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The impact of *Pseudomonas putida* UW3 and UW4 strains on photosynthetic activities of selected field crops under saline conditions

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ABSTRACT

This research was aimed to assess the photosynthetic activities of barley (*Hordeum valgare* L.), clover (*Trifolium repens* L.), and pearl millet (*Pennisetum glaucum* (L.) R. Br.) under different saline conditions with two strains of *Pseudomonas putida* (UW3 and UW4) treatments. An exceptional observation was revealed on barley biomass ratio (288.8%) that irrigated with brackish saline water (10,000 mg/L) with the presence of *P. putida* UW4 strain. In general, *P. putida* UW3 strain was significantly increased crops biomass ratio (249.4%, 202.1%, and 212.5%) for barley, pearl millet, and clover, respectively, which were irrigated with 10,000 mg/L brackish saline water. Plant root and shoot systems were significantly increased in their length and weight reflecting the improvement of plants' photosynthetic activities under salt stress conditions with the presence of *P. putida* strains. The results from pulse amplitude modulation fluorometry showed that the plants were recovered from the saline stress effect once *P. putida* strains were applied. The outcome of this research was highly recommended to apply *P. putida* strains (UW3 and UW4) with field crops for phytoremediation, in particular, where salinity (soil and/or brackish water) was environmentally challenging.

KEYWORDS

Brackish water; PGPRs; Phytoremediation; *Pseudomonas putida*

Introduction

Plants are varied from Halophytes to Glycophytes based on the ability to tolerate salt, compromising several crops such as oats, barley, wheat, tall wheatgrass, and many others (Niazi *et al.* 1992); Ashraf 2004). Halophytes tolerance mechanisms were classified based on their avoidance, adaptation, or accumulation of salts (Munns and Tester 2008), as well as their enzymes' stability (Das and Parida 2005).

Photosynthesis, the crucial physiological process in plants, was found to be impaired by salt stress, that causing closure of their stomata (Flexas *et al.* 2004; Baker 2008). The increased salt accumulate in plant's tissues would cause swelling of their thylakoids and chloroplast membrane distortion (MacNeill 2011). Salts stress will affect plants photosynthetic activities and can be measured through PAM fluorometry spectroscopy (Beer and Björk 2000; Meloni *et al.* 2003).

Plant growth promoting rhizobacteria (which are abbreviated as PGPRs) are naturally soil borne bacteria that found within the rhizospheric plant zone. These microbes are naturally motivated plants' growth through direct mechanisms by producing enhancement substances. Besides, PGPRs can facilitate plants acquisition of nitrogen and phosphorous, in addition to increase the concentration levels of plant hormone (Munees and Mulugeta 2014). These rhizobacteria were found decreasing the inhibitory effects of many pathways which affect the photosynthetic activities (Glick *et al.* 1998; Munees and Mulugeta 2014). In contrast, recent studies showed that plants growth could be enhanced under stresses using PGPR (Kende 1993; Qadir *et al.* 1996; Glick *et al.* 1998; Mayak *et al.* 2004; Wu 2009). It was found that in the presence of NaCl (up to 172 mM), the PGPR strains would enhance more resistance to salt stress conditions by increasing the expression of their amino cyclopropane-1-carboxylate (ACC) deaminase activity (Glick *et al.* 1998; Shan 2009).). Rhizobacteria would produce the ACC deaminase under salt stress conditions, which converted the plant roots' ACC, from stressed plants, into ammonia and α-ketobutyrate. The presence of PGPRs on the rhizospheric zone, the exuded ACC would be consumed by the bacteria, resulted in decreasing the ethylene amount in plant roots and thereby alleviates the salt-induced stress (Glick 2004; Wu 2009; MacNeill 2011; Munees and Mulugeta 2014).

In this research, two PGPRs strains (*Pseudomonas putida* [UW3 and UW4]), were applied on three selected field crops (barley [*Hordeum valgare* L.], clover [*Trifolium repens* L.], and pearl millet [*Pennisetum glaucum*]) to assess their interactions based on the recorded impacts on the plants photosynthetic activity which would be subjected to varied salt stress conditions.

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Material and methods

Bacterial viability test seeds treatment

Plant growth promoting rhizobacteria *P. putida*, strains UW3 and UW4 (courtesy donated by Professor Glick lab; at Waterloo University; in Canada) were used in this research study. Both rhizobacteria were cultured on solid and liquid tryptic soy broth (TSB) media and following the procedures of Lifshitz *et al.* (1987) and Penrose and Glick (2003) with some modification. Briefly, rhizobacterial suspensions for each strain were cultured at 30 °C for 26 h with shaking (80 rpm), and collected by centrifugation at 2000 rpm for 20 min. The pellets were washed with one volume of sodium pyrophosphate to remove secondary metabolites, and then resuspended in ddH_2O. Each strain was diluted to give 1.5 optical density (OD) for UW3 and 2.0 OD for UW4, using UV spectrophotometer (Spectro UV-Vis Dual Beam -8 Auto cell, UVS 2700) at wavelength 600 nm (Shan 2009; MacNeill 2011).

The viability of these strains under salt stress conditions was first ascertained by testing their ability to replicate in media with different salt concentrations. Therefore, the bacteria were cultured in TSB liquid media containing varied NaCl concentrations (2500, 5000, and 12000 ppm) that resembled brackish water. Inoculums were incubated at 30 °C for 8 h. The bacterial growth was measured by spectrophotometer absorbance (at $\lambda = 600$ nm) after 1 h, and in 2 h intervals. The cultures were stopped after 8 h. The absorbance of the bacterial growth in media without NaCl was used as a control in this test.

For packing seeds with the bacteria, methylcellulose adhesion white gel polymer was prepared. Briefly, 1.4% methylcellulose was added to ddH_2O, stirred for 1 h, and autoclaved for 20 min at 110 °C and 100 psi. The resulted white clear gel upon cooling was mixed with commercial nontoxic blue colorant (Color Coat Blue, Becker Underwood, Saskatchewan) at a ratio of 2%. The presence of colorant was necessary to meet safety regulations requiring all treated seeds to be visibly colored to avoid use for animal consumption.

The selected plant seeds (barley [*Hordeum vulgare* L.], clover [*Trifolium repens* L.], and pearl millet [*Pennisetum glaucum* (L.) Br.]), were brought from National Agriculture Research Center (NARC), Ministry of Agriculture, Jenin. Seeds surface were first sterilized in 70% ethanol for 2 min, then in 10% household bleach (Clorox, 6% sodium hypochlorite) for 10 min followed by three times washing with sterile distilled water. The slurry of the rhizobacteria and the blue methylcellulose polymer (at a ratio of 1:5) was applied to an equivalent volume of plant seeds at ratio of (1:10) in a sealed sterile plastic bag. That slurry was mixed for 1 min, before leaving them to dry in <1 h. The dried seeds were immediately transferred into other plastic bags, sealed, and stored at 4 °C for a maximum of 2 weeks prior to usage.

Seeds growth conditions

PGPR-treated seeds were planted in sterile (200 ml) pots with surface area of (0.114 m^2). Each pot was filled with sterile loamy soil and planted with 20 seeds for each treatment (Table 1). Pots (P1, P2, and P3) were used as a control in these experiments (untreated seeds). Pots (P4, P5, and P6) were for seeds that were treated with *P. putida*, strain UW3; while pots (P7, P8, and P9) were for those seeds that were treated with *P. putida*, strain UW4. All pots were irrigated with fresh and saline water (0.6% and of 1% of NaCl solutions).

Table 1. Experimental design conditions for each replicate (P1–P9).

	Irrigation		
Seeds treatment	FW	BW6K	BW10K
Untreated seeds (control)	(P1)	(P2)	(P3)
P. putida, UW3	(P4)	(P5)	(P6)
P. putida, UW4	(P7)	(P8)	(P9)

FW: fresh water irrigated replicates; BW6K: irrigated with brackish water [6000 mg/L]; BW10k: irrigated with brackish water [10,000 mg/L].

Plants were isolated from soil by aluminum trays, as well as the gravitational waters were collected for each replicate for leaked ions measurements. Seeds were planted in February 2014, and maintained in uncontrolled greenhouse structure (no human interference for the temperature or light intensity during the period of the experiments). They were placed in rows and greenhouse temperature was measured twice daily. These experiments were carried out in the successive years (2015–2016) where the results reliability had been confirmed.

At the early stage, and before seeds germination, all pots were irrigated with fresh water twice a day, and for 5 days. After that, each pot was irrigated with its corresponding irrigation water (Table 1) once daily. During their growth, plant's shoot lengths were measured weekly. After 30 days of planting, all plants were harvested and subjected to test analysis.

Measuring photosynthetic activity

Biomass was used to reflect photosynthetic activities of crops (Evans 2013). It was measured in dry mass ratio for each replicate to the total dry biomass of the control one (untreated seeds irrigated with fresh water), as the following formula:

$$\text{Biomass } (\%) = \left(\frac{\text{Total dry mass for each replicate}}{\text{Total dry of control}}\right) \times 100\%$$

The percentage would be used as an indication for plants growth activity of each replicate. Besides, roots and shoots dry masses were measured apart for evaluating the effects of each replica on plant growth development. In addition to that, root lengths and shoot lengths were measured for better understanding of effects of *P. putida* strains (UW3 and UW4) on the development of plant system's growth.

Measurement of photosynthesis with (PAM) fluorometry

Using pulse amplitude modulated fluorometry (PAM) (LUCAM, Fluor cam version 15.1.0), plant's photosynthetic activities were checked. The variable fluorescence (F_v) or yield was calculated as $[(F_m - F_o)/F_m]$ (Kitajima and Butler

1975), where F_m referred to the maximal fluorescence of dark adapted tissue and F_o for the minimal fluorescence. Optimal values for yield ranges from 0.5 to 0.75. Lowered value indicates that plant is stressed. The quantum yield (q_p) is referred to photochemical quenching and can be calculated as $q_p = [(F_m' - F_s)/(F_m' - F_o)]$, where F_s is the steady-state fluorescence. The value of q_p indicated PSII reaction center that were open and equal the approximate oxidation of PSII (Shan 2009). All that would be used to estimate the potential efficiency of PSII by taking dark-adapted measurements. Analysis was done on randomly chosen shoots from different replicates with no light interference, to ensure only fluorescence light were measured. Samples were adapted to dark for 20 min before pulse amplitude modulated analyses were carried out to ensure that PSII centers were open. The minimum fluorescence in darkness (F_o) was adjusted to 0.10–12 million ± 0.040. For the maximum fluorescence in darkness measurements (F_m), a single nonmodulated saturating 0.6 s light pulse was used. Then steady-state chlorophyll fluorescence (F_s) was measured after 30 s using nonmodulated 640–700 nm actinic radiation. After this step, plants were left for 14 min to ensure that fluorescence was reached to steady state. A single nonmodulated saturating 0.6 s light pulse was excited every minute to measure the F_m, in presence of actinic light. Then all parameters were measured and calculated.

Statistical data analysis

The experiments were designed as random block design, which included nine different treatments with three replicates for each crop. The obtained data were analyzed using the Microsoft excel sheet. Roots and shoots lengths and biomasses data were analyzed by t test at ($p < 0.1$) with random sampling method. The software package from Simple Interactive Statistical Analysis was used for statistical analyses (Uitenbroek 1997).

Results and discussion

Depletion of brackish water in environment causes osmotic stresses on plants and soil. That will limit the agricultural usage of those fields in the country. In fact, depletion of water resources is considered the key environmental challenges that require urgent actions to efficiently uses to cope with farmers increased demands (Marie and Vengosh 2001). Phytoremediation is one of the promising solutions that could be applied for water and land salt remediation (Brooks 1998). Phytoremediation can be enhanced with uses PGPR which showed satisfactory results in saline soils (MacNeill 2011). That was outlined by Glick et al. (1998) as reported that PGPRs were improved plant growth under stressful condition by lowering the ethylene stress hormone (Wu 2009; Munees and Mulugeta 2014). Therefore, this research focused on the assessment the impact of two strains of *P. putida* (UW3 and UW4) on promoting growth of widely planted field crops in the country.

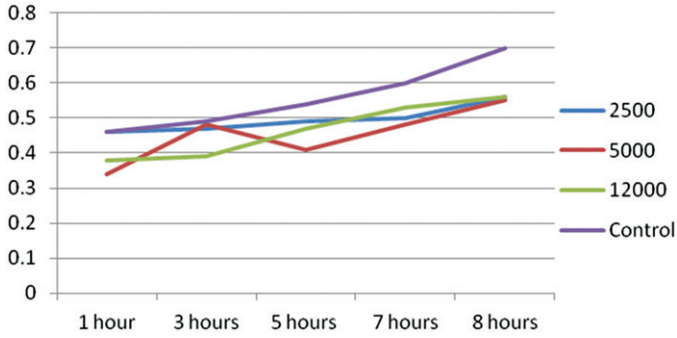

Figure 1. Average absorbance of *P. putida* strain UW3 that grown in varied concentrations of NaCl–TSB solution medium at $\lambda = 600$ nm. The absorbance of the bacterial growth in media without NaCl was used as a control.

First, the viability of these rhizobacteria strains under salt stress conditions was ascertained by testing their ability to grow under different salt concentration media (2500, 5000, and 12000 ppm NaCl).

After 8 h incubation of *P. putida* (UW3) that was cultured in TSB liquid media at 30 °C (Figure 1), showed that saline conditions were slightly lowered bacterial growth (0.56) compared to the control one (0.7). Different salt concentrations media showed slight variations in the bacterial growth at the first few hours of incubation, but stabilized after 7 h. The stability of growth for both strains was recorded after 8 h of incubation, confirming the salinity tolerance of this *P. putida* UW strains, as described for some other PGPRs (Shan 2009).

Biomass for measuring photosynthetic activity

The photosynthetic impacts of field crops: barley, clover, and pearl millet were assessed as biomass ratio of their dry weights. Plants were harvested after 1 month of their seeding and their dry biomass for their roots and shoots were measured and compared with untreated control plants as seen in Table 2.

It was obviously recorded the positive impact of the rhizobacteria *P. putida* on the total biomass of all tested crops (Table 2). The highest biomasses were recorded on barley seedlings (189.8 and 219.8 g) which were treated with *P. putida* strains UW3 & UW4, respectively; both were irrigated with brackish water of 10,000 mg/L NaCl. On barley, the obtained results gave advantages for the strain UW4. The responses of roots and shoots were in favor of the strain UW3 (3.41:2.92 g; and 0.68:0.47 g) for both pear millet and clover respectively; at the saline water treatments (10,000 mg/L NaCl). In other words, barley seedlings had the highest total biomass when treated with *P. putida* UW4 strain, meanwhile both pearl millet and clover had the best total biomass measurements with *P. putida* UW3 strain; all irrigated with 10,000 mg/L NaCl brackish water. Even though, *P. putida* UW3 increased the biomasses for barley roots and shoots (120.5, 69.3) for the same treatment.

Closer look to the plant root system, the highest root biomass was recorded on barley seedlings treated with *P. putida* strain UW4 (140.5 g), meanwhile the strain UW3 was found to enhance more roots biomasses on pearl millet and clover

Table 2. The average dry masses weights (g) of seedlings after 30 days of seeding for barley, pearl millet, and clover.

		Biomass (g)		
Field Crop	Replicate	Root	Shoot	Total
Barley	P1	40.5 ± 2.1	35.6 ± 1.9	76.1 ± 3.4
	P2	6.1 ± 0.9	0.7 ± 0.13	6.8 ± 1.1
	P3	9.3 ± 0.4	0.9 ± 1.9	10.2 ± 0.3
	P4	35.6 ± 1.6	83.2 ± 5.4	118.8 ± 5.7
	P5	93.4 ± 3.2	87.2 ± 4.3	180.6 ± 5.8
	P6	120.5 ± 10.5	69.3 ± 5.1	189.8 ± 15.5
	P7	45.6 ± 3.6	73.2 ± 1.9	118.8 ± 2.6
	P8	103.4 ± 3.9	77.2 ± 1.1	180.6 ± 4.7
	P9	140.5 ± 9.0	79.3 ± 1.8	219.8 ± 11.0
Pearl millet	P1	1.23 ± 0.23	0.45 ± 0.1	1.69 ± 0.24
	P2	0.56 ± 0.08	0.31 ± 0.1	0.88 ± 0.13
	P3	0.23 ± 0.11	0.14 ± 0.03	0.37 ± 0.13
	P4	1.35 ± 0.14	0.72 ± 0.08	2.08 ± 0.12
	P5	2.54 ± 0.20	0.73 ± 0.06	3.27 ± 0.29
	P6	2.79 ± 0.16	0.62 ± 0.06	3.41 ± 0.16
	P7	1.67 ± 0.16	0.53 ± 0.03	2.21 ± 0.2
	P8	1.99 ± 0.17	0.60 ± 0.86	2.59 ± 0.25
	P9	1.97 ± 0.28	0.96 ± 0.15	2.92 ± 0.31
Clover	P1	0.24 ± 0.02	0.08 ± 0.01	0.32 ± 0.03
	P2	0.30 ± 0.02	0.03 ± 0.01	0.33 ± 0.02
	P3	0.32 ± 0.03	0.07 ± 0.0	0.39 ± 0.03
	P4	0.36 ± 0.05	0.09 ± 0.0	0.45 ± 0.06
	P5	0.38 ± 0.01	0.04 ± 0.0	0.42 ± 0.02
	P6	0.46 ± 0.07	0.22 ± 0.03	0.68 ± 0.09
	P7	0.35 ± 0.03	0.16 ± 0.02	0.51 ± 0.03
	P8	0.42 ± 0.01	0.06 ± 0.04	0.48 ± 0.02
	P9	0.41 ± 0.04	0.06 ± 0.00	0.47 ± 0.05

(2.79, 046 g), and all irrigated with the same brackish water irrigated with 10,000 mg/L NaCl.

In general, untreated seeds with the rhizobacteria expressed low biomass of their roots proportionally with the increase in salt water as they were irrigated with in treatments (P2 and P3), but not when the rhizobacteria were applied to seeds. In fact, plants roots biomasses increased as the salt concentration of irrigation water was increase (P4, P7 & P5, and P8). That was reported on all three planted crops, with advantages to the strain UW4 on barley plant and to UW3 on pearl millets and clover.

Surprisingly, the shoot biomasses were expressed different growth. The highest shoot biomasses were recorded on barley seedlings that were treated with rhizobacteria strain UW3 and irrigated with 6000 mg/L brackish salt water (87.2 g). The same was on clover plants (0.73 g), but not for pearl millet seedlings, as the best results were obtained for those treated with UW4 and irrigated with 10,000 mg/L brackish salt water (0.96 g). In fact; with the strain UW3, all plants exhibited reduction in shoots biomasses as the salt concentration increased for seeds treated, but at the contrary with strain UW4. Even though, both *P. putida* strains were proved to improve plant total biomasses as the concentration of salts increased.

For better understanding of *P. putida* strains impact on plant growth development, total biomass percentages for each replicate were used as an indication for their growth improvement. The biomass ratio was referred to control biomass weight (P1) that was used as a reference point of their development (Figure 2). It was revealed that the best percentage of plant biomass was recorded on barley seedlings (288.8%) treated with UW4 rhizobacteria that irrigated with 10,000 mg/L brackish saline water, meanwhile pearl millet

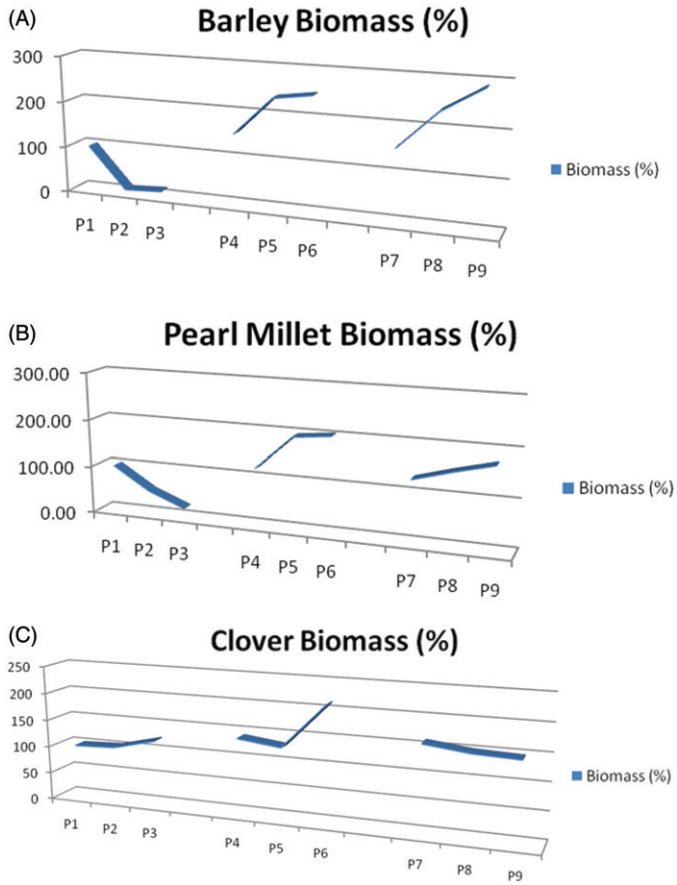

Figure 2. The graph illustrates the impact of *P. putida* strains on total biomass ratio at each different salt condition.

and clover seedlings were recorded best with *P. putida* strain UW3 and irrigated with 10,000 mg/L brackish water (202.07% and 212.5%, respectively).

The obtained results showed clearly the impact of *P. putida* strains on each plant with different salt stress conditions. For barley (Figure 2A), plant total biomass was reduced as salt concentration increases (P2 & P3). When the rhizobacteria had been applied to the seeds, their biomasses were increased (P4, P5, & P6), suggesting the influence of PGPRs on the plant growth. Salty conditions were in favor of increasing the growth of the plant with the presence of the rhizobacteria. That was as a result of the 1-aminocyclopropane 1-carboxylate (ACC) deaminase by lowering stress hormone (ethylene) levels (Chang *et al.* 2014; Glick 2014). The same was noticed with pearl millet and clover (Figure 2B,C). Clover showed withdrawal in growth in response to high salt concentration with *P. putida* strain UW4, but it was still improved with the other strain (UW3).

The roots length had been measured after 1 month of seeding as well as shoots for all replicates of plants (Figure 3). It was noticed that they proportionally increase with salt concentration compared with untreated once. The longest roots (305 mm) were recorded on barley seeds treated with UW3 strain and irrigated with 6000 mg/L salt brackish water. The same observations were noticed on pearl millet and clover plants (Figure 3). That was another indication of the establishment of symbiotic mutual relationships between the rhizobacteria and the plant's roots. Indeed, PGPRs were

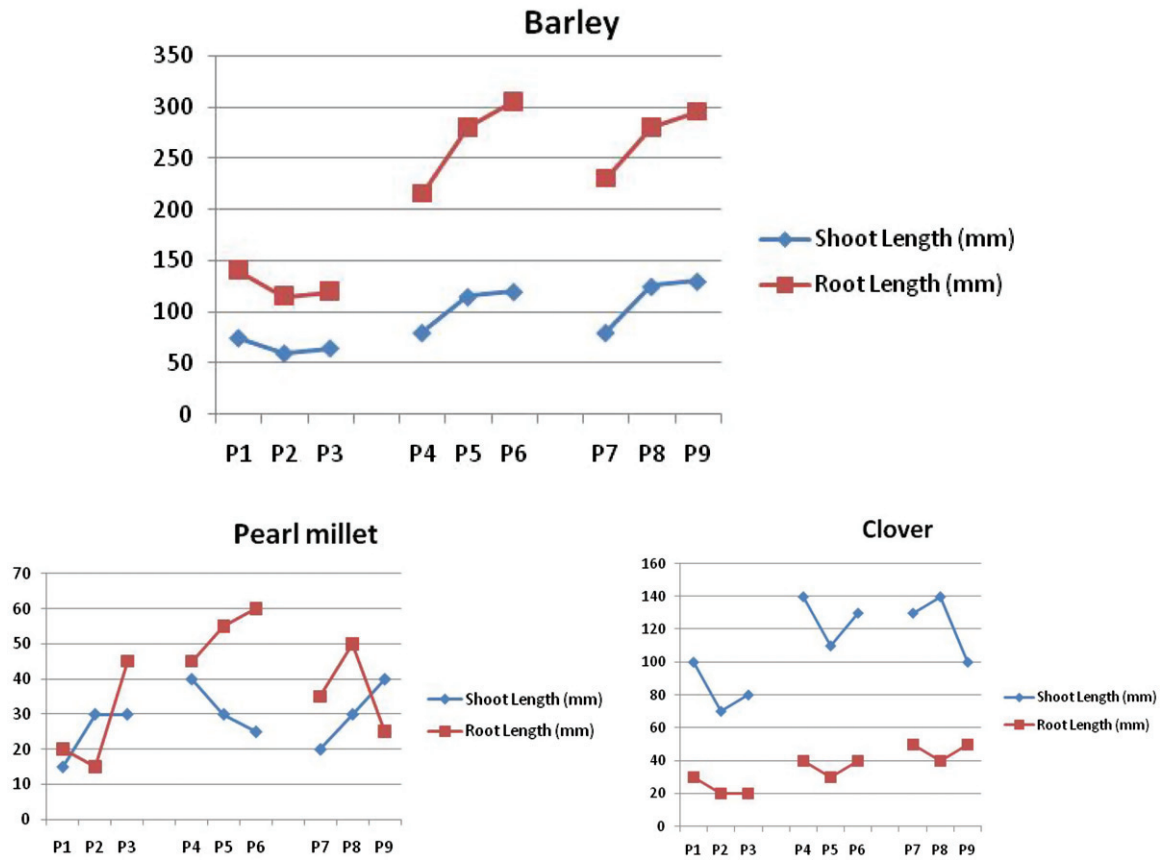

Figure 3. The graph showed the comparison among shoots and roots growth (in cm) after 30 days of barley, pearl millet, and clover planting.

reported to provide IAA in compensation with ACC, the precursor of ethylene (Glick 2014), which increase the roots length as far as the plant exposed to stress.

Shoot systems were also developed well in presence of rhizobacteria strains to record the highest shoot average (130, 40, and 140 mm) for barley, pearl millet, and clover, respectively. The UW4-treated barley seeds irrigated with 10,000 mg/L salt brackish water were recorded as a highest shoot, while pearl millet recorded the highest one with only seeds treated with UW3 and UW4 (Figure 3). Unexpectedly, pearl millet shoots were reduced as the salt concentration increased for rhizobacteria UW3-treated seeds, but for the UW4-treated ones did not. The best length of pearl millet was recorded for both treated seeds with UW3 and UW4, the last was irrigated with 10,000 mg/L salt brackish water (Figure 3). The shoots for the clover were the highest with UW3 and UW4, both were irrigated with 6000 mg/L salt brackish water. However, as shoots reflected by the root developments, these measures reflected only the vegetation development of plants compared with those untreated ones.

Root lengths were found to be affected by brackish water but were significantly increased once the rhizobacteria were applied on plants. That was noticed typically on barley plants but was erratic neither on pearl millet nor clover. That could be by unknown factors in soil which might alter the growth and function of the inoculated bacteria and, subsequently, on affect their growth (Chang *et al.* 2014).

Shoots were increased proportionally with the increase of salt concentration particularly on barley. That was in agreement with previously studies which indicated both UW3 and UW4 strains on plants (Huang *et al.* 2004; 2005; Cheng *et al.* 2007).

The salinity was inhibited plant growth on control replicates, decreasing shoot lengths and thickness that attributed to reduction of plant cell intercellular space. With the presence of *P. putida* strains, the bacterial produced ACC deaminase would oxidize ACC to ammonia and α-ketobutyrate, substances essential for bacterial growth. Hence ethylene hormone would be lowered in concentration, and plants overcome the stress effect (Jacobson *et al.* 1994; Glick *et al.* 1995; 1998; Grichko and Glick 2000; Li and Glick 2001; Hontzeas *et al.* 2004; Forni *et al.* 2017)

The use of *P. putida* strains was found to increase the phytoremediation capacity of plants and in particular the salt uptake (Jodeh *et al.* 2015). Moreover, the irrigated plants with 10,000 mg/L brackish water were accumulated salts inside their biomasses that can be measured in mass weights, more than those irrigated with 6000 mg/L. On the other hand, applying combinations of both *P. putida* strains (UW3 and UW4) did not enhance significant salt uptake more than those trials with either strain separately. However, leaves of barley plants that treated with *P. putida* strains showed taller, thicker, and green darker color compared to none treated ones (Figure 4). Some leaves were short and small, and some others exhibited yellowing with

Figure 4. The barley plant seeds treated with *P. putida* strains and irrigated with brackish water were taller leave (right) compared with those irrigated with fresh water (left). These leaves exhibited showed pale green color with necrosis at the leaves margins as a result of salt water.

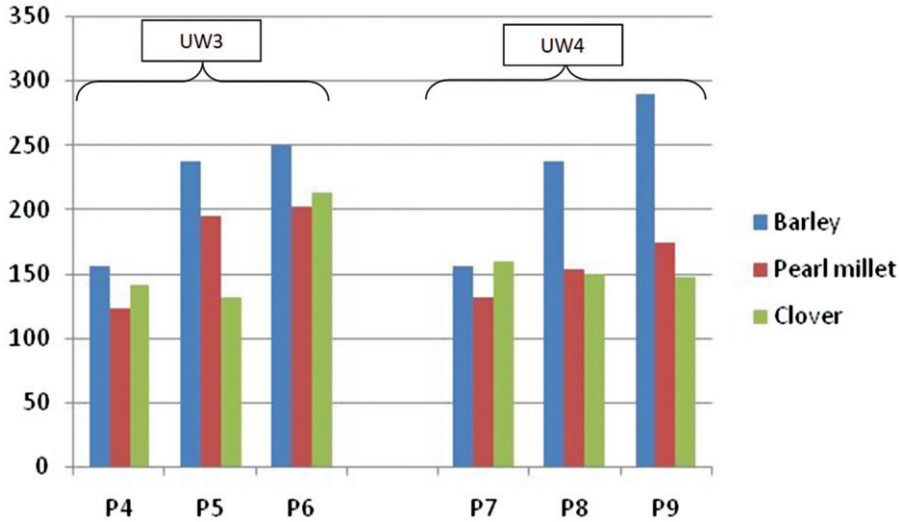

Figure 5. The impact of the *Pseudomonas putida* (UW3) on the photosynthesis activities of selected field crops (barley, pearl millet, and clover plants) expressed in biomass ratio measurements as shown in histogram is presented in (left) while the effect of *P. putida* (UW4) is presented in right.

few necrosis. The leaves lengths were noticed to be longer for those trials that irrigated with brackish water compared to those irrigated with fresh water, suggesting the impact of UW3 and UW4 to promote vigorous growth of plants under salt stress.

Besides, the roots were noticed to be longer than untreated ones, indicating the positive effects of *P. putida* strains on developing root system under salt stress conditions. It was evidently notable that both *P. putida* strains (UW3 and UW4) promoted plant's roots and shoots systems to overcome salt stress, the fact described in such rhizobacteria (Kong *et al.* 2015).

Comparing among these field crops, it was found that *P. putida* strain UW4 worked quite well on barely plants, while the other strain UW3 was giving satisfactory results on both pearl millet and clover (Figure 5). Some trials to mix both strains were failed to give significant improvements (unpublished data).

The responses of barley plants to the *P. putda* strains were found the best overall crops. Although the barley crop was considered as salt-tolerant plant, these results could be attributed to their large seed surface areas which could sustain more rhizobacteria than to pearl millet and clover. This was noticed for most plants that were tested with different rhizobacteria (Almaghrabi *et al.* 2013; Chang *et al.* 2014). It was believed that the surface area of barley seeds allowed more bacterial adhesion if compared with pearl millet and clover small seeds. Another reason might be related to species-specific differences in physiology and anatomy as well as specific differences in conditions required for optimal growth for pearl millet and clover plants, which surely differ from barley. It was suggested that Pearl millet and clover might react differently with PGPR strains other than UW3 and UW4.

Measurement of maximum photochemical efficiency

The maximum photochemical efficiency or yield of PSII (Y(II)) ratio was calculated (F_v/F_m), where typical value should be equal to 0.8 (MacNeill 2011). The parameters

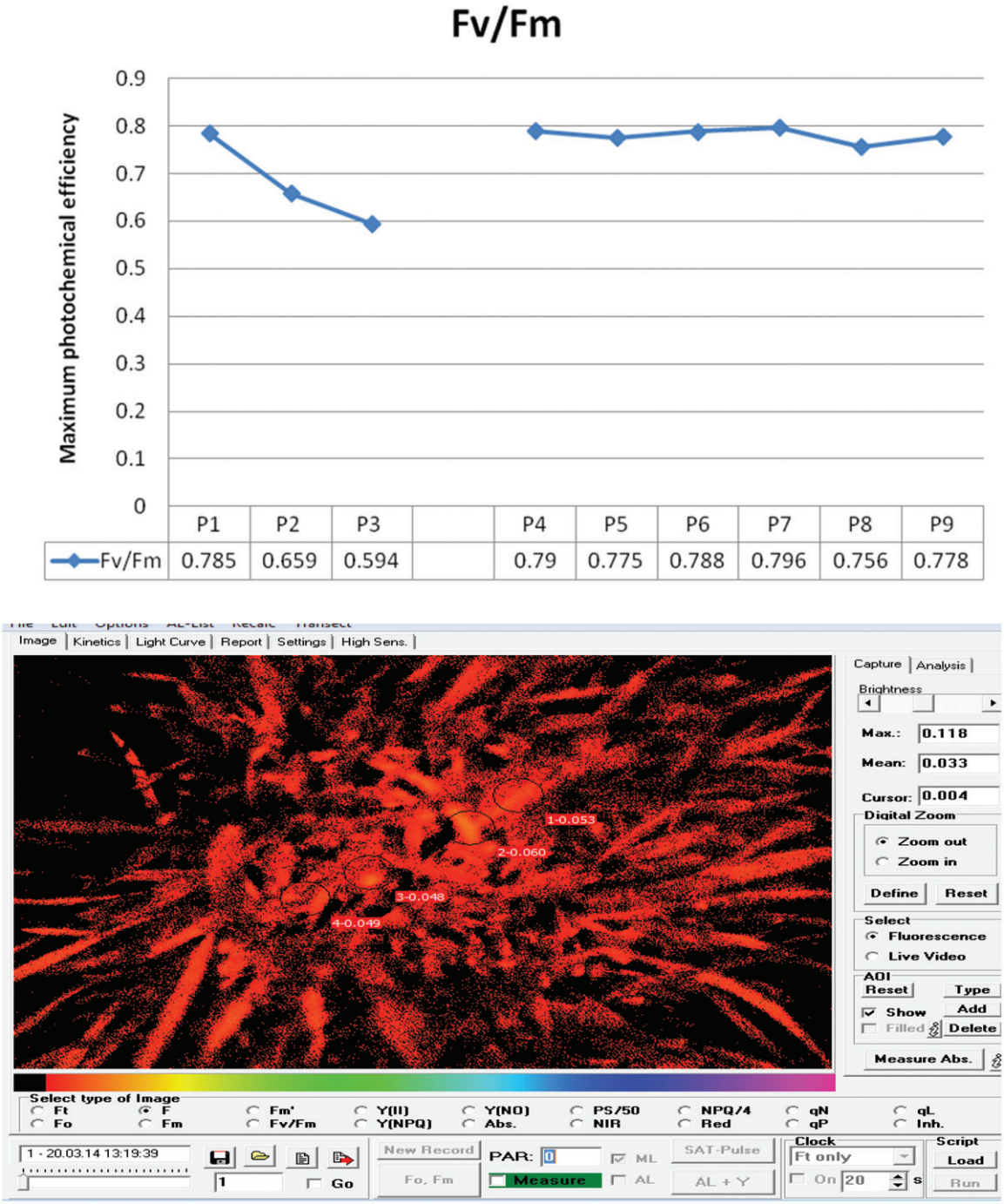

Figure 6. Comparison of maximum photochemical efficiency (F_v/F_m) among *P. putida* treated seeds of barley with untreated ones under different saline conditions using the PAM fluorometry. The photograph is illustrating the random selection of barley plant measurements.

(F_0), (F_m), and (F_s) in each spectrum were measured. The optimum values of (F_s) were measured on those plants (P4, P5, P7, and P8) irrigated with brackish water with *P. putida* strains (0.15–0.17). As expected, the values of plants irrigated with brackish water (P2 & P3) were larger (0.19–0.23) that mean plants were under salt stress.

The yield (Y(II)) of *P. putida* treated plants and irrigated with brackish water were found closely similar to those irrigated with fresh water fresh water (P1), suggesting that *P. putida* strains increased their capability to overcome stress and enhance normal photosynthetic activity. In fact, the presence of UW3 and UW4 enabled plants to sustain their photosynthesis even though they were stressed by different salt concentrations (Figure 6).

These astonishing results were in agreement with Shan (2009) study that showed some plant species as barley plant with PGPRs showed high performance of photosynthesis activity in saline soil. The same founding was with MacNeill (2011) on different plants species as barley, oats, and tall wheatgrass treated with PGPR and grown in saline soil field, high performance of their photosynthesis activity.

These experimental trials were showed the rhizobacteria as *P. putida* which are naturally living organisms and environmentally friendly (Olanrewaju *et al.* 2017) could be

possible to useful in cleaning soil and water from pollutants in combination with field crops. On the other hand, these bacteria made such crops could benefit from salty soil and/or saline water (Glick 2012) and used for animal feedings. This would be the natural way to provide more foods with environmental impact. It was also advisable to apply these bacteria with salt-tolerant cereals and forage plants for cleaning environment from saline water as well as feeding crops.

Conclusion

P. putida strains (UW3 and UW4), had showed significant improvements on plants (barley, pearl millet, and clover) biomasses under saline stress conditions, suggesting their potential use in any phytoremediation process. The research experiments showed an advantage of barley over the other crops (pearl millet and clover) with *P. putida* strain UW4, even though the strain UW3 resulted well with all crops. Pulse amplitude modulated fluorometry (PAM) indicated that plants increased their photosynthesis rate under stress due to the presence of these rhizobacteria. In addition to that, the significant increase in plants biomass would suggest to be used as forage foods for animals. The results of this research were highly recommended to apply that in open field at large scale.

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