EFFECT OF PGPR STRAINS ON SUNFLOWER GROWTH AND NUTRIENT CONTENTS UNDER SALINITY STRESS

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ABSTRACT:- Plant growth promoting rhizobacteria (PGPR) are potentially useful for stimulating plant growth under harsh environment. A pot trial was conducted to examine the performance of four isolated strains in five treatments viz., KS 44, KS 7, KS 41, KS 42 and KS mix under four different artificially developed salinity levels (5, 8, 10 and 12 dS m^{-1}) at National Agricultural Research Centre, Islamabad. Hybrid seeds (SMH 0917) of sunflower (*Helianthus annuus* L.) were used. After six weeks of sowing, plants were harvested and data regarding root length, plant height, root dry weight and shoot dry weight were recorded. The plant growth improved significantly with PGPR strain under salt stress. The root length, plant height, root and shoot weight increased up to 40%, 40%, 167% and 255%, respectively over un-inoculated at 12 dS m^{-1} salinity level. Whereas an increase of 38%, 54%, 109%, and 117% in root length, plant height, root weight and shoot weight, respectively was observed over un-inoculated at 10 dS m^{-1} salinity level. Improvement of 25%, 47%, 112%, and 98% in root length, plant height, root weight, shoot weight, respectively was recorded over control at 8 dS m^{-1} salinity level. Similar trend was observed at 5dS m^{-1} salinity level. The phosphorus and potassium content significantly increased because of bacterial inoculation. The strain KS 7 indicated the maximum phosphorus contents at 5dS m^{-1} which was 313% higher as compared to control. Similarly K+ content significantly increased at all salinity levels. This study concluded that PGPR isolate KS 44 is the most effective strain followed by KS 7 among all tested strains in improving sunflower growth and P, K content under salinity stress.

Key Words: Helianthus annuus; Hybrid; Strains; Plant Growth-promoting Rhizobacteria; Salinity; Agronomic Characteristics; Pakistan.

INTRODUCTION

Plants face mainly two types of stresses due to salinity, one is water deficiency (osmotic effect) because of high concentration of salts around root rhizosphere, that makes it difficult for plant to intake water and the second is to buildup salts within plant body up to toxic level (toxic effect). Osmotic effect occurs immediately within hours while salts accumulation take many hours and days (Munnas and Tester, 2008). The enhanced level of salts around roots, effect plant metabolism causing reduction in plant growth and yield (Tank and Sarf, 2010). Increased Na+ concentration in plant tissues cause low K+: Na+ ratio. However, for normal functioning of plants low Na+ and high K+ up take is required (Marschner, 1995). Under salinity stressed environment, reduction in phosphorus

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uptake has also been reported by Martenez and Lauchli (1994). Efficient rooting system is required for better nutrients and water uptake under saline conditions. Salinity not only causes osmotic effect, specific ion toxicity, nutritional imbalance, but also disrupt hormonal balance (Arbona et al., 2005).

. Low concentration of ethylene (C_2H_4) is required for several physiological processes like ripening of fruit, seed germination, root elongation, organ senescence (Bleecker and Kende, 2000). Salinity stress increases ethylene level in rhizosphere and acts as negative plant growth regulator (Holguin and Glick, 2001; Zapata et al., 2004; Arbona et al., 2005). The bacteria present in root rhizosphere synthesize indole acetic acid (IAA) in response to root exudates. The plant having endogenous IAA also takes up some of the bacterial synthesized IAA. It is used in cell proliferation, elongation as well as induces synthesis of 1-Amino-cyclopropane-1-carboxylate (ACC) synthase. This enzyme plays an important role for biosynthesis of ethylene under stress. Bacteria, having ACC deaminase present in rhizosphere, attack on ACC and hydrolyze into ammonia and α-ketobutyrate thus decrease ACC level outside the plant. The inhibitory effect of ethylene is reduced causing more root proliferation and better plant growth (Glick et al., 1995). This pot study was designed to test the performance of selected bacterial strains (KS 44, KS 7, KS 41, and KS 42) having ACC deaminase activity for improving sunflower growth at different salinity levels.

MATERIALS AND METHOD

Sunflower root samples were collected from different sites of salt affected region of central Punjab to isolate bacterial strains. Thirty six isolates were biochemically characterized on the basis of IAA production and phosphate solubilization (Kiani et al., 2015). Four promising PGPR strains (KS 44, KS 7, KS 41and KS 42) were selected for this study, which also have IAA and P solubilization ability.

Inoculum Preparation

The selected bacterial strains were inoculated separately in 200 ml flask having Luria Bertani (LB) broth. These were incubated in shaker for three to four days at 28±2°C. Later, transferred to carrier of biofertilizer at Soil Biology and Biochemistry Laboratory, NARC, Islamabad. Surface sterilization of sunflower seeds (Hybrid SMH-0917) were made with $HgCl_2$ (2%) before inoculation with slurry.

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Pot Study

A pot study was carried out at NARC, Islamabad during 2014. A composite soil sample was taken for physiochemical analysis. Each pot was filled with 5kg soil. The soil was sandy clay loam in texture with, electrical conductivity 1.0 dS m^{-1}, pH 7.89, OM 0.86%, ,extractable K 3.25 meq l^{-1}, available P 3.95 mg kg^{-1} Ca^{++}+Mg^{++} 6 $meql^{-1}$, Na^{+} 12 $meql^{-1}$ and saturation 32.5%. Four salts (NaCl, Na_2SO_4, $CaCl_2$ and $MgSO_4$) with 3:4:2:1 ratio, respectively were used to develop salinity levels 5, 8, 10, 12 dS m^{-1}. Autoclaved soil was used to fill up 72 pots. NPK fertilizers were applied (100:60:50 kg ha^{-1}) and carefully mixed in each pot. One pot was used as control (un-inoculated) for each salinity level. These pots were shifted to glass house and arranged according to CRD experimental design along with three repeats. After germination, irrigation was

applied when required. After six weeks of sowing, plants were harvested and data were recorded for root length (cm), plant height (cm), root dry weight (g) and shoot dry weight (g). P and K of plant samples were measured by using method as described by U.S. Salinity Laboratory Staff (Richard, 1954). The phosphorus content of plant was also measured (Ryan et al., 1996). Following treatments were used T_1= un-inoculated, T_2= KS 44, T_3= KS 7, T_4= KS 41, T_5= KS 42 and T_6=KS mix. Data were analyzed by ANOVA and comparison of treatment means were made by using LSD at P = 0.05 (Steel et al., 1997). The analyses were conducted using the software statistic 8.1.

RESULTS AND DISCUSSION

The data showed that root length continuously decreased with increasing salinity levels. It was 6.56 cm at 5 dS m^{-1} and decreased to 4.13 cm at 12 dS m^{-1} under un-inoculated conditions (Table 1). While those treatments where seeds were inoculated with bacterial strains showed enhanced root length at all salinity levels as compared to respective control. The bacterial strains KS 44 and KS 7 produced root length of 7.9cm and 7.5cm at salinity level 5 dS m^{-1} that was 21% and 14% higher as compared to respective control, while at 8 dS m^{-1} root length was 6.8cm and 6.7cm which was 25% and 24% higher as compared to control. Whereas at 10 dS m-1, 7.9cm and 7.0cm i.e., 38% and 30% higher over control, while at 12 dS m^{-1} increase of 40% and 28% was observed, respectively over control. Treatments which were inoculated with bacterial strains KS 41 and KS 42 indicated root length of 7.0cm and 7.5cm i.e., 8% and 14% increase over control at 5 dS m^{-1}, whereas 5.3cm and 6.3cm at 8 dS m^{-1} with improvement of 4% and 19%, respectively over un-inoculated. Likewise inoculation with KS 41 and KS 42 enhanced root length 8% and 26% at salinity level 10 dS m^{-1}, while an enhancement of 5% and 25%, respectively over control was recorded at 12dS m^{-1}. The increase in root length due to mixture treatment (KS mix) at salinity levels 5, 8, 10 and 12 dS m^{-1} was 10%, 3%, 28% and 19%, respectively over control. The maximum increase of 40% and 38% was observed at EC level 12 and 10 dS m^{-1}, respectively due to inoculation with KS 44, followed by KS 7(30%, 28%) at said salinity (Figure 1).

Similarly continuous reduction in plant height was observed with increase in salinity levels in control (un-inoculated) i.e., 42.16 cm at 5 dS

Table 1. Effect of inoculation of PGPR strains on plant growth parameters at four salinity levels

Treatment	5 dS m^{-1}	8 dS m^{-1}	10 dS m^{-1}	12 dS m^{-1}
Root length plant^{-1} (cm)				
Control	6.6^{abcde}	5.1^{fgh}	4.9^{gh}	4.1^{hi}
KS 44	7.9^{a}	6.8^{abcd}	7.9^{a}	6.9^{abc}
KS 7	7.5^{ab}	$6.\ 8^{abcd}$	7.0^{abc}	5.70^{cdefg}
KS 41	7.0^{abc}	5.3^{efgh}	5.3^{ghi}	4.3^{ghi}
KS 42	7.5^{ab}	6.3^{bcdef}	6.7^{abcde}	5.5^{defg}
KS mix	7.2^{ab}	5.2^{efgh}	6.8^{abcd}	5.1^{i}
Plant height plant^{-1}(cm)				
Control	42.2^{ghijk}	40.0^{ijkl}	36.0^{kl}	34.3^{l}
KS 44	63.7^{a}	58.7^{ab}	55.7^{bcd}	48^{defgh}
KS 7	56.3^{bc}	57.3^{abc}	52.3^{bcde}	47.7^{efgh}
KS 41	44.2^{ghij}	49^{defg}	49^{efgh}	47^{efgh}
KS 42	54^{bcde}	53.6^{bcde}	51.3^{cdef}	45.3^{fghi}
KS mix	47.3^{efgh}	51.3^{cdef}	41.3^{hijk}	38.0^{jkl}
Root dry weight plant^{-1}(g)				
Control	1.2^{b}	0.5^{de}	0.4^{e}	0.36^{e}
KS 44	1.9^{a}	1.1^{b}	0.9^{bc}	0.96^{bc}
KS 7	1.6^{a}	1.0^{b}	0.8^{bcd}	0.70^{cde}
KS 41	1.0^{b}	0.6^{cde}	0.5^{de}	0.4^{e}
KS 42	1.8^{a}	0.9^{bc}	0.6^{cde}	0.65^{cde}
KS mix	1.0^{b}	0.6^{efg}	0.62^{cde}	0.46^{e}
Shoot dry weight plant^{-1}(g)				
Control	7.3^{efg}	7.0^{efg}	6.3^{gh}	3.0^{fgh}
KS 44	21.2^{a}	14.0^{cd}	13.8^{cd}	10.8^{de}
KS 7	19.0^{ab}	13.3^{cd}	10.8^{de}	8.6^{efg}
KS 41	7.8^{efg}	8.03^{efg}	6.6^{fgh}	5.9^{efg}
KS 42	15.3^{bc}	10.4^{def}	8.2^{efg}	7.8^{gh}
KS mix	8.8^{efg}	9.21^{efg}	9.1^{efg}	5.7^{gh}

m^{-1} and decreased to 34.33 cm at 12 dS m^{-1} (Table 1). While, inoculated treatments showed improvement in plant height at all salinity levels as compared to respective control. Seeds inoculated with isolates KS 44 and KS 7 were 63.7cm and 56.3cm in height i.e., 51% and 34% increase over control, respectively at 5 dS m^{-1}, while 58.7cm and 57.3cm height was recorded at 8 dS m^{-1} with an improvement of 47% and 43% respectively over control. At 10 dS m^{-1}, plant height was 55.7 cm and 52.3 cm i.e.54% and 45% increase. While at 12 dS m^{-1} this increase was of 40% and 39%, respectively over control. Similarly KS 41 and KS 42 inoculated seeds gave plant height of 44.2 cm and 54 cm i.e., an increase of 5% and 28% at 5 dS m^{-1}, while 49 cm and 53.6 cm with an improvement of 22% and 34%, respectively over control at 8 dS m^{-1}. At10 dS m^{-1} plant height 49 cm and 51.3 cm was recorded with an increase of 36% and 43%, whereas an increase of 38% and 32% was noted, respectively, over control at 12 dS m^{-1}. While at 5, 8, 10 and 12 dS m^{-1} mixture treatment

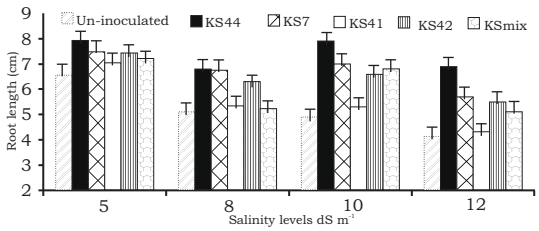

Figure 1. Percent increase in root length over respective control at different salinity levels

showed an increase of 12%, 28%, 15% and 11%, respectively over un-inoculated. The highest increase (51% and 54%) in plant height was observed at EC levels of 5 and 10 dS m^{-1} over un-inoculated due to isolate KS 44 (Figure 2).

All inoculated treatments increased root dry weight at all salinity levels

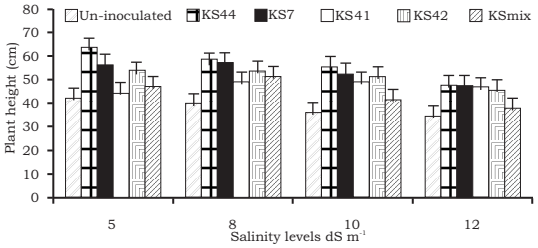

Figure 2. Percent increase in plant height over respective control at different salinity levels

over un-inoculated. At 5 dS m-1 bacterial strains KS 44 and KS7 exhibited root dry weight 1.9 g and1.6 g that was an increase of 61% and 36%, respectively over control, at 8 dS m^{-1} root dry weight was 1.1g and 1.0g with an improvement of 112% and 104%, respectively over control. Whereas at 10 dS m^{-1}, 0.9g and 0.8g with an improvement of 109%, 100%, respectively over control. While at 12 dS m^{-1} an improvement of 167% and 94%, respectively was recorded over un-inoculated treatment. The bacterial strain KS 41 and KS 42 recorded root dry weight 1.0 g and 1.8 g that was 53% higher than control respectively at 5 dS m^{-1}, while it was 0.6 g and 0.9 g at 8 dS m^{-1} with increase of 21% and 77%, at 10 dS m^{-1}, 0.5 g and 0.6 g i.e., an improvement of 16% and 53% at 10 dS m^{-1}, while at 12 dS m^{-1} an increase of 11% and 81% were recorded respectively, over un-inoculated. However, at 8, 10 and 12 dS m^{-1} KS mix treatment exhibited an improvement of 21%, 44% and 28%, respectively over control. The highest increase of 167% and 112% in root dry weight was recorded at 12 and 8 dS m^{-1}, respectively, over control due to KS 44 (Figure 3).

Similar trend of increase and decrease in shoot dry weight was observed in inoculated and un-inoculated treatments. At salinity level 5 dS m^{-1}, shoot dry weight was 7.30 g

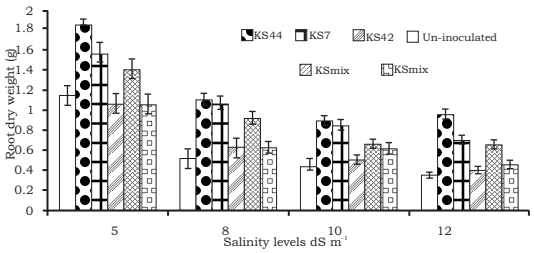

Figure 3. Percent increase in root dry weight over respective control at different salinity levels

that decreased to 3.03 g in control at 12dS m^{-1} (Table 1). Whereas PGPR inoculated treatments showed increase in shoot dry weight at all salinity levels as compared to their control. At salinity level 5 dS m^{-1}, bacterial strains KS 44 and KS 7 produced 21.2g and 19g shoot dry weight i.e., increase of 191% and 162%, respectively over control, 14g and 13.3g at 8dS m^{-1}, and with increase of 98% and 89%, respectively. Whereas 13.8g and 10.8g shoot dry weight was calculated at 10 dS m^{-1}, an improvement of 117% and 71% over control, 255% and 184% increase, respectively over control was recorded at 12 dS m^{-1}(Figure 4).While isolate KS 41 and KS 42 produced shoot dry weight 7.8 g and 13.5 g at 5 dS m^{-1} with an increase of 7% and 109% respectively, whereas 8.03 g and 10.04 g at 8 dS m^{-1} with an improvement of 14% and 47% respectively over un-inoculated. Likewise KS 41 and KS 42 showed an increase of 5% and 30% at

Figure 4. Percent increase in shoot dry weight over respective control at different salinity levels

10 dS m^{-1}, 93% and 161%, respectively over control at EC level 12 dS m^{-1}. The mixture treatment indicated an increase of 20%, 30%, 44% and 84%, respectively over control at 5, 8, 10 and 12 dS m^{-1}. The bacterial strains KS 44 exhibited the highest increase of 255% and 191% in shoot dry weight at salinity level 12 and 5 dS m^{-1}, respectively over control (Figure 4).

PGPR inoculated treatments showed an increase of phosphorus contents in inoculated plants as compared to control at all salinity levels (Table 2). The PGPR strain KS 7 showed 313% higher P content at 5dS m^{-1} as compared to control. Mixture treatment exhibited minimum phosphorus content at 12dS m^{-1} among inoculated treatments. Whereas K^+ content mostly increased significantly at all salinity levels. The mixture treatment showed highest potassium content at 5 dS m^{-1}, which was 138% higher as compared to un-inoculated. Similarly PGPR inoculated plants showed low Na^+ contents as compared to un-inoculated. The highest K^+:Na^+ ratio was observed with KS 41 at 5, 8 and10 dS m^{-1}, while KS 44 showed maximum K^+/Na^+ ratio at 12 dS m^{-1}(Table 2).

The inoculated treatments exhibited improvement in all plant growth parameters (root length, plant height, root dry weight and shoot dry weight) over un-inoculated treatments at all salinity levels (5, 8, 10 and 12 dS m^{-1}). Whereas reduction in all agronomic parameters were recorded with increase in salinity levels (i.e., from 5 to12 dS m^{-1}). The higher concentration of salts in root rhizosphere increased ethylene level that adversely affected plant growth. These PGPR strains might have ACC deaminase activity hydrolyze ACC (precursor of ethylene) into ammonia and α-ketobutyrate and thus

Table 2. PGPR inoculation effect on P, K contents and potassium/sodium ratio in shoot of *Helianthus annuus* L. (42 days old) under 5,8,10,12 d Sm^{-1} EC levels

Isolate	Phosphorus (%)				Potassium (%)				K^+/Na^+ ratio			
	5	8	10	12	5	8	10	12	5	8	10	12
Control	0.3g	0.2 j	0.15k	0.06m	2.47fg	2.30fg	1.04lm	0.82m	7.48e	1.59d	1.04e	0.50c
KS 44	0.68d	0.81hij	0.23hij	0.13kl	3.72cd	3.23e	1.81hij	1.53jk	8.08d	2.76c	2.55b	1.22ab
KS 7	1.24a	0.70d	0.39f	0.12kl	2.58 f	1.83hij	1.75 ij	1.27kl	3.53f	1.08f	2.11c	1.38a
KS 41	0.94b	0.47e	0.30 g	0.07m	4.09b	4.08bc	3.52de	2.17gh	11.69a	2.83b	2.67a	0.71c
KS 42	0.69d	0.25hi	0.26gh	0.14kl	3.86bcd	1.88hij	1.63ijk	0.81m	8.50c	1.15e	2.04d	0.99b
KS mix	0.50e	0.21ij	0.12kl	0.1lm	5.87a	3.80bcd	1.93hi	1.33kl	10.67b	3.05a	2.10c	0.47c

Means followed by same letter(s) do not differ significantly at P ≤ 0.05.

decreased ethylene level and improved plant growth. Similar findings have been reported by Husen et al. (2009) and Saleem et al. (2007). The conversion of ACC into α-ketobutyrate and ammonia by PGPR having ACC deaminase activity had more strong positive effect on root and shoot growth in sunflower. Significant increase in plant growth at 10 and 15 dS m^{-1} over un-inoculated with bacterial isolates W 17 and W 2 showing1-aminocyc-lopropane-1-carboxylate activity has been reported by Nadeem et al. (2010). These results were in consonance to the finding of Shahzad et al. (2010) and Nadeem et al. (2013). An important trait of salt tolerant plants is more potassium and less sodium uptake. Greenway and Munns, (1980); Jeschke, (1984) reported that higher K^+:Na^+ ratio in plants mainly contribute in salt tolerance. Present treatments also showed higher K^+:Na^+ ratio over control at all salinity levels. Ashraf et al. (2004) reported that bacteria producing exopolysaccharide, restricted the Na^+ uptake and improve plant growth as compared to control. Similar results were mentioned by Yue et al. (2007) that PGPR inoculation increased plant growth in cotton under salt affected condition and this increase might be due to high K^+ and low Na^+ uptake in addition to other factor. The performance of bacterial isolate KS 44 was much higher followed by isolate KS 7 as compared to all other tested isolates. More P content was observed in inoculated treatments as compared to control. This was possible due to production of organic acid in rhizospheric vicinity (Saleemi, 2011). Although mixture treatment (KS mix) also enhanced growth of all parameters at all salinity levels but this increase was less than individual performance of KS 44 and KS 7. Non-compatible combination of these isolates might be the reason.

It is concluded that PGPR KS 44 confirmed as the most promising strains followed by KS 7 among all tested isolates. These two strains should be further tested in field trials at different salinity levels.

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AUTHORSHIP AND CONTRIBUTION DECLARATION

S. No	Author Name	Contribution to the paper
1.	Dr.Muhammad Zahid Kiani	Concieved the idea, Data collection and Introduction
2.	Dr.Tariq Sultan	Technical input at every step
3.	Dr.Arshad Ali	Overall management of the article
4.	Dr.Ghulam Qadir	Result and Discussion
5.	Dr.Imdad Ali Mahmood	Methodology, Did SPSS analysis, Conclusion
6.	Dr.Tauseef Tabassam	Data entry in SPSS and analysis
7.	Dr.Muhammad Arshad Ullah	References
8.	Mr.Nasir Abbas	Methodology, Did SPSS analysis, Conclusion

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