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Research Article

Growth Promotion of *Glycyrrhiza glabra L.* by Salt-Tolerant Plant Growth Promotion Rhizobacteria under Saline Conditions

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ABSTRACT:

Salinity stress is one of the most serious factors limiting the productivity of agriculture. Plant growth promotion rhizobacteria (PGPR) which produce phytohormones is one of the options to mitigate salt stress in plants and improve their growth and improvement under saline conditions. We study the effect of salt-tolerant *P.putida*NUU8strain on plant growth of *Glycyrrhizaglabra L.* under saline soils. The treatment inoculation of *P. putida*NUU8strainstatistically significantly increased roots and shoots length plant^{-1} over the control under a pot experiment. The results showed that inoculation of *Glycyrrhizaglabra with* of salt-tolerant *P.putida*NUU8can enhance salt tolerance and plant growth under soil saline conditions. In our previous study we reported that the salinity did not inhibit the IAA production by strain. Strain *P. putida*NUU8appeared to produce IAA in media contained NaCl up to 9 % and it was able to growth at high salt condition.Salt-stressed *Glycyrrhizaglabra* inoculated with the *P. putida*NUU8sharply increased than uninoculated plants. Inoculation of *P. putida*NUU8 strain significantly improved the root length 56% and shoots lenth 49% of *Glycyrrhizaglabra*compared with uninoculated control.

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Key words: Glycyrrhizaglabra, salinity, PGPR, IAA, phosphate-solubilization

[I] NTRODUCTION

Soil salinity is one of the major abiotic stresses affecting plant productivity [1]**.**Reduction in germination, plant growth and development by increasing salinity levels has been described by many authors [2-4]. Legumes have been suggested as appropriate crops for the enhancement of bioproductivity and the reclamation of marginal lands, they also enrich soil nitrogen in symbiotic association with *Rhizobium*[5]. Nodulation and nitrogen fixation in legume-*Rhizobium* associations are adversely affected by salinity, which can preclude legume establishment and growth or reduce crop yield [6]. *Glycyrrhizaglabra*L. is one of the important medicinal plant, belonging to the family *Fabaceae (Leguminosae)* and commonly called as 'liquorice'. Medicinally, it is used internally for addison's disease, bronchitis, arthritis, asthma, and allergic complaints [7]. Roots of *Glycyrrhiza*plants are important crude drugs, and their major sweet constituent, glycyrrhizin is used in large quantities as a well-known natural sweetener and as a pharmaceutical [8]. Plant growth promoting rhizobacteria (PGPR) may improve plant growth and yield by direct and indirect mechanisms [9].Thus bacterial inoculation that produces phytohormones is one of the options to mitigate salt stress in plants and improve their growth and development under saline conditions [10-11]. The present study was carried out to elucidate the role of salt-tolerant PGPR, *Pseudomonas putida* NUU8 in alleviating salinity stress in *Glycyrrhizaglabra*grown under soil saline conditions in the greenhouse.

[II] MATERIALS AND METHODS

2.1. Plant and bacteria

Glycyrrhizaglabra seeds used in this study was provided by the Termiz State University, Uzbekistan. PGPR *P. putida* NUU8 strain were obtained from the culture collection of the Department of Microbiology and Biotechnology, National University of Uzbekistan. *P. putida* NUU8 were grown in KB broth [12].

2.2. Salt tolerance test

NaCl added into trypticase soy agar medium and various concentrations of NaCl (1 to 10%) were added to the medium and the test bacterial strains were streaked.

2.3. P-Solubilization

Phosphate-solubilization test was conducted qualitatively by plating the bacteria in agar containing precipitated tricalcium phosphate. The medium was a modification of Pikovskaya medium [13], consisted of 10 g glucose, 5 g tribasic phosphate ($Ca_5HO_{13}P_3$), 0.5 g $(NH_4)_2SO_4$, 0.2 g KCl, 0.1 g $MgSO_4.7H_2O$, trace of $MnSO_4$ and $FeSO_4$, 0.5 g yeast extract, and 15 g agar, 1-10% NaCl in 1,000 ml distilled water. Bacterial culture was streaked on the surface of replicated agar plates. The presence of clearing zone around bacterial colonies after overnight incubation was used as indicator for positive P-solubilization.

2.4. IAA production

Bacterial cultures were grown for 48 h on their respective media at 28±2 °C. Fully grown cultures were centrifuged at 3000 rpm for 30 min. The supernatant (2 ml) was mixed with two drops of orthophosphoric acid and 4 ml of the Salkowski reagent (50 ml, 35% of perchloric acid, 1 ml 0.5 M FeCl3 solution). Development of pink colour indicates IAA production [14].

2.5.Greenhouse pot experiment

Seeds were sorted to eliminate broken, small and infected seeds and sterilized for 5 minutes with concentrated sulphuric acid, followed by 70% ethanol for 3 min and rinsed five times with sterile, distilled water. *P. putida* NUU8 strain used for inoculation of sterile *G. glabra* seeds. For the seed inoculation, *P. putida* NUU8 were grownin KB broth [12]. One ml of each culture was pelleted by centrifugation and cell pellets were washed with 1 ml phosphate buffered saline (PBS; 20 mM sodium phosphate, 150 mMNaCl, pH 7.4) and re-suspended into PBS. The suspension used for the inoculation was adjusted to the final concentration of approximately 10^8 CFU mL^1. The cell suspensions containing two strains were prepared by mixing them in a ratio 1:1 and vortexes vigorously to achieve a homogenous suspension. Uniform seeds were first placed with sterile forceps into bacterial suspension for 10 minutes and were then cultivated into plastic pots of 40 diameter, containing 400 gr of salinity soils. Soil samples were collected from Sherobod district, Surkhandarya province in Uzbekistan. Each pot watered every 3 days. Plants were grown for 4 weeks in a greenhouse. Treatments were completely randomized, with 4 replicates per treatment. At harvest, after 4 weeks, the length of shoots and roots, the fresh weight of whole plants were measured.

Analysis of variance was performed using the Excel program package version 11 for Windows 2007 (Microsoft Corporation), Student's *t*-test and least significant differences (LSD) were applied to compare means at $P<0.05$.

[III] RESULTS

3.1. Salt tolerance

P. putida NUU8 showed normal growth on trypticase soy agar medium containing 1 to 9% NaCl as compared to control medium. Above 9% NaCl concentration in medium, the survival number of *P. putida* NUU8 slowly decreased. Strain of *P. putida* NUU8 can tolerate 9 % NaCl**[Table. 1].**The resultsof this studyshowed that*P. putida* NUU8 straintolerated 9% NaCl in medium.

3.2. IAA production and P-Solubilization

Bacteria which are able to produce IAA and P-Solubilization under saline conditions may supply additional phytohormone to the plant, thus may help stimulate root growth and reverse the growth inhibiting effect of salt stress to a certain extent in both shoot and root growth. In our previous study we reported that the salinity did not inhibit the IAA production by strain. Strain *P. putida* NUU8 appeared to produce IAA in media contained NaCl up to 9 % and it was able also to growth at high salt condition **[Table. 1].***P. putida* NUU8 produced P-solubilization in medium contained 1 to 10% NaCl. As concentration of NaCl improved from 1 to 10% NaCl in medium, *P. putida* NUU8 showed P-solubilization production **[Table 1].**

Bacterial test strain	Salt tolerance[a,b]	Phosphate solubilization[a,c]	IAA production[a,b]
P. putida NUU8	+	+	+

Table 1. Effect of NaCl on IAA and P-solubilization production of *P. putida* NUU8

a) All tests conducted with addition of 1-10%NaCl

b) Highest salt concentration (8 and 9 %) which did not inhibit growth

c) Highest salt concentration (10 %) which did not inhibit growth

3.2. Plant growth promotion

An effect of inoculation of salt-stressed *Glycyrrhizaglabra* seedlings with *P. putida* NUU8 on plant growth was preliminary tested under greenhouse conditions. Salt-stressed *Glycyrrhizaglabra*inoculated with the *P. putida* NUU8 sharply increased than uninoculated plants. Inoculation of *P. putida* NUU8 strain significantly improved the root length 56% and shoot lenth 49% of *Glycyrrhizaglabra*compared with uninoculated control[Table 2].

Treatments	Root length[a]	Shoot length[a]	Fresh weight[b]
Control	15.2 ± 0.8	12.2 ± 0.8	0.4 ± 0.08
P. putida NUU8	23.8 0.6*	18.2 0.8*	0.6 ± 0.05*

Table 2. Effect of *P. putida* NUU8on the growth of *Glycyrrhizaglabra*

[a] Shoot and root length cm; [b] Fresh weight, gram/plant; ± SD. Plants were grown for 4 weeks in greenhouse, values represent means for six plants (N = 6), *significantly different at $P<0.05$

[IV] DISCUSSION

Different halotolerant bacteria were able to withstand high salt concentration (1.75 M NaCl) and were able to facilitate plant growth promotion in the presence of growth inhibitory levels of salt [15].Salt-tolerant PGPR alleviated quite successfully the reductive effect of salt stress on percentage of germination of wheat, soybean probably through their ability to produce IAA in saline condition [16-11]. Furthermore, it has been reported that IAA production by PGPR can vary among different species and strains and that it is also influenced by culture condition, growth stage, and substrate availability[17]. Other research group suggested the ability of the selected halotolerant PSB to provide available phosphorous for plants grown in saline soils [18]. Several species of fluorescent *Pseudomonas* such as *P. fluorescens* NJ101[19], and*P. Aeruginosa* [20], were reported as good phosphate solubilizers. Several phosphate-solubilizing rhizobacteria could also promote plant growth by rendering phosphate into solution more than they need for their metabolism, and the surplus can be absorbed by plant [21]. Salinity affected shoot growth more than root growth, as was also reported for beans [22]. Selvaraj and Sumithra[23]. mentioned that inoculation of *G.glabra*with *Glomusaggregatum*along with PGPR's enhanced its growth, biomass and yield. Single inoculation with *G. aggregatum*also significantly enhanced the total dry weight of liqourice plants followed by dual inoculation with *G. aggregatum*+ *B. coagulans*. Rani et al [24]under salt stress, PGPR have shown positive effects in plants on parameters such as germination rate, tolerance to drought, weight of shoots and roots, yield and plant growth.

[V] CONCLUSION

P. putida NUU8 strain, especially P-solubilization and IAA producing bacteria, can induce soil salinity tolerance and growth promotion in *Glycyrrhizaglabra* under greenhouse conditions.

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