were arranged in the greenhouse in a complete randomized design with 4 replications and irrigated along the experimental period to manitain 70 % of soil water holding capacity. After 30 days, plants were gently uprooted and separated into roots and shoots and determined for length and dry weights in addition to chemical constituents. Those are chlorophll a, chlorophyll b and carotenoides as well as phenolic compounds (Porter and Villar, 1997), proline contents (Bates *et al.*, 1973) and sodium (Allen *et al.*, 1974). This is beside the oxidative enzymes, catalase and peroxidase activities.

Table 2. Layout of maize experiment including the applied treatments

Treatment	Tap water	Salted water	Full N	Half N	*B. megaterium*	*L. sphaericus*	*D. spiroides*	*D. circinale*
1	•		•					
2		•		•				
3		•		•	•			
4		•		•		•		
5		•		•			•	
6		•		•				•

Wheat-microbiota interaction

Saline soil (EC, 6.50 dSm^{-1}) taken from El-Hussinia Plan was distributed in plastic pots (25 cm diameter and 25 cm depth) as 5 kg pot^{-1}. Superphosphate and potassium sulphate were added in quantities equivalent to the recommended rates of 150 and 50 kg $acre^{-1}$ respectively and thoroughly mixed with soil. Simultaneously, urea was applied to soil in amounts representing 100 and 50 % of recommended N. Seeds of wheat cvs. Egypt 1 and Sakha 93, whether inoculated or not, were sown @ the rate of 20 seeds pot^{-1}. For inoculation, a composite inoculum of *Lysinibacillus sphaericus* and *Dolichospermum spiroides* in equal portions was prepared and used for seed soaking. Three treatments were prepared for each cultivar as follows: 1) 100 % N, 2) 50 % N plus humic acid (0.1 % w/w) and 3) 50 % N with inoculation. Pots were irrigated with tap water when needed to maintain the moisture level @ 70 % WHC. The experimental layout was a complete randomization with three replicates. At the end of the experiment (90 days), plants were gently uprooted without tearing the root system as possible and determined for plant length, dry weight, chlorophyll a, b and carotenoides. Plant contents of Na^{+}, K^{+}, Ca^{++} and Mg^{++} were determined adopting the procedures of Allen *et al.* (1974). Nitrogen contents of soils and plants (Cottenie *et al.*, 1982) were estimated as well for N-balance calculations.

N_2-fixation potential in saline soil

Two samples representing non-saline (EC, 3.10 dSm^{-1}) and saline (EC, 6.50 dSm^{-1}) soils were selected to assess the nitrogen fixation potential using acetylene reduction assay (ARA) using the procedure of Fayez and Vlassak (1984). 125 ml flasks containing 50 g air dried soil portions either as such or amended with glucose (1 % w/w) or powdered barley straw (1 % w/w; C/N ratio, 53.4) were prepared. Moisture content was kept at 70 % WHC and incubation took place @ 30 °C. ARA was measured after 2, 6 and 10 days of incubation. In addition, three carbon sources (cellulose, malate and sucrose) added to soil @ 1 % (w/w) were tested for their influence on ARA under flooded and non-flooded conditions. One set of soil samples placed in 125 ml flasks were flooded (1.0 cm standing water) and the others were held non-flooded (70 % WHC). Flasks were incubated @ 30 °C for 10 days. All the aforementioned treatments were replicated three times. The cotton plugs of flasks were replaced by rubber caps and 10 % of the gas phase were replaced by C_2H_2, then flasks were reincubated for 4 hr. Thereather, C_2H_4 produced was measured by injecting 1 ml gas sample into gas chromatograph equiped with coiled glass column packed with Porapak R.

Statistical analysis

Data were statistically analyzed for least significant differences according to Huang and Chen (2008). Linear regressions and correlation coefficients among a number of variables were considered as well.

Results

Isolation and identification of halophilic PGP microbiota

Eight isolates representing bacteria or cyanobacteria were single-colony and- filament isolated and purified. The biochemical potential of the isolates (Table, 3) indicated their ability to slightly reduce acetylene (< 13 nmoles C_2H_4 ml^{-1} h^{-1}), produce IAA, solubilize phosphate and tolerate up to 6 % NaCl in culture media. But all failed to produce siderophores or hydrogen cyanide.

Based on their biochemical potential, the potent 2 bacterial isolates (BA1, BE2) were determined for phylogenetic position by 16S rRNA gene sequence. This analysis indicated that one bacterial isolate was identified as *Bacillus megaterium* sharing 98 % identity with its closent phylogenetic relatives. The other isolate was genetically identical to *Lysinibacillus sphaericus* with 92 % similarity. Similarly, the superior 2 cyanobacterial isolates (CD1, CG2) were identified as *Dolichospermum circinale* and *Dolichospermum spiroides* with 97 % similarity.

Appreaciable quantities of the plant growth promoting substances; indole acetic acid, gibberllins and cytokinins were produced by the identified isolates as follows (in respective order) : a) *B. megaterium* (66.1, 9.7, 276.9 ppm), b) *L. sphaericus* (72.8, 9.6, 533.0 ppm), c) *Dolichospermum circinale* (27.2, 3.3, 203.4 ppm) and d) *Dolichospermum spiroides* (67.1, 11.6, 247.9 ppm).

Table 3. Biochemical activities of bacterial and cyanobacterial isolates

Isolate code	ARA (nmoles C_2H_4 ml^{-1} h^{-1})	IAA	P-solubilization (clear zone diameter, cm)	Siderophores	HCN	NaCl (%)
Bacteria						
BA1	0.17	+	1	-	-	6
BB1	-	+	3	-	-	6
BC1	-	+	4	-	-	5
BD1	-	+	1.5	-	-	4
BE2	0.11	+	1	-	-	6
BF2	-	+	1	-	-	6
BG2	-	+	2	-	-	4
BH2	-	+	1	-	-	6
Cyanobacteria						
CA1	3.32	+	1	-	-	3
CB1	0.16	+	3	-	-	2
CC1	2.02	+	4	-	-	3
CD1	12.51	+	2	-	-	3
CE1	3.05	+	1	-	-	3
CF2	5.50	+	1	-	-	2
CG2	6.72	+	2	-	-	3
CH2	2.84	+	1	-	-	3

Maize seed germination in salted water

The germination attributes (Table, 4) of maize seeds cvs. TWC 329 and TWC 353 were assessed in distilled water received increased concentrations of NaCl (0-500 mM). For both cultivars, the number of germinated seeds proporationly increased with time (data not shown). Seeds of cv. TWC 329 did successfully withstand the increased salt concentrations up to 350 mM with germination rate of 100 %. This was not the case for cv. TWC 353 where 300 mM Nacl was sufficient enough to reduce the germination to 73.3 %. Raising salinity level > 400 mM adversely affected seed germination, an effect that was more obvious with cv. TWC 353. Seeds of both cultivars failed to germinate at 500 mM NaCl.

Table 4. Germination indexes determined for maize seeds after 7 days of germination in presence of increased concentrations of sodium chloride

NaCl (mM)	Total germination (%)	GVC (%)	GRI (%)	MGT (days)
TWC 329				
50	100	57.1	17.1	2.9
100	100	57.1	17.1	2.9
150	100	57.1	17.1	2.9
200	100	54.3	16.3	3.3
250	100	44.8	13.4	3.4
300	100	29.5	8.9	3.3
350	100	25.7	7.7	3.4
400	60	14.3	4.3	3.1
450	33.3	6.7	2.0	3.1
500	0.0	0.0	0.0	0.0
TWC 353				
0	100	57.1	17.1	3.0
50	100	55.2	16.6	2.9
100	100	53.3	16.0	3.1
150	100	51.4	15.4	2.9
200	100	43.8	13.1	3.3
250	100	29.5	8.9	3.1
300	73.3	11.4	3.4	3.3
350	73.3	10.5	3.1	3.8
400	26.7	3.8	1.1	3.5
450	6.7	1.0	0.3	4.1
500	0.0	0.0	0.0	0.0

The calculated germination velociety coefficient (GVC) records of cv. TWC 329 (6.7-57.1 %) were 27.4 % in average higher than the correspondings of cv. TWC 353 (1.0-57.1 %). The GVC estimates dramatically decreased as the salt level increased. Actually, the GVC gives an indication of the germination rapidity, it increases when the number of germinated seeds increases and the time required for germination decreases. The germination rate index (GRI) that reflects the percentage of germination on each day of the germination period decreased as well with

increasing the salt quantity. The GRI estimates were lower for cv. TWC 353 (0.3-17.1 %) compared to those of cv. TWC 329 (2.0-17.1 %). Conversely, the time required for germination (MGT) increased as NaCl level in water increased. Respective periods of 2.9-3.4 and 2.9-4.1 days were scored for cvs. TWC 329 and TWC 353.

Maize-salinity interaction in gnotobiotic model experiment

Table (5) presents the shoot and root traits of the 15-day old maize cv. TWC 329 seedlings grown in gnotobiotic system in presence of PGP bacterial and/or cyanobacterial isolates together with increased NaCl concentration. Apart from microbiota, salt stress adversely affected shoot leaf area, reductions of 3.3-32.1 % were attributed to increased salinity from 1.50 to 6.75 dSm^{-1}. Further reductions of > 75 % were recorded @ the highest NaCl level of 9.50 dSm^{-1}. When introduced into salted growth medium, bacterial and cyanobacterial candidates did restrict the injurious effect of salinity where reduction percentages in leaf area for inoculated plants were falling in the range 7-65 % against 77 % for uninoculated ones. Among the inocula applied, the composite inoculum of cyanobacteria deemed the superior with an average leaf area of 584 mm^2.

The detrimental influence of high salinity markedly reflected on the whole plant development resulting in shorter shoots, reductions of 19.3-62.8 % were attributed to the highest NaCl concentration. The tallest maize shoots (21.7 cm) were those of plants received the cyanobacterium *Dolichospermum circinale* followed by those inoculated with the dual culture of cyanobacteria (19.1 cm). Bacilli strains ranked thereafter with shoot length of 12.9 cm in average.

As expected, reductions in shoot fresh weights were proportional to the increased salt concentration, *i.e.* the higher the salt level the lower fresh weight. Cyanobacterial inoculants overcame those of bacteria in supporting better shoot vigor, estimates of 430-1220 mg $plant^{-1}$ were scored for the former and 450-910 mg $plant^{-1}$ for the latter.

Fluctuations in shoot dry weights among the various treatments were more or less akin to those of fresh weights. Regardless the depressive effect of high salinity, cyanobacterial mixed culture promoted the shoot biomass yield even in presence of the highest NaCl concentration with an average increase of *ca.* 18 % over uninoculated plants. Bacterial inocula had no stimulating influence in this respect. The quadriple culture of all was not that supportive for shoot biomass production with an average of 103 mg $plant^{-1}$.

For uninoculated maize, salt treatment reduced the root length, an effect that was more sever with the highest NaCl level of 9.50 dSm^{-1}. *Bacillus* and *Lysinibacillus* strains failed to

Table 5. Shoot and root characteristics of 15-day old gontobiotic-grown maize seedlings as affected by salinity and PGPR isolates

Treatments	Shoot (per plant)					Root (per plant)		
	NaCl (dSm^{-1})	Leaf area (mm^2)	Length (cm)	Fresh weight (mg)	Dry weight (mg)	Length (cm)	Fresh weight (mg)	Dry weight (mg)
Uninoculated	1.50	663	24.2	1280	268	15.5	860	42.1
	6.75	641	21.0	1150	133	10.0	680	29.0
	9.50	151	12.0	850	111	8.2	260	28.2
B. megaterium	1.50	364	16.5	910	197	9.6	650	27.0
	6.75	322	13.0	800	134	10.0	440	16.0
	9.50	377	11.1	820	87	6.7	450	7.4
L. sphaericus	1.50	571	18.2	820	125	11.2	600	11.0
	6.75	540	11.1	770	134	7.1	450	15.0
	9.50	531	12.3	450	75	7.7	270	7.0
B. megaterium* + *L. sphaericus	1.50	500	19.2	810	146	8.8	880	12.0
	6.75	385	7.2	800	68	7.2	400	15.0
	9.50	189	7.6	810	48	9.8	360	13.0
D. spiroides	1.50	471	27.7	930	197	14.3	930	46.2
	6.75	320	23.3	770	134	13.0	770	46.0
	9.50	167	10.3	430	94	3.2	430	29.1
D. circinale	1.50	586	25.9	1220	132	14.1	1210	51.0
	6.75	309	21.2	1100	128	12.3	730	21.0
	9.50	477	17.9	550	126	12.9	710	26.2
D. spiroides* + *D. circinale	1.50	696	23.3	1210	274	18.3	1030	37.0
	6.75	501	19.7	1210	201	12.2	780	39.1
	9.50	556	14.4	960	127	15.5	420	10.6
Mixture of all	1.50	235	11.4	1020	151	13.3	480	10.0
	6.75	159	9.2	850	74	12.5	300	31.1
	9.50	305	9.2	670	84	13.3	280	11.5
LSD (0.05)		73	2.8	113	59	2.0	66	3.4
CV (%)		11.6	4.4	9.5	2.1	6.9	12.5	5.1

overcome the detrimental influence of high salinity. In contrast, both *Dolichospermum spiroides* and *Dolichospermum circinale* did alleviate, to an extent, the toxicity of excess salinity. In general, fluctuations in root fresh weights among the various treatments were in trends similar to those recorded with root length.

Raising the salinity of cultivation media to 9.50 dSm^{-1} conspicuously decreased the root biomass, an effect that was inoculum-dependent, cyanobacteria acted more active than bacteria. An average increase percentage of *ca*. 22 was attributed to *Dolichospermum spiroides* inoculation. In few cases, the highest NaCl concentration rarely suppressed root dry matter production.

The photosynthetic pigments and oxidative enzymes of maize shoots were among the criteria that obviously affected by elevating the salt content of the growth medium (Table 6). Scantly salted media of all treatments supported the highest chlorophyll accumulation in the cereal shoot tissues. Raising the NaCl concentration to 6.75 dSm^{-1} decreased chlorophylls a and b pools by 11.7- 64.9 and 9.3- 85.5 % respectively. Salinity of 9.50 dSm^{-1} was sufficient enough to result in the lowest estimates of the photosynthetic appratus. In the majority of cases, microbial inocula, whatever they are, failed to overcome the detrimental influence of salinity on chlorophyll production. The dual culture of *Dolichospermum circinale* and *Dolichospermum spiroides* succeeded to compensate an extent of salt toxicity. Carotenoides of plant shoots changed among the applied treatments in trends almost similar to those of chlorophylls. Again, the composite bioformulation of cyanobacteria nicely acted in comparison with the bacterial one.

On the contrary, both catalase and peroxidase activities significantly increased as the NaCl level in growth medium increased. Increases in inoculated treatments, in most cases, were more pronounced with catalase (146.8-398.3 %) against 21.4-223.4 % for peroxidase. Apart from salt content, catalase enzyme responded to microbial inoculation much better than peroxidase. Increases over control of 17.1-60.0 % in catalase activities were attributed to inoculation, the cyanobacterium *Dolichospermum spiroides* was the pioneeric. No apparent increases in peroxidase activity were recorded for inoculated treatments.

Table 6. Shoot physiological properties of maize seedlings grown in salted media in presence of bacterial and cyanobacterial inocula

Treatments	NaCl (dSm^{-1})	Ch-a (µg/g DW)	Ch-b (µg/g DW)	Carotenoides (µg/g DW)	Catalase (mM/min/FW)	Peroxidase (Units/mg FW)
Uninoculated	1.50	22.57	11.91	2.86	129.5	2.02
	6.75	19.94	10.12	2.55	167.3	3.73
	9.50	0.12	0.06	0.00	491.0	12.17
B. megaterium	1.50	4.92	2.94	1.02	66.1	5.16
	6.75	5.48	3.09	0.57	106.6	4.32
	9.50	0.46	0.28	0.07	229.5	8.47
L. sphaericus	1.50	13.36	9.82	1.73	130.3	4.13
	6.75	10.38	5.46	1.41	255.8	7.88
	9.50	0.95	0.50	0.16	536.4	5.64
B. megaterium* + *L. sphaericus	1.50	6.76	2.96	1.56	120.5	2.45
	6.75	2.37	0.43	0.54	368.8	3.68
	9.50	0.28	0.20	0.06	297.7	1.21
D. spiroides	1.50	9.55	6.65	1.19	105.8	2.64
	6.75	8.28	6.03	0.95	627.6	3.37
	9.50	8.14	4.80	0.71	527.2	6.24
D. circinale	1.50	19.38	12.77	1.81	109.6	2.62
	6.75	7.00	4.22	0.88	103.2	3.01
	9.50	6.50	5.13	0.74	74.5	2.48
D. spiroides* + *D. circinale	1.50	19.32	12.60	1. 88	103.1	2.01
	6.75	14.61	8.59	1.54	113.4	2.31
	9.50	10.93	6.45	1.37	254.4	3.11
Mixture of all	1.50	10.84	5.01	1.40	103.2	2.89
	6.75	7.14	6.92	0.51	417.5	1.65
	9.50	5.45	3.48	0.79	479.7	5.34
LSD (0.05)		2.9	1.4	0.8	67.6	3.0
CV (%)		8.6	3.4	9.1	5.2	11.6

Regardless of inocula applied, maize biomass negatively correlated with salinity level of gnotobiotic cultivation system; in contrast, catalase activity positively correlated. This is expressed in the linear regressions illustrated in Figure (1) and the calculated coefficient of determination. Table (7) presents the correlation matrix of maize shoot characteristics. All the

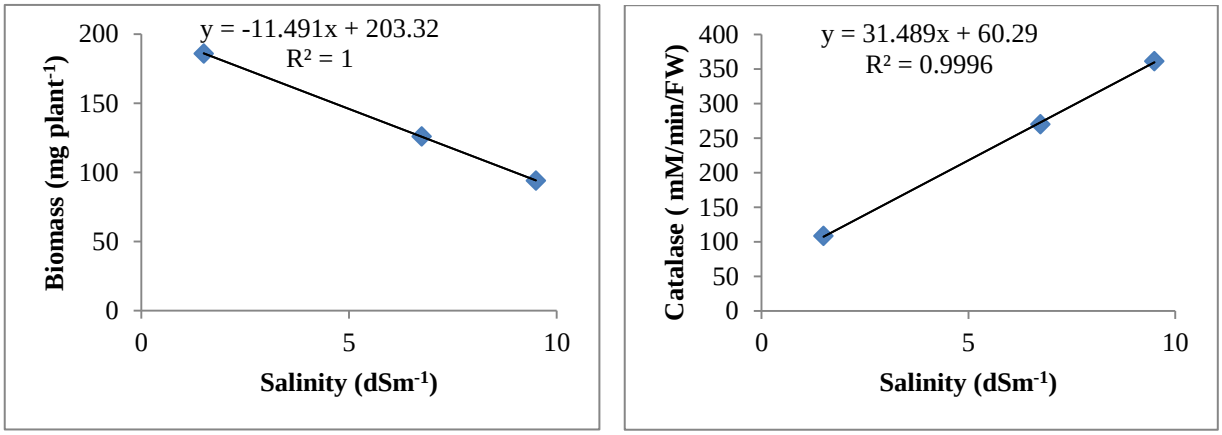

Fig. 1. Linear regressions indicating the relationships between salinity of cultivation medium and either maize shoot biomass or catalase activity.

Table. 7. Correlation matrix (R-values) of maize shoot traits under the effect of salinity-microbiota interactions

Traits	LA	L	FW	DW	Ch-a	Ch-b	Carot.	Cat.	Perox.
LA	-	0.99*	0.90^{ns}	1.00**	0.96^{ns}	0.97^{ns}	0.98^{ns}	-1.00**	-0.89^{ns}
L		-	0.95^{ns}	0.99*	0.99*	0.99*	1.00**	-1.00**	-0.94^{ns}
FW			-	0.91^{ns}	0.99*	0.98^{ns}	0.97^{ns}	-0.92^{ns}	-1.00**
DW				-	0.97^{ns}	0.97^{ns}	0.99*	-1.00**	-0.91^{ns}
Ch-a					-	1.00**	1.00**	-0.97^{ns}	-0.99*
Ch-b						-	1.00**	-0.98^{ns}	-0.98^{ns}
Carot.							-	-0.99*	-0.97^{ns}
Cat.								-	0.91^{ns}
Perox.									-

LA, leaf area; L, length; FW, fresh weight; DW, dry weight; Ch-a, chlorophyll a; Ch-b, chlorophyll b; Carot., carotenoids; Cat., catalase and Perox., peroxidase.
*, significant ($p < 0.05$); **, highly significant ($p < 0.01$); ns, non-significant.

cereal growth and photosynthetic traits positively correlated under the effect of salinity and microbial inoculation, although the calculated correlation coefficients were not statistically significant in > 50 % of cases. On the other hand, the oxidative enzyme activities (catalase and peroxidase) negatively interacted with all the other assessed attributes, correlations in a number of cases reached the significance level of $p < 0.01$.

Development of maize irrigated with saline water

Irrigation with salted water (EC, 6.75 dsm^{-1}) significantly reduced the root length of cv. TWC 353 but not cv. TWC 329. All the introduced inocula successfully overcame the deleterious impact of water salinity (data not shown).

Root biomass severely reduced due to irrigation with saline water (Fig 2-A), respective reduction percentages of 20.8 and 46.2 were estimated for cvs. TWC 329 and TWC 353. Bacterial strains deemed more supportive for root dry matter production than cyanobacteria, average increases of 60.4 % for the former and 22.2 % for the latter were scored over tap water-irrigated plants. Roots of 100 % N-supplied-tap water-irrigated plants contained the lowest quantities of phenolic compounds (Fig 2-B). Significant increases were obtained with saline water irrigation particularly in inoculated plants. Fluctuations in root proline contents (Fig 2-C) were more or less akin to those of phenols.

Sodium uptake of root systems of both maize cultivars obviously increased due to irrigation with saline water (Fig 2-D). Salted water-received cvs. TWC 329 and TWC 353 plants, whether inoculated or not, contained 112.5 and 158.8 % respectively more sodium than those irrigated with tap water.

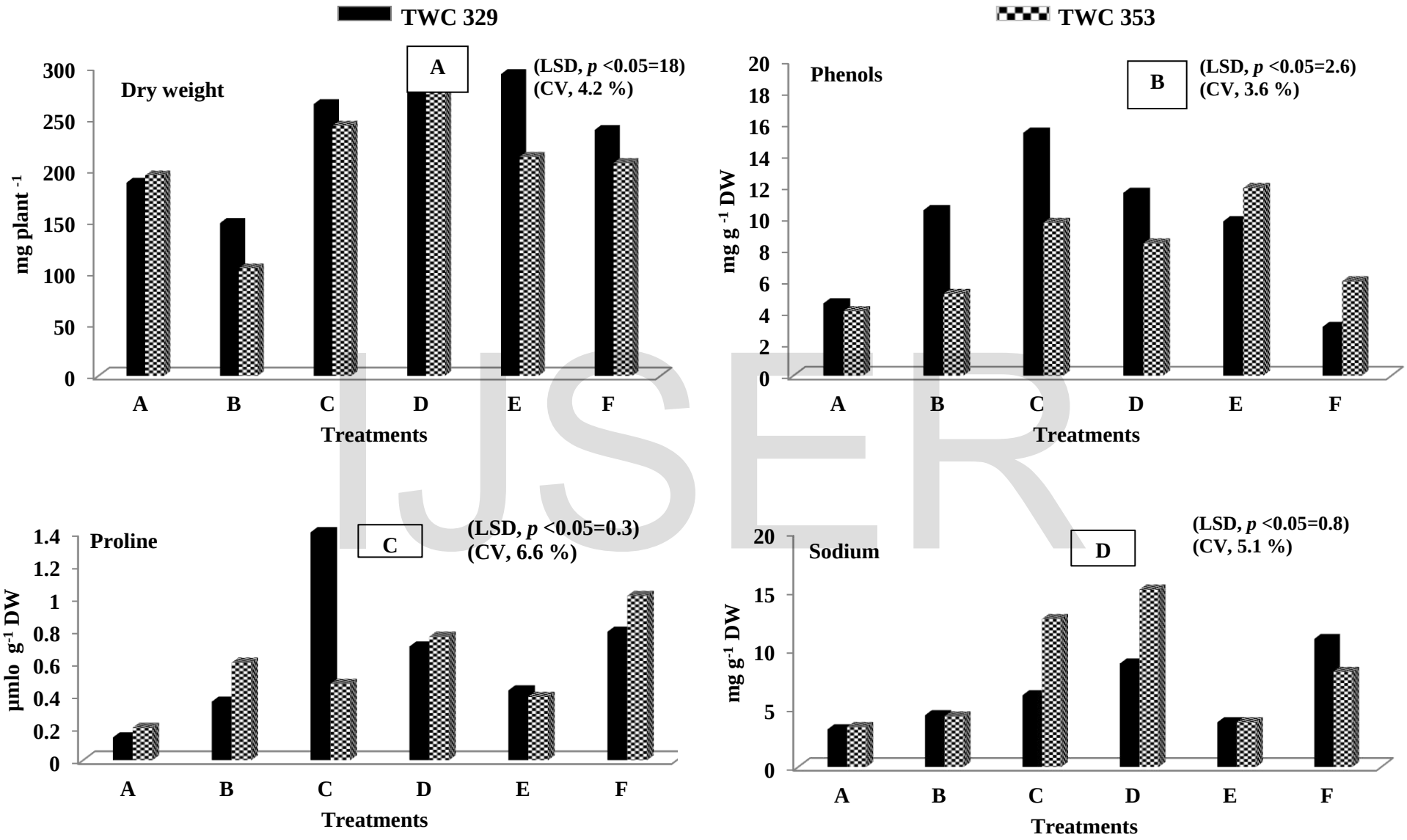

Fig. 2. Root parameters of maize cultivars TWC 329 and TWC 353 of the different treatments. A) 100 kg N + tap water, B) 50 % N + saline water, C, D, E and F represent 50 % N + saline water simultaneously with *B. megaterium, L. sphaericus, D. spiroides* or *D. circinale* inoculation respectively.

Shoot growth and biochemical characteristics of maize of the different treatments are present in Table (8). Bacterial and cyanobacterial inoculation, even with saline water irrigation, resulted in *ca*. 8 and 4 % increases in shoot heights of cvs. TWC 329 and TWC 353 respectively (data not shown). In absence of inoculation, irrigation with saline water decreased shoot lengths by *ca.* 5 and 10 %.

Compared to full N-tap water-received plants, those dressed with 50 % N and irrigated with saline water produced up to 52.5 % less shoot biomass. PGP microbial inoculation did successfully alleviate the harmful effect of water salinity. *Bacillus megaterium* was the superior particularly in case of cv. TWC 329 with the highest increase percentage of 80.5.

Irrigation with saline water markedly reduced cholorphylls a and b of both maize cultivares. Inoculation diminished to a great extent the toxicity of water salinity, an effect that was more pronounced with *B. megaterium* and *L. sphaericus*. Apart from N level and microbiota, cv. TWC 353 accumulated higher quantities of total chlorophyll (average of 4.16 µg g^{-1} DW) compared to cv. TWC 329 (3.35 µg g^{-1} DW). In 80 % of case, water salinity increased the maize shoot carotenoide contents, this was rather obvious for cv. TWC 353.

In absence of inoculation, salinity stress reduced shoot contents of phenols, this was more sever for TWC 353 with reduction of *ca.* 60 %. Introduction of bacterial inocula, in particular, conspicuously compensated the detrimental influence of irrigation water salinity. Irrespective of maize cultivar, increases attributed to bacteria approximated 95 % against 80 % for cyanobacteria. Proline, as a compatible solute in osmotic adjustment, increased in shoot with salinity. Again, bacterial and cyanobacterial inoculation supported higher accumulation of the chemical compound in plant tissues.

The patterns of changes in catalase and peroxidase activities varied among the treatments. In general, cv. TWC 329 exhibited higher catalase (492.2 mM/min/FW) and lower peroxidase (3.27 units mg^{-1} FW) compared to cv. TWC 353 (467.5 and 4.95). Irrigation with saline water together with PGP microbial inoculation increased the enzymatic activities.

Na^+ concentrations were greatly higher in shoots of uninoculated plants irrigated with saline water. Increases over those full N dressed-tap water-irrigated plants were 153.9 and 214.3 % for cvs. TWC 329 and TWC 353 respectively. Extraordinary increases in the ion pool were attributed to inoculation. Bacterial cultures overcame the cyanobacterial correspondings, average Na^+ of 12.1 mg g^{-1} DW was recorded for the former and 7.4 mg g^{-1} DW for the latter.

Table 8. Shoot growth parameters and biochemical profile of maize cultivars inoculated with PGP isolates and irrigated with salted water

Treaments	Dry weight (mg plant^{-1})	Chlorophyll a (µg g^{-1} DW)	Chlorophyll b (µg g^{-1} DW)	Carotenoids (µg g^{-1} DW)	Phenols (mg g^{-1} DW)	Proline (µmol g^{-1} DW)	Catalase (mM/min/FW)	Peroxidase (units mg^{-1}FW)	Sodium (mg g^{-1} DW)
				cv. TWC 329					
100 % N + tap water	323.0	5.08	2.26	1.17	7.4	0.62	496.0	1.36	1.3
50 % N+ saline water	158.0	2.24	1.21	0.97	4.4	0.64	456.0	2.60	3.3
50 % N+ saline water:									
+ ***B. megaterium***	583.0	5.92	2.67	1.75	7.2	0.95	513.0	5.13	14.8
+ ***L. sphaericus***	527.0	6.43	3.14	1.30	6.5	2.13	500.0	4.91	10.8
+ ***D. circinale***	322.0	ND	1.24	1.81	6.1	2.83	481.0	4.04	5.0
+ ***D. spiroides***	477.0	ND	ND	0.80	ND	0.75	507.0	1.59	6.8
				cv.TWC 353					
100 % N+ tap water	318.0	3.56	1.37	1.05	7.2	0.56	454.0	2.56	1.4
50 % N+ saline water	151.0	2.21	2.39	1.84	2.9	0.94	487.0	5.90	4.4
50 % N+ saline water:									
+ ***B. megaterium***	333.0	6.59	3.18	1.73	6.0	2.03	462.0	8.78	10.4
+ ***L. sphaericus***	338.0	6.83	3.11	1.79	8.7	1.89	465.0	4.10	12.2
+ ***D. circinale***	235.0	7.08	3.50	1.78	6.5	1.37	463.0	6.75	8.3
+ ***D. spiroides***	271.0	6.69	3.45	1.92	7.1	0.75	474.0	1.62	9.5
LSD (0.05)	89	1.01	0.82	0.40	2.9	0.81	22	1.9	1.4
CV (%)	11.2	3.9	6.7	7.6	4.8	8.6	10.1	3.2	4.6

Wheat-PGPR interweave in saline soil

Variance analyses proved the validity of PGPR inoculation in supporting wheat development (Table, 9). This is expressed in increased length (33 %), biomass (71 %) and spike dry weight (253 %). The beneficial effect of inoculation extended as well to chlorophyll a (20 %), chlorophyll b (25 %) and carotenoides (27 %). This indicates that the introduced microorganisms successfully compensated the 50 % reduction in N fertilization. Incorporation into soil of the organic material (humic acid) in presence of 50 % N was not that supportive. Inoculated plants accumulated considerable quantities of N in their tissues, respective increase percentages of 17.1 and 32.4 over uninoculated ones were scored for cvs. Egypt 1 and Sakha 93.

The effect of the biopreparation was more conspicuous on nutrient pool of both wheat cultivars (Fig. 3). Regardless of cultivar, increases in Ca^{++}, Mg^{++} and K^{+} contents approximated 36, 68 and 25 % respectively. Interestingly, the introduced inoculum succeeded to reduce the quantities of Na^{+} taken up by plants grown in such saline soil (EC, 6.75 dSm^{-1}), reductions in N^{+} contents of 29.5 and 17.8 % were scored for cvs. Egypt 1 and Sakha 93 respectively. Furthermore, the K^{+}/Na^{+} ratios were modified to the proper limits that allow the plant establishment in salt-stressed media. Widder K^{+}/Na^{+} ratios of 3.56 and 3.11 for cvs. Egypt 1- and Sakha 93- inoculated plants respectively against 2.01 and 2.18 for uninoculated ones were calculated.

Nitrogen balance measurements (Table, 10) revealed that negligible amount of N (1.2 mg pot^{-1}) was introduced via N_2-fixation into full N-dressed wheat soil system, more N quantities of 4.9 and 8.7 mg pot^{-1} were gained with humic acid - supplied cvs. Egypt 1 and Sakha 93 respectively. The highest net N income of 23.1 mg pot^{-1} was estimated for cv. Sakha 93 received the dual bacterial culture, an amount that equivalent to 4.62 kg N $acre^{-1}$.

N_2-fixation potential as affected by soil salinity

Very low acetylene reducing activities were estimated for unamended soils, 9.3-13.8 nmoles C_2H_4 g^{-1} h^{-1} scored for non-saline soil against 3.1-6.7 nmoles C_2H_4 g^{-1} h^{-1} for saline one (Table, 11). The enzyme activity increased due to addition of 1 % glucose particularly in non-saline soil. The quantities of ethylene produced in 1 % barley straw-amended soil were much higher than those of unamended correspondings. Apart from amendments and incubation

Table 9. Growth and photosynthetic pigments of 90-day old wheat cultivated in saline soil and received humic acid and PGPR bioformulation

Treaments	Length (cm)	Dry weight (mg plant^{-1})	Spike DW (mg plant^{-1})	N-content (mg plant^{-1})	Chlorophyll a (µg g^{-1} DW)	Chlorophyll b (µg g^{-1} DW)	Carotenoids (µg g^{-1} DW)
				cv. Egypt 1			
100 % N + tap water	44.0	1280.0	190.0	3.91	2.40	1.67	1.13
50 % N + Humic acid	25.7	410.0	150.0	2.88	1.49	1.70	1.23
50 % N+ *L. sphaericus* + *D. spiroides*	42.0	2190.0	670.0	4.58	2.88	2.08	1.44
				cv. Sakha 93			
100 % N + tap water	33.0	1390.0	410.0	3.67	2.68	1.96	1.04
50 % N + Humic acid	35.7	2040.0	380.0	4.09	2.07	1.88	1.23
50 % N+ *L. sphaericus* + *D. spiroides*	43.9	2018.0	610.0	4.86	2.80	2.23	0.93
LSD (0.05)	6.4	109	118	0.51	0.42	0.39	0.32
CV (%)	2.9	7.1	6.9	3.4	7.6	5.6	4.1

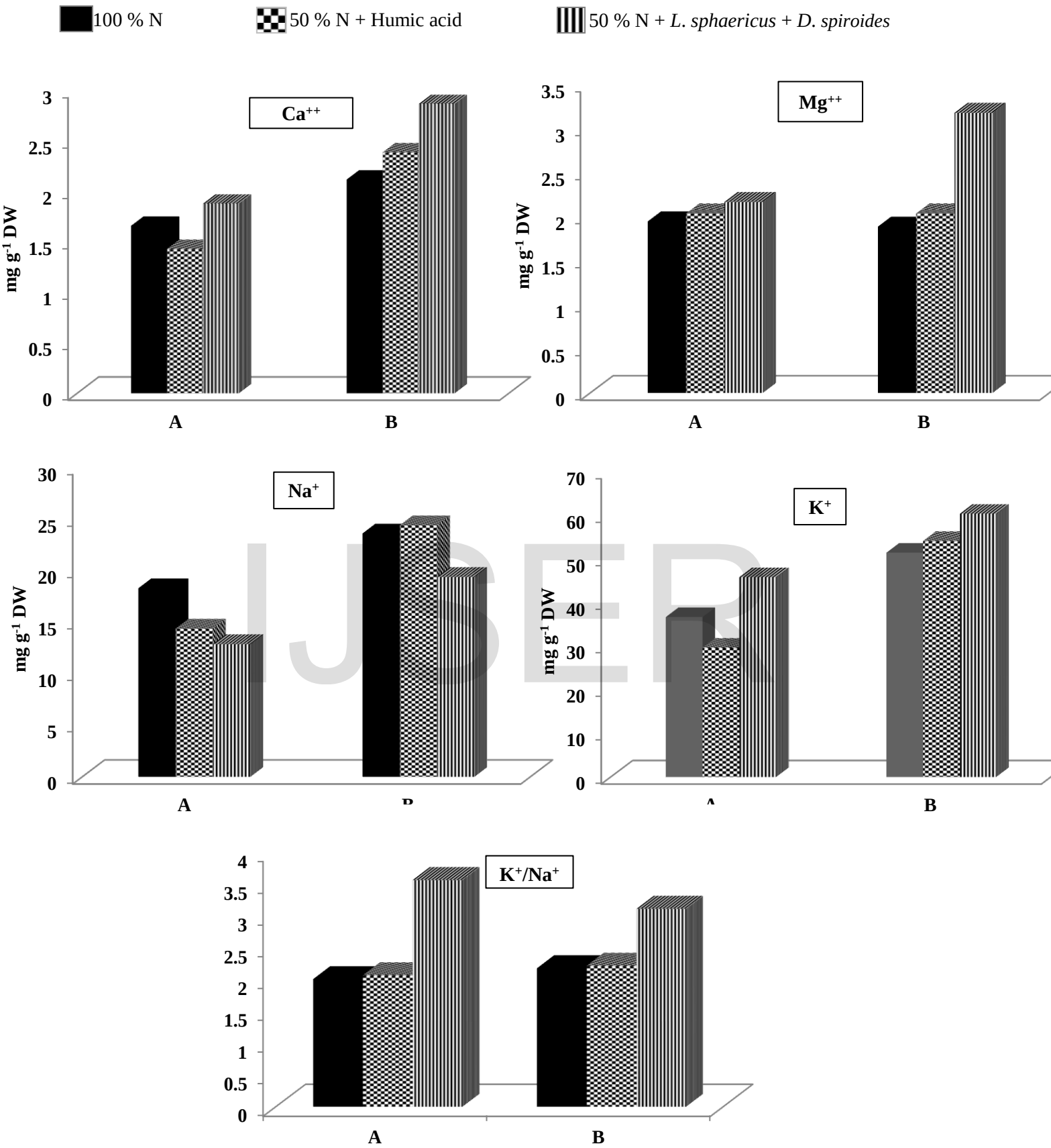

Fig 3. Chemical constituents of wheat cultivars Egypt 1(A) and Sakha 93 (B) as affected by organo-, bio-treatments in saline soil.

Table 10. Net N budgets (mg pot^{-1})* of the different wheat-PGPR-soil systems

	cv. Egypt 1			cv. Sakha 93		
	100 % N	**50 % N+ Humic acid**	**50 % N+ *L.sphaericus* + *D. spiroides***	**100 % N**	**50 % N+ Humic acid**	**50 % N+ *L. sphaericus* + *D. spiroides***
Initial:						
Seeds**	24	24	24	28	28	28
Soil	4700	4700	4700	4700	4700	4700
Total	4724	4724	4724	4728	4728	4728
After 90 days:						
Plants	78.2	57.6	91.6	73.4	81.8	97.2
Soil	7647.0	4671.3	4655.5	4654.2	4654.9	4652.7
Total	4725.2	4728.9	4747.1	4727.6	4736.7	4749.9
N-balance	+1.2	+4.9	+23.1	-0.4	+8.7	+21.9

*****Pot contains 5 kg soil; TN content of soil is 0.094 %.
**20 seeds pot^{-1}, seed N contents are 1.2 and 1.4 mg seed^{-1} for cvs. Egypt 1 and Sakha 93 respectively.

Table 11. Periodical fluctuations in acetylene reducing activities (nmoles C_2H_4 g^{-1} h^{-1}) of the different treatments

Treatment	Incubation periods (days)		
	2	6	10
Non-saline soil:			
Nil	9.3	13.8	11.4
1 % glucose	47.3	715.3	1012.4
1 % barley straw	19.8	86.5	118.7
Saline soil:			
Nil	3.4	3.1	6.7
1 % glucose	14.2	78.4	321.6
1 % barley straw	1.9	48.4	81.7

LSD (0.05), 42.7; CV(%), 11.6.

periods, acetylene reducing activities of non-saline soil exceeded those of saline one by > 260 %.

When kept water-flooded, the unamended soils scored higher C_2H_2 reduction activities, increases over un-flooded soils of 194.8 and 60.4 % were recorder for non-saline and saline soils respectively (Table, 12). A storm of C_2H_4 production was obtained in sucrose-enriched non-saline soil, > 1600 nmoles $g^{-1}h^{-1}$ were produced. This was not the situation in saline soil where the activity hardly exceeded 100 nmoles C_2H_4 $g^{-1}h^{-1}$. Under flooded conditions, greater ARAs were estimated for cellulose-received saline and non-saline soils compared to those maintained un-flooded. The ARA in malate-amended soils seemed to be almost similar under both water regimes. Again, salinity adversely affected the quantities of C_2H_2 reduced, average reduction percentages of *ca.* 56 and 52 were recorded under aerobic and anaerobic conditions, respectively.

Table 12. Acetylene reducing activities (nmoles C_2H_4 g^{-1} h^{-1}) of amended soils under aerobic and anaerobic conditions

Treatment	aerobic	anaerobic
Non-saline soil:		
Unamended	9.6	28.3
Cellulose	124.4	182.5
Malate	51.8	94.7
Sucrose	1628.3	94.7
Non-saline soil:		
Unamended	10.1	16.2
Cellulose	76.4	98.9
Malate	34.6	29.5
Sucrose	699.9	23.8

LSD (0.05), 67.3; CV (%), 8.8.

Discussion

Many tools of modern science have been extensively applied for crop improvement under salt stress of which the plant growth promoting rhizobacteria (PGPR) have become paramount importance in this respect. It is hypothesized that a bacterium natively occurring in naturally salinated soil and coevolved over long time with salt resistant plants could be a suitable alleviator of salt stress on a given crop. In this study, a number of PGPR was isolated from saline habitats, identified and experimented for cereal seed germination, plant growth and N_2-fixation potential under salt stresses. Four isolates, 2 bacteria and 2 cyanobacteria, successfully tolerated high NaCl concentrations and exhibited multiple plant growth promoting traits were subjected to 16S rRNA gene sequence analysis. The isolates were identified as *Bacillus megaterium*, *Lysinibacillus sphaericus*, *Dolichospermum spiroides* and *Dolichospermum circinale* In this context, Rohben *et al.* (2009) isolated bacterial members belonging to the genera *Bacillus, Gracilibacillus, Halobacillus, Halomonas, Halovibrio, Oceanobacillus, Piscibacillus, Salicola, Salinicoccus, Thalasobacillus* and *virgibacillus* from Saltan lake of Iran. In fact, halophilic microorganisms have different mechanisms to respond in salt-stressed environments at molecular level. They have the ability to establish biofilms containing extracellular polymeric substances with high water content (Xiang *et al*., 2008), and inducing competitive advantage to other bacteria under salt stress. Osmotolerance helps the effective colonization of root as well. Osmotolerance is induced either by the production of exopolysaccharides, accumulation intracellular osmolytes or biofilm formation. Compatible solutes are used for osmotic adjustment of bacterial cells and protect the cells against desiccation, high temperature and oxygen radicals (Fernandez- Aunion *et al*., 2010).

Crop establishment comprises principally three processes; germination, emergence and early seedling growth. In the present study, maize seed germination dramatically injured in presence of salt particularly with increasing the concentration. Ozturk *et al*. (2009) found that increasing the salt level decreased the number of seeds germinated and postponed initiation of the process beside decreasing length of radical and plumule got reduced too. Such effects might be caused by the high osmotic pressure of the solution slowing down the intake of necessary water for germination and by the toxic influence of high salt concentration on the embryo. High salt level inhibits the mobilization of the seed reserves and the growth of embryonic axis. At the first, the salt stress induces rapid osmotic changes which affect the root growth within few minutes, and thereafter the disruption of the shoot development (Munns and Tester, 2008). It is also possible

that under high salt concentrations, the naturally present hormones in seeds may be suppressed (Afzal *et al*., 2005).

Quite marked difference in maize development in gnotobiotic system were observed depending upon NaCl concentration and microbial isolate introduced into cultivation media. The leaf area dramatically reduced as the salt level increased, reductions of > 75 % were scored. Neumann (1993) reported that the reduction in leaf growth of plants exposed to salinity has been attributed to reduced turgor or reduction in extensibility of expanding cell walls. This inhibition in short-term may be due to water stress, while on long-term, leaf growth is affected by ion toxicity when the ions move through the transpiration stream and accumulate in the leaves (Ambede *et al.,* 2012), which eventually leads to increased leaf mortality and senescence. The author added that, reduction in leaf area is an adaptation mechanism to reduce ion uptake by roots, the plant development is adversely affected since low leaf areas contribute to less photosynthesis and hence less biomass accumulation. Bacterial and cyanobacterial inoculation greatly diminished adverse impact of high salinity on leaf growth where low reductions in leaf areas were estimated in salinated growth media in presence of the microorganisms. The cereal growth promotion due to inoculation with PGPR in salted environments was also obtained by Suares *et al.* (2015).

Decreases in maize fresh weights with increasing salinity are possibly attributed to the accumulation of inorganic ions (Na^+) which result in decreased water content of plant tissues or accumulation of compatible solutes (Wu *et al.,* 2015). Inoculation with bacterial and cyanobacterial isolates improved the fresh weights of the cereal in highly salted media, an effect that was microbe-dependent. As reported by Yang and Lu (2005), bacterial inoculation improved the water status of plants and thus increased the plant vigor. Morever, improved water status of inoculated plants causes stomatal opening and increased CO_2 assimilation (Dubey, 2005).

The reductions in shoot and root dry weights of cereals tested in the present study due to increased salinity might be a result of combination between osmotic and specific ion effects of Na^+ and Cl^- (Turan *et al.,* 2007). Decreased shoot biomass yield of both maize cultivars was associated with reduced leaf areas leading to reduced photosynthesis and accumulation of dry matter. Reduction of plant biomass yield reflects the increased metabolic energy cost and reduced carbon gain (Netondo *et al.,* 2004). It also mirrors soil impact on plant tissues, reduction in photosynthetic rates par unit of leaf area and attainment of maximum salt concentration tolerated

by the fully expanded leaves (Munns *et al.,* 2006). Shoot and root damages caused by ion toxicity or osmotic effects or both may have contributed to the observed sharp drop in dry weights preceding the death of highly-stressed plants. Inhibition of long distance trampart of nutrient ions by salinity has been proposed to explain the reduced nutrient contents in the plant organs due to displacement of K^+ and Ca^{++} by Na^+ on the membranes, hence reduced shoot biomass. Root elongation rate is reduced by salinity due to reduced rates of cell production and growth, reduced final length of epidermal cells and shorter apical meristem (Zadeh and Naeini, 2007).

Root- to shoot ratio of inoculated maize plants under salt stress increased compared to uninoculated plants, the calculated ratios were falling in the range 0.16-0.25 for uninoculated plants increased up to 0.39 for inoculated ones. In absance of microbiota, the ratio increased from 0.16 to 0.25 as the NaCl level in the gnotobiotic cultivation media increased (Fig. 4). Ratios for maize and wheat of the pot experiments similarly behavied in respones to salinity and PGPR inoculation. This is probably due to the importance of root biomass to allow proper development and water uptake. The root-to-shoot increases under salt stress have been observed as well on wheat by *Pseudomonas putida* (N21), *Pseudomonas aerugniora* (N39) and *Srvatia proteamaculans* (Zahir *et al*., 2009), on red pepper seedlings by *Bacillus licheniformis*, *Bacillus iodinum* (Siddikee *et al.*, 2011) and on barley by *Pseudomonas corrugate* CMH3 and *Acienetobacter* sp. CMH2 (Chang *et al.,* 2014) and *Hartmannibacter diazotrophicus* (Suares *et al.,* 2015). PGPR promote root development providing more surface area enhancing both water and nutrients uptake from soil. Morphological changes in shoot and root elongation in certain plant species by different ACC deaminase producting PGPR strains were reported by Bhattacharyya and Jha (2012).

Actually, the reduction in chlorophyll content due to salinity stress in the present study is expected since its stability depends on membrane stability, which under saline conditions seldom remain intact (Khan *et al*., 2009). Accumulation of endogenous proline in plants, in the present study, markedly increased due to salinity stress of either soil or irrigation water. Extraordinary increases in plant proline contents were attributed to PGPR inoculation. Here, Afrasayab *et al*. (2010) mentioned that inoculation oppressed NaCl to mentain normal metabolic activities required for growth, thereby sustain in defending salt stress from plants. Endogenous osmolytes include various compounds like proline, glycine, betaine, choline, salicylic acids, brassinosteroids,

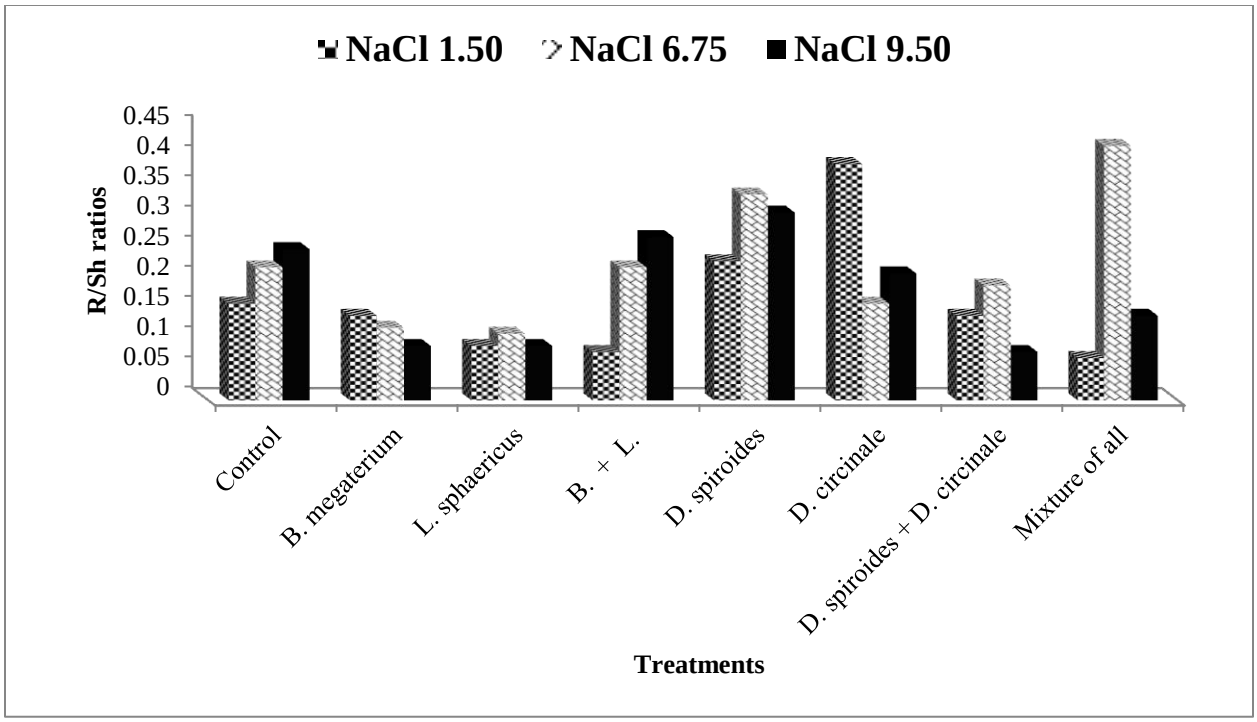

Fig. 4. Root/ Shoot ratios (on dry weight bases) of gnotobiotic model-grown maize seedlings of inoculation and salinity treatments.

silicates and total soluble sugars, *etc.* (Munns, 2002). These compounds play a fundamental role in osmotic adjustment under saline conditions and create the hindrance in the way of ion toxicity for plants. Thus, elevated levels of endogenous osmolytes in inoculated plants compared to uninoculated ones under salt stress indicate salt tolerance status of plants. Generally speaking, it is believed that the accumulation of proline may help to maintain the relatively high water content necessary for growth and cellular function.

In the present study, maize phenols obviously increased as the salt content of the growth medium increased. In conformity with these findings, Sharma and Ramawat (2014) found that, total phenols, total protein, proline and catalase activity in callus of the halophytes; *Salsola baryosma*, *Trianthema triquetra* and *Zygophyllum simplex* greatly increased as soil salinity increased. Chenane *et al*. (2015) reported increased total phenolic content of wheat by sea water treatment.

While Na^+ contents in cereal plants increased, the contents of K^+ and K^+/ Na^+ ratio decreased by salinity. Apart from inoculation, respective increase rates were 2.1-11.0 times for the former and 1.2- 4.8 times for the latter. These findings support those of Carpici *et al*. (2010). Soil

salinity leads to dehydration and osmotic stress, resulting in stomatal closure, reduced carbon dioxide supply, and high production of reactive oxygen species (ROS) such as superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-) (Mittler, 2002), thereby causing irreversible cellular damage. In order to keep the balance between ROS production and scavenging, plants develop scavenging system against ROS, involving both enzymatic and non-enzymatic systems. Major ROS- scavenging enzymes include superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and glutathione peroxidase (GPX) (Pang and Wang, 2008). In the present study, the increased catalase and peroxidase enzyme activities in presence of salt indicate that plants did protect themselves against oxidative stress, increases in such activities were more obvious with the salt-tolerant maize cv. TWC 329. In conformity with these results, Zhang *et al*. (2013) found that catalase and peroxidase activities of cotton leaves gradually increased as soil salinity increaced. The salt tolerant cultivar CCRI-44 hardly affected by salinity and the enzyme activities increased. Reductions in the majority of assessed maize traits of cultivar TWC 329 due to salt stress were comparatively lower than those recorded for cultivar TWC 353 indicating that the former deemed more salt tolerant. The characteristics of a salt-tolerant variety induce Na^+ exclusion, K^+/Na^+ discrimination, retention of ions in the leaf sheath, tissue tolerance, ion partitioning into different-aged leaves, osmotic adjustment, transpiration efficiency, early vigour and early flowering leading to shorter growing season, the latter increasing water use efficiency. The major increases in salt tolerance of modern cereal cultivars, in paricular, will come from introducing new genes by either crossing with new donor germplasm or by tranformation with single genes (Munns, 2005). Munns and Tester (2008) added that some species tolerate salt stress by avoiding uptake of certain ions by tolerating high ion concentrations in the tissue. Differences among genotypes in respect to salt tolerance could be due to genotype, experimantal duration, growth stage and soil phsico-chemical properties.

As mentioned by Niu *et al*. (2012), salt accumulation in plant tissues greatly depends on salinity of irrigation water, leaching fraction and frequency of irrigation. As indicated in this study, irrigation of maize plants with saline water led to salt accumulation in the root zone. In reality, salinity of irrigation water would not by as high as 8.0 dSm^{-1}, near to that use in this study (6.75 dSm^{-1}). The reason for choosing this EC was to distinguish the salt tolerance among the genotypes in a realtively short term, 30 days in this case. Among the two cultivars tested, TWC

329 seemed more tolerant to irrigation water salinity, a result obtained in presence or absence of microbial inocula.

Results of N_2-fixation potential as affected by soil salinity proved that acetylene reducing activities, in general, were lower in saline soil (up to 700 nmole C_2H_4 g^{-1} h^{-1}) compared to those measured for the non-saline one (> 1600 nmoles C_2H_4 g^{-1} h^{-1}). Several studies did domenstrate the detrmiental effect of high salinity on growth and N_2-fixation efficiency of soil N_2-fixers (Mordai *et al*., 2011; Severin *et al.,* 2012). In both saline and non-saline soils, the addition of carbohydrates substantially supported N_2-fixation, the highest rates were scord in glucose-amended soils. N_2-fixation in barley straw-rececived soils increased with time perhaps due to the microbial decompostion of straw resulting in release of suitable byproducts necessary for the activity of authochthonous N_2-fixing populations. The effect of barley straw amendment was rather low in saline soil. Significantly high ARA were recorded in flooded soils supplemented with cellulose particularly the non-saline one. Indeed, the anaerobic decomposition products of organic matter leads to substrates that support N_2-fixation (Soussi *et al*., 2001; Rezazadeh *et al*., 2014). It is an interesting observation that acetylene reducing activities were significantly greater in non flooded sucrose-amended soil either saline or not. This could be attributed to the higher invertase activity in non-flooded soils leading to increase glucose availability which in turn supported higher N_2-fixation. Chendrayan *et al*. (1980) found that soil invertase acivity was many fold higher in non-flooded soil even after 40 days incubation compared to flooded conditions.

Assuming the C_2H_2 reduction to N_2-fixation conversion factor of 3 proposed by Hardy *et al*. (1973), the highest figure of 1628.2 nmoles C_2H_4 g^{-1} h^{-1} recorded for sucrose-enriched non-saline soil respresents a net N gain of 38.7 kg $acre^{-1}$. Meanwhile, 669.9 nmoles C_2H_4 g^{-1} h^{-1} measured for saline soil means that 15.9 kg $acre^{-1}$ of N were added to soil due to sucrose supplementation. In fact, the croping systems applied in irrigated soils of Egypt guarantee a good supply of easily decomposable plant residues and water in addition to temperature in the range of 20-35 through the year. Such conditions provide an environment favourable for N_2-fixation. Intensive cropping and application of crop residues besides the flooding irrigation system adopted in Nile Dellta soils at 10-day interval ensure continuous supply of mositure, all of all, supporting better growth and activities of N_2-fixing residents (Hegazi *et al*., 1979).

Muhling and Lauchli (2002) summarized the overall toxic effect of salinity as the salt taken up by the plant concentrates in the old leaves, and continued transport of salt into transpiration leaves over a long period of time eventually results in very high Na^+ and Cl^- concentrations and the leaves die. The cause of the injury is probably due to the salt load exceeding the ability of the cells to compartmentalize salts in the vacuole. Salts then would rapidly build up in the cytoplasm and inhibit enzyme activity. Alternatively, they might build up in the cell walls and dehydate the cell. Besides, to improve seed performace and provide faster and synchronized germination in salt stressed environments, seed priming or osmo-conditioning is highly recommended (Abraha and Yohannes, 2013). It is an easy, low cost and non-risky technique and recently being used to overcome salinity problem in agricultural lands. It intails the partial germination of seed by soaking in either water or in a solution of salts for a specified periods of time following drying just prior to radical emerges. This condition stimulates many of the metabolic processes involved with the early phases of germination and it has been noted that seedlings from primed seeds emerge faster, grow more vigorously and perform better in adverse conditions. Further studies are required as well to investigate the impacts of seed priming on the late growth and yield stages of cereals. Additional advanced research is needed to explore priming induced alteration of physiological and biochemical attributes both at seed and whole plant levels.

Conclusion

In view of over increasing global populations, in addition to arable land, saline land needs to be cultivated for increased yield output. Besides, the plant-beneficial rhizobacteria may decrease the global dependence on hazardous agrochemicals which destabilize the agro-eco system. This work accentuates the perception of the PGPR in saline environments. Developing salt-tolerant crops is still in pipeline and therefore, the only viable alternative seems to be the use of salt-tolerant biofertiliziers to promote growth and productivity of crops. The widespread utilization of the multi-functional PGPR particulary in stressed environments, will necessitates that a number of issues be addressed. In the first, going from laboratory and greenhouse experiments to field trials to large scale commercial field use will reqiure a number of new approaches for the growth, storage, shipping, formulation and application of these microbiota. Second, it will be indispensable to educate the public on the use of PGPR in agriculture. Much popular mythology is directed toward thinking about bacteria only as agents of diseases. The

misconception needs to be corrected before the public accepts the deliberate release of benefical microbes into the environment on a large scale. Third, scientists need to prove to both public and to regulatory agencies worldwide that genteically engineered PGPR do not present any new hazards or risks. Fourth, scientists are required to determine whether future research should be directed toward developing PGPR that are rhizopheric or endophytic. In addtion, further improvements in salt tolerance of crops will undoubtedly result from close interaction and cooperation among molecular geneticists and physiologists and benefit from timely feedback from plant breeders and microbiologists.

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