

The Study of Electrolyte Leakage from Barley (*Hordeum vulgare L*) and Pearlmillet Using Plant Growth Promotion (PGPR) and Reverse Osmosis

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Abstract The effect of water stress induced on cell membrane stability was examined in two plants, (*Hordeum vulgare L*) and Pearlmillet using plant growth promotion (PGPR). Brackish water as byproduct from reverse osmosis plant (RO) after desalination process, considered as unfriendly environmental impact and affects agriculture growth. It contains significant concentrations of dissolved salts ions such as Na⁺, Cl⁻, Ca²⁺, Mg²⁺, K⁺, SO²⁻, and CO₃²⁻ as major ions. Total dissolved salts (TDS) of these ions ranged from (5000 mg/L -10000 mg/L). Salt ions accumulation was found to be increased in shoots of barley and pearlmillet (159.09mmol, 179.73mmol) /0.114m² of pots while TDS for decant water decreased to reach (0.101 mg/L). Electrolyte leakage assay showed that plant treated with PGPRs resulted in same values for trials treated with fresh water and less electrolyte leakage from membrane equal to 304 mg/L. The novel results of this research study that carried for the first time where PGPRs *Pseudomonas putida* (UW3 and UW4) had been used for improving the phytoremediation activities of two salt tolerant plants: Barley (*Hordeum vulgare L.*) and Pearlmillet plants had showed a very clear and significant improvements of high salt uptake and thus high phytoremediation activities of these plants once they were treated with PGPRs.

Keywords: Barly, Pearlmillet, phytoremediation, reverse osmosis, bacteria, brackish

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1. Introduction

Water is considered to be a basic and vital component of the social, economic, and political fabric of Palestine. Its sector represents the basic foundation for sovereignty and attachment to our land. The limited sources are classified into surface and ground water. Recent depletion of water resources and deterioration has become the key of environmental challenges. These challenges require urgent action to treat water to an appropriate quality and quantity, in order to meet the needs of disposal and beneficial reuses [1,2].

Many techniques and operations have been implemented to treat wastewater and saline water in Palestine. Four RO plants exist in Jericho for the treatment of brackish and brackish water. This operation has created side products such generated brackish water. Disposal of the water caused salinity of soil, and has inhibited plant growth. To minimize the effect of brackish water disposal into the environment, many researchers have been assigned to finding economical and effective methods for treating it through many feasible processes [3].

Phytoremediation technique uses plants to take up ions into their biomass, then above ground the biomass can be harvested. Still, nowadays the Phytoremediation process isn't used widely, due to its high salinity, which inhibits the plant growth of even tolerant plant species [4].

In this study, phytoremediation technique was implemented for the treatment of brackish water generated from the reverse osmosis plant, using the Barley Plant (*Hordeum vulgare L.*) and Pearlmillet Plant. These plants germinated with PGPR. Some of the trials with PGPR were imbibed with hydrogen peroxide to study the effect of antioxidant resistance damage caused by the production of reactive oxygen radical under salt stress.

In (2003) Tchobanoglous et al [5]. context was provided about brackish management and it examined a broader context of brackish treatment. The treatment technologies included a membrane filtration process such as reverse osmosis; an ion exchange process such as electrolysis or weak acid cation and an exchange or evaporation process such as brackish concentrators.

Hamed et al [6], mentioned remediation methods for salt impacted soils including excavation, leaching, electronic restoration and phytoremediation. Phytoremediation was enhanced with PGPR showing satisfactory results in the

infiltration of soils salinity by sequestered ions in the biomasses of plants.

The impact of brackish water is the most severe environmental stress on plants. The common ions stressing and inhibiting plant growth are sodium and chloride. When these ions enter the soil and surround the rhizosphere part of root, It caused differences between water potential in roots above water potential in soils. This change lowered the movement of water from soil into rhizosphere, limiting water and nutrient uptake [7,8,9].

Sodium ions are the primary cause of disorders concerning enzyme activation to protein synthesis. It is considered more toxic than the chloride ions.

Moreover, sodium has numerous physiological effects. It causes deficiencies of other nutrients by interfering with ion transporters for K^+ . which are essential to activate more than 50 enzymes and the synthesis of protein, which plays a role in cellular functions. The competition results in an overabundance of sodium in tissue compared to potassium, and enters in coordination with t-RNA, resulting in inhibited protein synthesis, leading to disruption of these cellular functions [10,11,12].

Chloride ion is required in plants to some limited levels as a vital ion. It's involved in photosynthetic mechanisms, in adjusting osmotic potential, and maintains electrical charge throughout the membrane [11].

Plants are divided into two groups according to their ability to tolerate salt which are: Halophytes and Glycophytes. Halophytes are more adapted to salt stress than Glycophytes. Differences between these groups are in the stability of their enzymes and physiological processes; even Halophytes are inhibited at some point of high salt concentration [9].

We want to study the effect of PGPR on plant cells' integrity: salt ions entry damage on cell membrane, and the increase of permeability will be studied. Measurements of NaCl accumulations in plants will be made and compared with control plants.

In this research, Phytoremediation will be implemented as a method for the treatment of generated brackish water by using selected tolerant plants species germinated with PGPRs at Palestine.

2. Material and Methods

2.1. Selecting and Culturing PGPR

In this study two salt tolerant plants species selected [Barley plant (*Hordeum vulgare* L.) and Pearl millet were used for ion leakage studies using phytoremediation technique. In order to increase their liability and tolerance to salty conditions, trials tested by incorporating them with plant growth promoting rhizobacteria (PGPR): UW3 and UW4 (*Pseudomonas putida*). These strains were used in coating seeds separately, or in combination and they were grown in Tropic Soy Growth (TSB) media. The media for UW3 growth was the only one that contained 100 mg/L of Ampicillin antibiotic (AMP). Solid media had been prepared by addition of 7.5 g of agar for preparation of solid plates. Bacterial strains were cultured on solid and liquid media at 30°C for overnight. Some of these prepared bacteria were transferred to sterile falcon tubes with addition of glycerol layer (1:1) volume and stored at -80°C as stock liquid solutions.

For liquid cultures preparations, bacterial inoculums had been transferred to 50 mL falcon tubes containing proper TSB media and incubated at 30 °C with shaking at 200 r.p.m in rotator shaker (orbital shaking incubator, labtech, LSI-3016 A) for 26 hour.

2.2. Seed Treatment with PGPR

Cultures for each strain were transferred to two 50 mL falcon tubes separately, followed by centrifugation at 2000 r.p.m for 20 minutes using (Universal 320 R). The pellets were soaked in (10 mL) of distilled deionized(dd) H_2O and the optical density (OD) had been measured for each strain at wavelength 600 nm by UV- spectrophotometer (Spectro UV-Vis Dual Beam -8 Auto cell, UVS- 2700) to have 1.5 (OD) for UW3 which is perfect germination and 2.0(OD) for UW4 include for perfect germination [13].

For adhesion process of bacterial cells to the seeds surfaces, methylcellulose white gel polymer was prepared. Briefly, 7 g of methylcellulose powder was dissolved in 500 mL of distilled – deionized water (dd H_2O); stirred for one hour until most of clumps had been dissolved. Then they were autoclaved for 20 minutes at 110°C and 100 psi using auto cleave (EQUUS steam sterilization auto cleave). The resulted polymer was clear white gel upon cooling.

The bacterial-methylcellulose polymers incorporated with (2.5:1) volume for pearl millet seeds and up to (7:1) volume for barley seeds. It is worth to mention that plant seeds had been disinfection previously by soaking in bleach sodium hypochlorite (1% M) for 10 minutes, followed by three times washing with (dd H_2O).

After seeds treatments with PGPR, they were dried for 5 minutes at room temp before they were transferred into sealed autoclaved plastic bags, and then stored at 4°C for one week prior usage.

2.3. Measurement of Soil Salinity

Soil samples were selected to be loam soil collected from An-Najah field campus in Palestine. The soil samples were filled in bags and autoclaved (EQUUS steam sterilization auto cleave) to ensure removal of any bacterial and/or fungi infections. Soils were allowed to dry to remove moisture, and sieved using 10 mm particle size sieve.

Electrical conductivity was measured for randomly chosen samples. Measurement based upon EC_e (soil saturated with water) and $EC_{1:2}$ (1:2 represent ratio of soil to water extract). These measurements were carried out according to published procedures by Chang (2007). Measurements for two parameters were performed in triplicates. $EC_{1:2}$ measurement done by addition of 15 g of sterile-soil to 30 mL of distilled –deionized water (dd H_2O) in 50 mL sterile falcon tube. The mixtures were shaken on rotator shaker (Orbital shaking incubator, lab tech, LSI-3016 A) at 200 r.p.m for 30 minutes to make them homogenous mixtures, and centrifuged at 2000 r.p.m for 10 minute (Universal 320 R). The electrical conductivity was measured for supernatant using electrical conductivity meter instrument (4510 – conductivity meter, Jen way).

For EC_e (soil saturated with water) measurements; 50 g of sterile soil was mixed with sufficient dd H_2O in 100 mL beaker till reach saturation. Where saturation, point indicated by shining appearance of the paste. The paste allowed to settle down at least 4 hours to ensure the

saturation criteria. The mixture then centrifuged at 2000 r.p.m for 10 minutes.

Soil samples were filled in plastic pots of 17x16x15 cm with 12 medium holes at bottom for drainage. Each pot was filled with 350 gram of sieved soil.

All pots were planted in early February, 2014; and maintained in miniature greenhouse built in backyard of my house. All pots were placed inside in rows to make it easy for irrigation. This was to mimic the climate condition in Jericho. Greenhouse temperature was measured twice daily. No human interference for the temperature or light intensity during the period of the experiments.

Before germination all pots were irrigated with fresh water twice daily for five days. Pitchers used with holes to regulate operation of irrigation.

The control seeds pots used in this experiments are one pot was irrigated with fresh tape water; the second one was irrigated with brackish water of 6000 mg/L; and the last one was irrigated with brackish water of 10000 mg/L. Each pot contained an average of twenty seeds of each barley and Pearlmillet Plants.

The same control samples used for seeds germinated with pseudomonas putida (UW3).

2.4. Salt Accumulation in Plants

Salt accumulation test was used in this study to determine the effectiveness of phytoextraction mechanism of the tested plants. It was used to determine how much of salt ions have been eliminated from brackish water. This method was carried for all trials by taking roots and shoots of plants after 30 days and washing them with tap H₂O and air dried for 5 days.

Shoot tissues were analyzed for Na⁺ concentration by taking 1.0 g of plant shoot tissues into 50 mL Taylor tube. Adding 10 mL of concentrated nitric acid to tube for digestion and leaving them overnight. The tube was heated at 125°C for 4 hours then cooled and diluted to 12.5 mL with concentrated nitric acid and 50 mL of distilled water for further analysis using ICP-MS for sodium and a titration method with AgNO₃ was applied for chloride.

2.5. Assessment of Plant Cell Membrane Stability Using the Electrolyte Leakage Methods

For each trial fresh shoot samples (1 g fresh weight) of similar size were cut into approximately 3 cm long segments, washed with ddH₂O, and dried. Segments were submerged in 10 mL of ddH₂O in a 20 mL test tube and placed into vacuum desiccators. Each sample was subjected to a vacuum at a rate of 100L/min for 2 hours. Then electrical conductivity (EC) value of the solution were measured at room temperature of 23±1 °C using an electrical-conductivity meter.

3. Results and Discussion

3.1. Measurement of PGPR Growth under Saline NaCl Solutions

Different concentrations of NaCl – TSB solution were prepared to test performance of PGPR salt tolerance in two plant species: “Barley and Pearlmillet”. The results are shown in Figure 1.

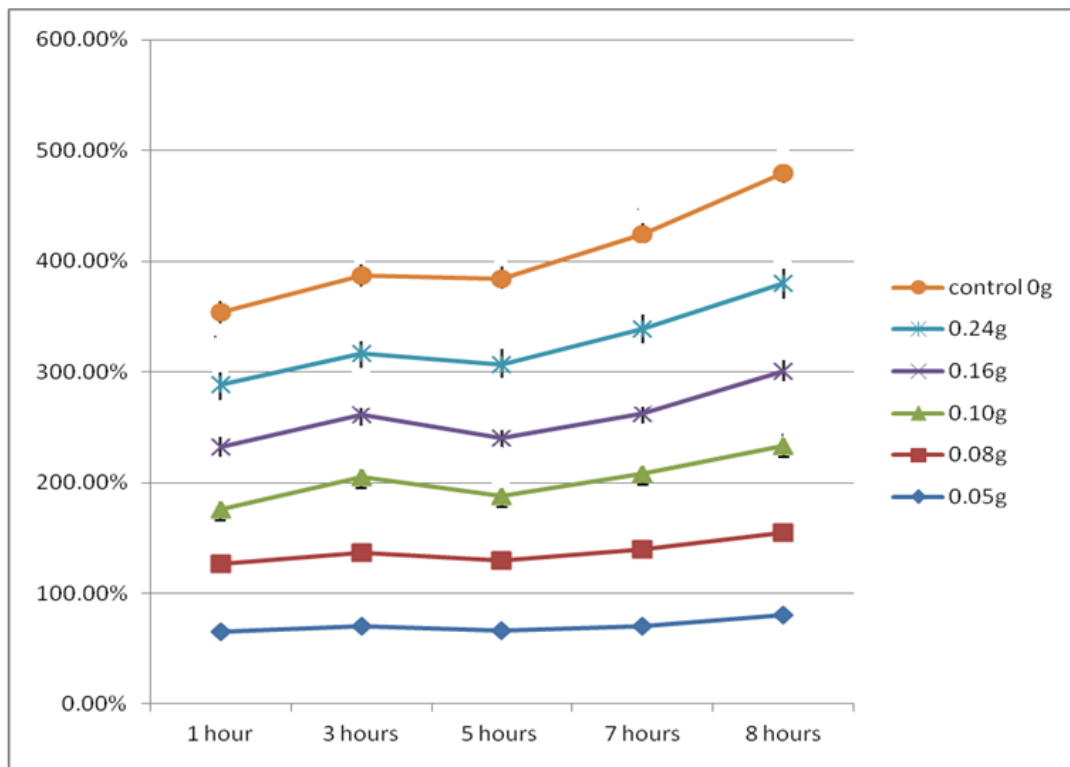


Figure 1. The percentage of control absorbance for UW3 at $\lambda = 600$ nm in NaCl - (TSB) solution at different salt weights

The ratio measurement of control sample is equal to the absorbance of bacteria grown in saline for each weight

divided by absorbance control (0 g NaCl) at 8 hours, where control (0 g NaCl) is equal the absorbance of

bacteria UW3 grown in control (0 g NaCl) at each time divided by absorbance of the bacteria grown in control(0 g NaCl) at 8 hours.

The measurements were taken for 8 hours, after which maximum efficiency was reached, and then became constant after 8 hours. OD measurements and % of control are shown in Figure 1 which shows that UW3 germination was increased under saline conditions at different time intervals, until it reached maximum levels and became constant without any incensement, after 8 hours.

This increase indicated that salinity tolerant performances of PGPRs were increased [13], and the growth had been shown for TSB medias containing:0.08g, 0.10g, and 0.24g to be as 74.55%, 78.31%, and 79.68 %, respectively. Also, the lowest measurement of bacterial growth was obtained for a 0.16 g salt containing media

(66.88%), and may be related to some performance of germination of bacteria in the tube.

The UW3 strains were chosen only for these measurements, since there is no differences between UW4 and UW3.

This test can be applied in future research to study if the performance of PGPR increases with time, which will indicate more biomass production.

3.2. Soil Electrical Conductivity

Measurements of soil salinity were used in experiments to study changes in EC when irrigated with brackish water. Experimental measurements of (TDS) for random samples of autoclaved loam soil are shown in Table 1.

Table 1. Experimental measurements of (TDS) for random samples of autoclaved loam soil, each parameter was performed in triplicate.

Name of parameter	Trial 1 TDS (mg/L)	Trial 2 TDS(mg/L)	Trial 3 TDS(mg/L)	Average	Standard deviation
EC _e	70.0	72	67.2	69.7	2.4
EC 1:2	47.7	44.7	46.3	46.2	1.5

Measurements of soil salinity as TDS after 30 days of cultivation period at temp 17°C, using Eq. 1.

$$\text{TDS}(\text{mg/L}) = \text{EC}(\text{dS/m}) \times 640 \quad (1)$$

where EC used between 0.1 and 5.0 ds/m.

The texture of the soil sample used in this study was similar to the texture existing in the Jericho area, which is a loamy texture. In order to be implemented, this study received field trials in the Jericho area. Each trial sample was done in triplicate with 30 days between each trial.

The results are shown in Figure 2. In the barley plant trials, the plants were treated with PGPRs and irrigated with brackish water, where their EC and TDS values before and after 30 days showed no obvious changes in measurement and their values were close to control trial irrigated with fresh water. This indicates the accumulation of salts in biomass, and trials treated with H₂O₂ were slightly similar to trials irrigated with brackish water. We concluded that PGPR enhanced more salt uptake into plant biomass.

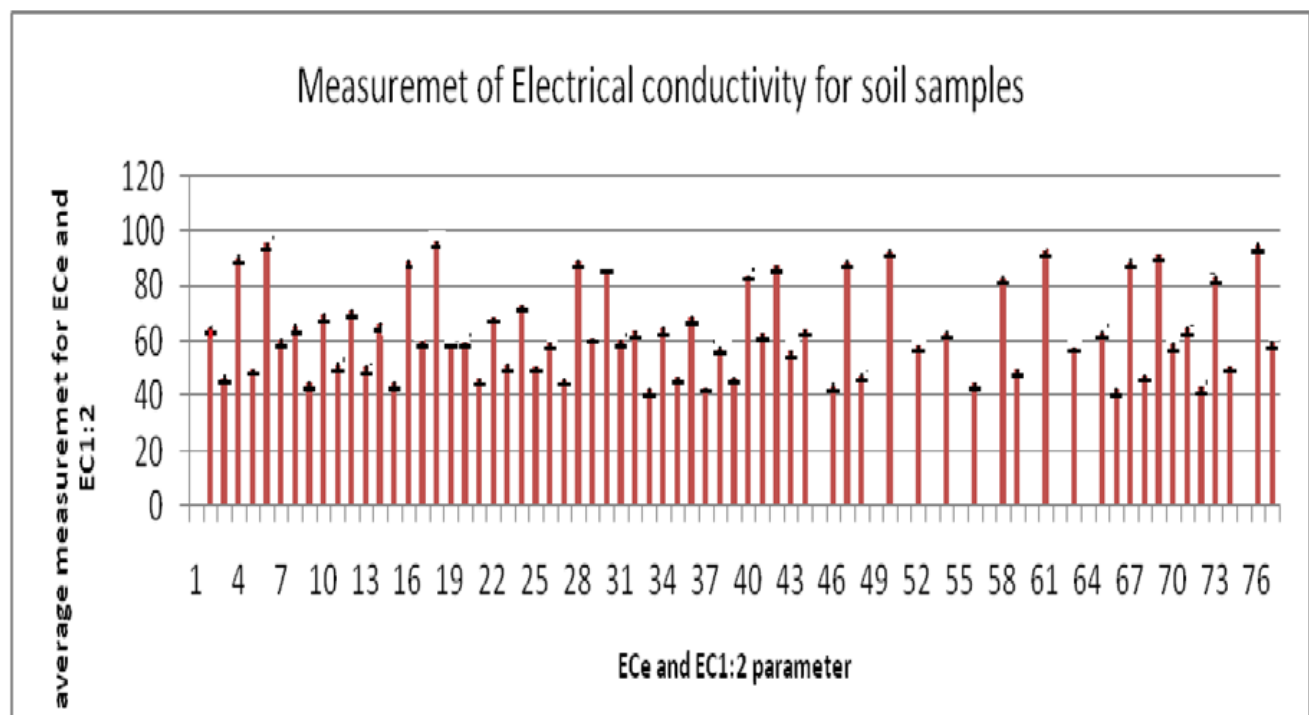


Figure 2. Measurements of soil salinity as EC after 30 days of cultivation period at temp 17 °C

In the case of Pearlmillet plant trials, results were not promising in promoting plant growth, even for trials with PGPRs, their values were still less than the values for barley plant trials. This can be related to some specific response of the plant due to these microbes.

3.3. Brackish Water Parameters Measurements

TDS measurements for decent water trails were shown in Figure 3. For trials treated with PGPRs their

measurements values were less than those of control trials. This indicated that PGPR helped in increasing the phytoextraction mechanism for salt uptake by leaf and stem succulence. Trials included the combination of UW3

and UW4, which showed no significance over treated trials separately as shown in TDS measurement. This means that the same salt accumulated in the plants tissues.

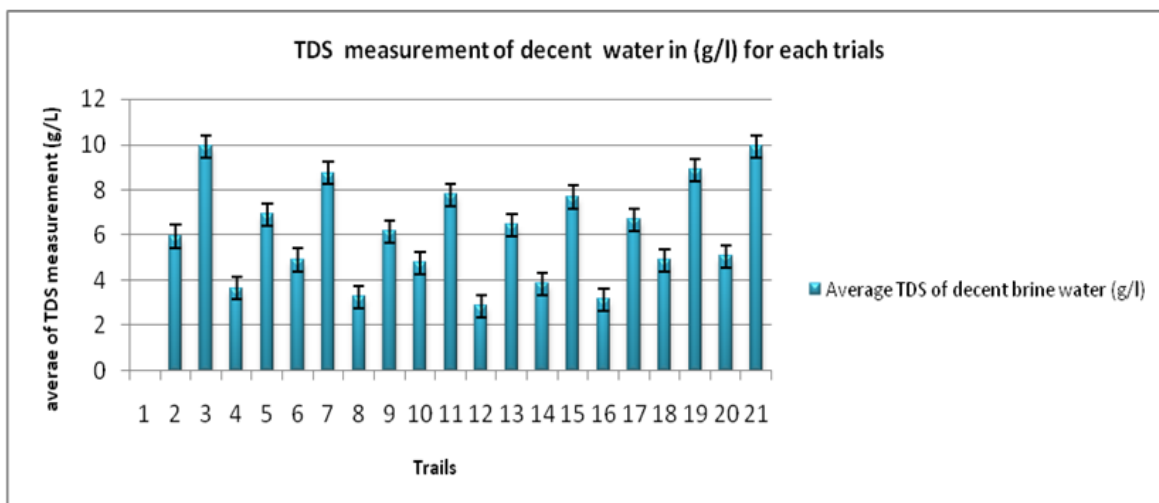


Figure 3. Salinity measurements as TDS for two synthetic brackish water samples before and after irrigation include for decent water (gravitational water), it detects any contaminant ions that could be leached out. These measurements were included also for determination of how much leaching water could be arrived to ground water and cause salinity

TDS for decent brackish water of trials of barley seeds with H₂O₂ irrigated with 6000 mg/L and 10000 mg/L brackish water gave 4.89 g/L and 8.87g/L respectively, compared to control (5.94 g/L and 9.92 g/L). This means that the only tolerance mechanisms that could happened, while hydrogen peroxide aid plant overcome oxidative stress through participation in cell signaling, (MAPK) nitrogen –activated protein kinase represents a central for mediating cellular responses to multiple stressors.

TDS measurements for Pearlmillet plant trials results showed no obvious significant combination of both strains to raise salt accumulation of plant to slat and increase plant growth promotion over separate treatment.

Barely plant responded more to PGPR than Pearlmillet plant; this attribute could be due to the large surface area of barley seeds compared to Pearlmillet seeds, so more bacteria strains have adhered to the surface of barley seeds. Another reason may be related to some difference in physiology and anatomy, as well as specific differences in conditions required for optimal growth for Pearlmillet plants (which differ from Barley plants). This may indicate also that Pearlmillet plants may need different

PGPR strains (other than UW3, UW4) for their optimal growth condition.

3.4. Salt Accumulation in Plant

The salt accumulation test was used in to determine the effectiveness of the phytoextraction mechanism of the tested plants. It was used to determine the amount of salt ions that have been eliminated from brackish water. This method was carried out over trials by taking roots and shoots of plants. All trials are shown in Table 2.

For the weight of salt accumulation of Na/ Cl ions (mg/g dry weight) compared to the theoretical weight are shown in Figure 4.

Plant shoot tissue that was analyzed for ion accumulation (Table 2 and Figure 4) showed a total ion weight in total dry mass (g) for barley seeds treated with UW3. Trial of barley seeds treated with UW4, and both UW3 + UW4, irrigated with 6000 mg/L of brackish water were (2382.1 mg, 1872.6 mg, and 2478.4 mg) compared to control (13.2 mg).

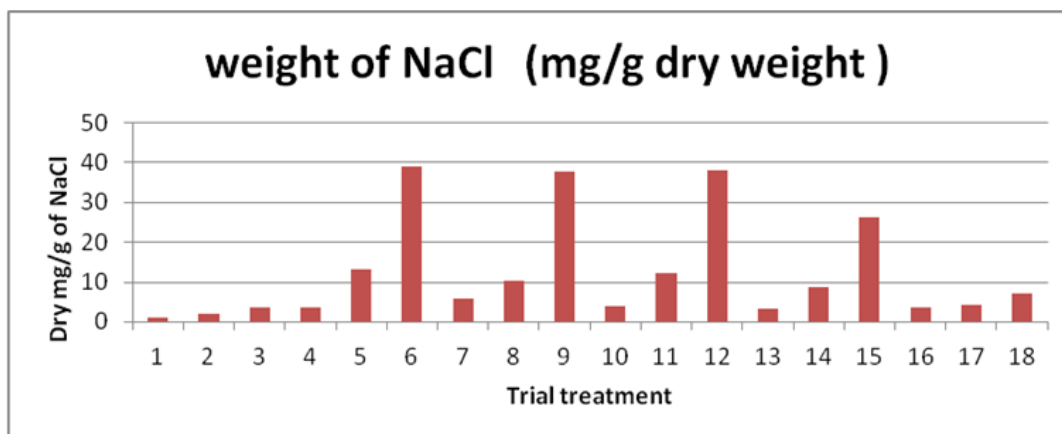


Figure 4. Measurement of salt accumulation of NaCl ions (mg/g dry weight) in barley plant root tissue

Table 2. Measurement of salt accumulation of Na Cl ions (mg/g dry weight) in Barley Plant shoots tissue

Trial number	Treatment	Na (mg/g dry weight)	Cl (mg/g dry weight)	NaCl (mg/g dry weight)	Total Dry mass(g)	weight total ion in total dry mass (mg)/0.114m ² of pot	Concentration of mmol / 0.114m ² of pot	Ratio of Cl/Na
1	Control Barley irrigated with fresh water	0.659	0.457	1.116	76.1	84.926	1.826	0.693
2	Control Barley irrigated with 6000 mg/L of brackish water	0.956	0.975	1.931	6.835	13.198	0.283	1.019
3	Control Barley irrigated with 10000 mg/L of brackish water	1.974	1.564	3.538	10.258	36.292	0.780	0.792
4	Treated Barley seeds with UW3 irrigated with fresh water	2.378	1.326	3.704	118.8	440.035	9.4631	0.557
5	Treated Barley seeds with UW3 irrigated with 6000 mg/L of brackish water	7.666	5.524	13.19	180.6	2382.114	51.228	0.720
6	Treated Barley seeds with UW3 irrigated with 10000 mg/L of brackish water	23.65	15.324	38.978	189.8	7398.024	159.097	0.647
7	Treated Barley seeds with UW4 irrigated with fresh water	3.475	2.436	5.911	118.8	702.226	15.101	0.601
8	Treated Barley seeds with UW4 irrigated with 6000 mg/L of brackish water	6.146	4.223	10.369	180.6	1872.641	40.271	0.787
9	Treated Barley seeds with UW4 irrigated with 10000 mg/L of brackish water	26.81	11.014	37.828	219.8	8314.594	178.808	0.910
10	Treated Barley seeds with UW3 + UW4 irrigated with fresh water	3.008	0.786	3.794	97.5	369.915	7.9551	0.261
11	Treated Barley seeds with UW3+ UW4 irrigated with 6000 mg/L of brackish water	9.147	3.020	12.167	203.7	2478.417	53.299	0.330
12	Treated Barley seeds with UW3+UW4 irrigated with 10000 mg/L of brackish water	28.05	10.004	38.058	219.6	8357.536	179.731	0.356
13	Treated Barley seeds with UW3+H ₂ O ₂ irrigated with fresh water	2.078	1.341	3.419	88.8	303.607	6.529	0.645
14	Treated Barley seeds with UW3+ H ₂ O ₂ irrigated with 6000 mg/L of brackish water	5.457	3.224	8.681	180.6	1567.788	33.715	0.690
15	Treated Barley seeds with UW3+ H ₂ O ₂ irrigated with 10000 mg/L of brackish water	18.35	7.972	26.327	189.80	4996.864	107.459	0.434
16	Treated Barley seeds with H ₂ O ₂ irrigated with fresh water	2.378	1.326	3.704	69.100	255.946	5.504	0.557
17	Treated Barley seeds with H ₂ O ₂ irrigated with 6000 mg/L of brackish water	2.765	1.524	4.289	7.735	33.175	0.713	0.551
18	Treated Barley seeds with H ₂ O ₂ irrigated with 10000 mg/L of brackish water	4.954	2.324	7.278	63.192	459.911	9.890	0.469

Measurements for the trials of barley seeds treated with UW3, UW4, and both UW3+ UW4 irrigated with 10000 mg/L of brackish water (7398.0 mg, 8314.6 mg, and 8357.5 mg) were compared to control barley irrigated with 10000 mg/L of brackish water (36.3 mg).

Measurements for trials of barley seeds with UW3+ H₂O₂ irrigated with 6000 mg/L of brackish water, irrigated with 10000 mg/L of brackish water (1567.8 mg, 4996.9 mg) compared to control barley irrigated with 6000 mg/L of brackish water (13.2 mg) and control barley irrigated with 10000 mg/L of brackish water (36.3 mg). The measurements of salt ion uptake analyses were higher for trials with PGPR compared with trials without PGPR.

NaCl accumulation in plant tissue for total dry mass ranged from 36.3-8357.5 mg, and the ratio of Cl/Na 0.6-1.01 for experimental results compared to theoretical atomic weight equaled 1.5. These results indicated that accumulations of Cl⁻ ions in plant tissue were uneven where Na⁺ accumulations were greater than Cl⁻,

suggesting that plants utilize more Cl⁻ for their biosynthesis.

Moreover, these concentrations of salt have no effect in using these plants as forage food for animals, when compared with the theoretical ratio.

3.5. Assessment of plant cell membrane stability using the electrolyte leakage methods.

This method describes assessing membrane permeability in relation to salt stress. In this study increases in salt affect plant membrane permeability, where measurements of electrolyte leakage methods as TDS in (mg/L) in barley plant root tissue trials are shown in Table 3 and Figure 5.

The measurements of ion leakage plant tissue are a method for assessing membrane permeability in relation to salt stress. In this study increases in salts affect plant membrane permeability, as indicated by higher ion leakage.

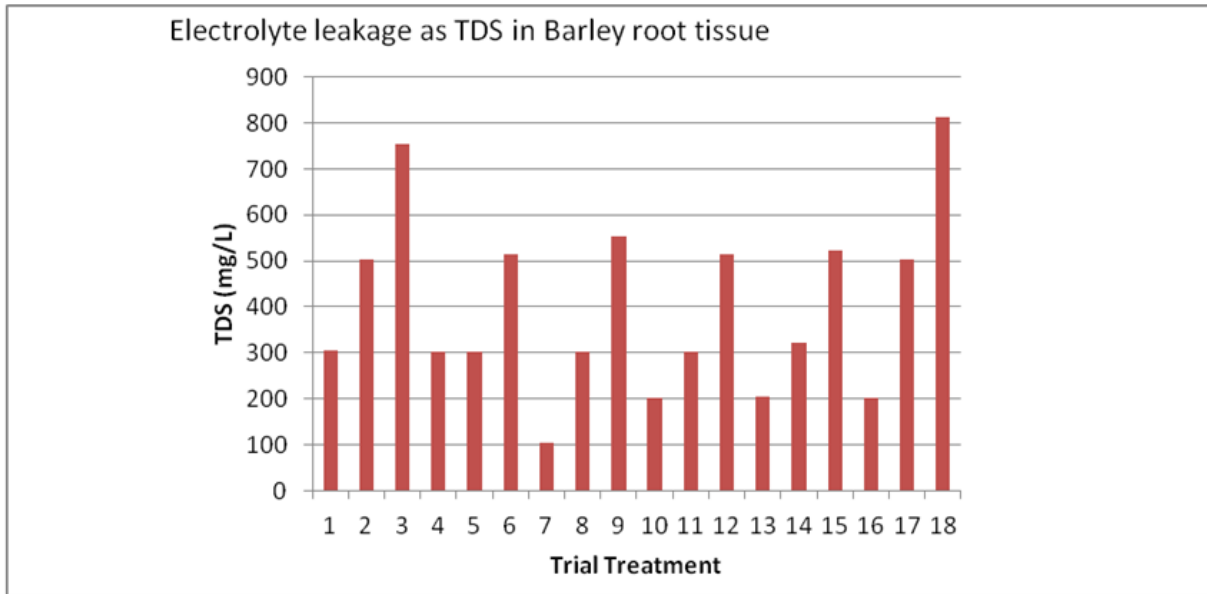


Figure 5. Measurements of electrolyte leakage methods as TDS in (mg/L) in barley plant root tissue trials

Table 3. Measurements of electrolyte leakage methods as TDS in mg/L in barley plant root tissue trials

Num	Treatment	TDS mg/L	Significant result
1	Control Barley irrigated with fresh water	304	Sig
2	Control Barley irrigated with 6000 mg/L of brackish water	503	--
3	Control Barley irrigated with 10000 mg/L of brackish water	754	--
4	Treated Barley seeds with UW3 irrigated with fresh water	302	Sig
5	Treated Barley seeds with UW3 irrigated with 6000 mg/L of brackish water	302	Sig
6	Treated Barley seeds with UW3 irrigated with 10000 mg/L of brackish water	513	Sig
7	Treated Barley seeds with UW4 irrigated with fresh water	104	Sig
8	Treated Barley seeds with UW4 irrigated with 6000 mg/L of brackish water	303	Sig
9	Treated Barley seeds with UW4 irrigated with 10000 mg/L of brackish water	554	Sig
10	Treated Barley seeds with UW3 + UW4 irrigated with fresh water	202	Sig
11	Treated Barley seeds with UW3+ UW4 irrigated with 6000 mg/L of brackish water	302	Sig
12	Treated Barley seeds with UW3+UW4 irrigated with 10000 mg/L of brackish water	513	Sig
13	Treated Barley seeds with UW3+H ₂ O ₂ irrigated with fresh water	204	Sig
14	Treated Barley seeds with UW3+ H ₂ O ₂ irrigated with 6000 mg/L of brackish water	323	Sig
15	Treated Barley seeds with UW3+ H ₂ O ₂ irrigated with 10000 mg/L of brackish water	524	Sig
16	Treated Barley seeds with H ₂ O ₂ irrigated with fresh water	202	Sig
17	Treated Barley seeds with H ₂ O ₂ irrigated with 6000 mg/L of brackish water	502	--
18	Treated Barley seeds with H ₂ O ₂ irrigated with 10000 mg/L of brackish water	813	--

Results revealed that salinity had increased the amount of electrolyte leakage from the plant cell membrane in general for control trials and one treated only with H₂O₂, and salinity made cell membrane more permeable, which when observed in results compared to control fresh water. Even though plant cell membranes in trials treated with PGPRs, were found having less electron leakage, compared to control one treated irrigated with brackish water. In this tale, implicate PGPR in protection of plant cell membranes was possible by promoting the synthesis of lipids, that considered as structural constituents of most cellular membrane [14,15,16,17].

4. Conclusion:

- Specifically, trials treated with PGPRs had showed significant improvements in salt accumulation for the plants (Barley and Pearl millet) that were used in these experiments, indicated that these two plants successfully can be used in the phytoremediation process in combination of the PGPRs (*Pseudomonas pituda* UW3 and/or UW4), with an advantage of Barley over Pearl millet Plant.
- Results had showed that these PGPRs increased the cell membrane stability as demonstrated by less electrolyte leakage from plant cells relative to plants that were not treated with PGPR.
- Results from pulse amplitude modulated fluorometry (PAM) studies indicated that these plants when treated with PGPR had an increased photosynthesis rate thus prevented salinity damage to photosystems compared to those untreated ones.
- Biomass measurements showed a significant mass increase for those plants treated with PGPRs compared with the controls (untreated); in which biomass production could enhance phytoremediation

efficiency, as well as be used as forage food for animals.

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References

- [1] Marie, A., and Vengosh, A. Source of salinity in ground water from Jericho area, Jordan Valley. *Ground Water*.39, 240-248. 2001.
- [2] Y. Nassar. Virtual water as a policy instrument for achieving water security in Palestine. *Water Resources in the Middle East*.2, 141-146.2007.
- [3] Sachit, D. E., Veenstra, J. N., "Analysis of reverse osmosis membrane performance during desalination of simulated brackish surface waters. *Journal of Membrane Science*, 453, 136–154.2014.
- [4] USEPA. (2000). Government report: Introduction to phytoremediation. The U.S.Environmental Protection Agency.
- [5] Tchobanoglous, G., Franklin, L. Burton, H., and Stensel, D. (2003). *Wastewater Engineering (Treatment Disposal Reuse)*. Metcalf & Eddy, Inc. (4th ed.). McGraw-Hill Book Company. U.S.A.
- [6] Hamed, O. A., Hassan, A. M., Al-Shail, K., Farooque, M. A. Performance Analysis of a Trihybrid NF/RO/MSF Desalination Plant, *Desalination and Water Treatment*, 1(1-3), 215-222. 2009.
- [7] Guan, R., Chen, J., Jiang, J., Liu, G., Liu, Y., Tian, L., Yu, L., Chang, R., and Qiu, L., "Mapping and validation of a dominant salt tolerance gene in the cultivated soybean (*Glycine max*) variety Tiefeng 8. *The Crop Journal*, 2(6). 358-365.2014.
- [8] Ashraf, M., "Some important physiological selection criteria for salt tolerance in plants. *Flora*, 199(5), 361-376. 2004.
- [9] Das A. and Parida, K. Salt tolerance and salinity affect on plants a review. *Ecotoxicology and Environmental Safety*. 60(3), 324-349. 2005.
- [10] Blaha, G., Stelzl, J., Spahn, C. M., Agrawal, R. K., Frank, J., Nierhaus, K. H. Preparation of functional ribosomal complexes and effect of buffer conditions on tRNA positions observed by cryoelectron microscopy. *Methods in Enzymology*, 317, 292-309.2000.
- [11] Blumwald, E., and Aharon, G. Sodium transport in plantcells. *Biochimica et Biophysica Acta* 1465, 140-151. 2000.
- [12] Carden, D. E., Walker, D. J., Miller, A. J. Single-cell measurements of the contributions of cytosolic Na⁺ and K⁺ to salt tolerance. *Plant Physiology* 131. 676-683. 2003
- [13] Saharan, B. S., and Nehra, V. *Plant Growth Promoting Rhizobacteria: A Critical Review Life Sciences and Medicine Research*, Volume 2011: LSMR-21. 2011.
- [14] Gopal, S., Chandrasekaran, M., Shagol, C., Kim, K., and Sa Spore, T. Associated Bacteria (SAB) of Arbuscular Mycorrhizal Fungi (AMF) and Plant Growth Promoting Rhizobacteria (PGPR) Increase Nutrient Uptake and Plant Growth Under Stress Conditions. *Korean J. Soil Sci. Fert.* 45(4), 582-592. 2012
- [15] Ahemad, M., and Kibert, M. Mechanisms and application of plantgrowth promoting rhizobacteria: current perspective. *J. King Saud University-Science*. 26 (1): 1-20. 2014.
- [16] Naidoo, G., and Somaru, R. Morphological and physiological responses of the halophyte, *Odyssea paucinervis* (Staph) (Poaceae), to salinity. *Flora* 203, 437-447. 2008.
- [17] Ekmekçi, Y., Tanyolaç, D., and Ayhan, B. Effects of cadmium on antioxidant enzyme and photosynthetic activities in leaves of two maize cultivars. *Journal of Plant Physiology*. 165, (6), 6600-6611. 2008.