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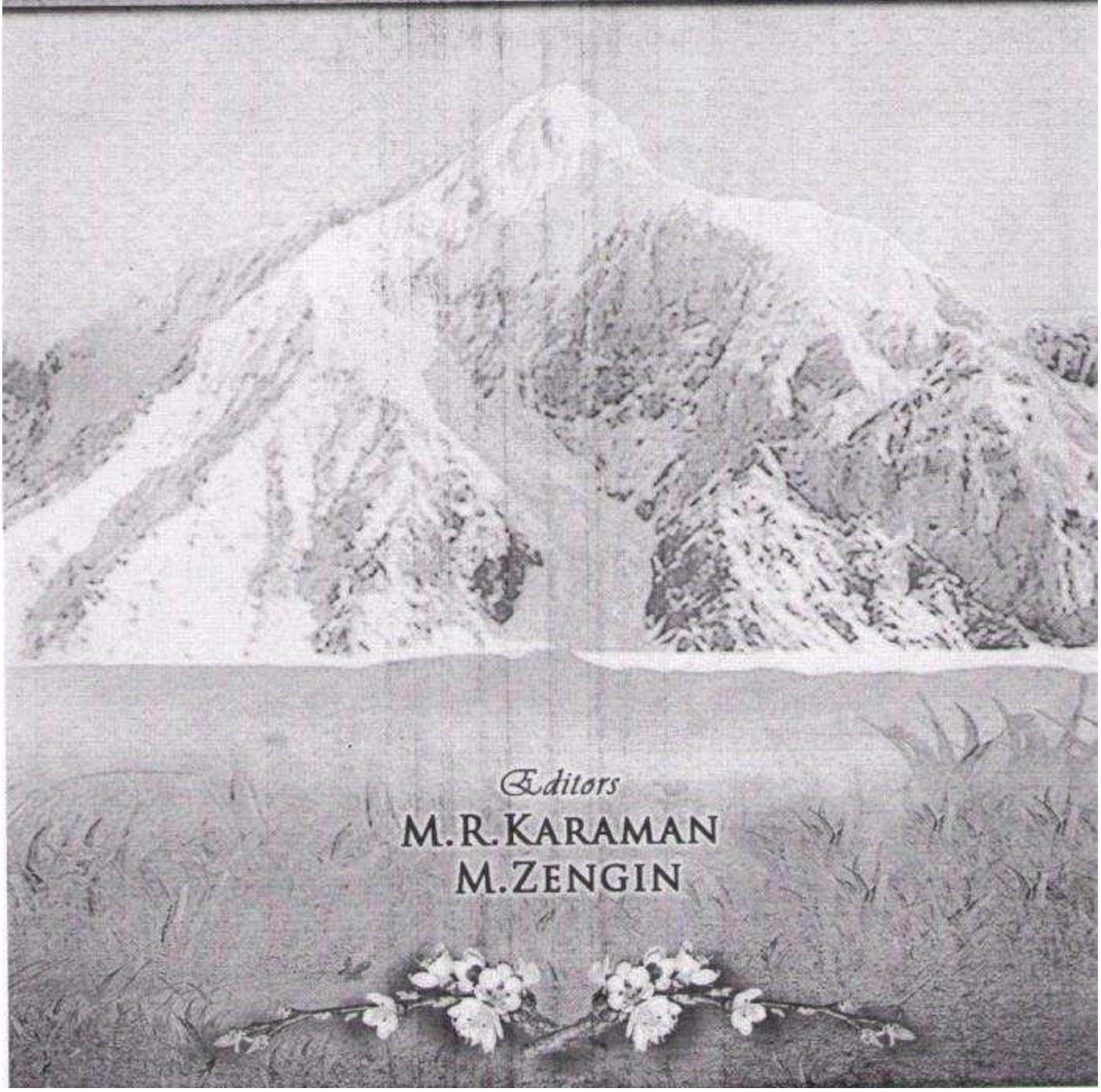

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Improvement of Seedling Establishment of Soybean Using IAA and IAA Producing Bacteria Under the Saline Conditions

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Abstract: The comparative effect of auxin (IAA) and IAA producing bacteria on the amelioration of salt stress on seedling growth of soybean under saline condition were studied. Seedling growth of soybean was inhibited at 100 mM NaCl condition and salt stress reduced the length of root up to 56%. In our observation, the plant growth regulator IAA did reverse the growth inhibiting effect of salt stress to a certain extent in both shoot and root. The IAA producing bacterial strain *Pseudomonas putida* TSAU1 significantly increased seedling root growth up to 29% in non-salinated condition and up to 86% at 100 mM NaCl compared to control plants. Among the treatments, bacterial strain and 0.001 µM IAA recorded significantly higher shoot length and dry weight of seedling over the control. It was concluded from the present study that the application of the low concentration of auxin (IAA) or IAA producing bacteria may improve growth of soybean seedling which would ultimately leads to induce soil salinity tolerance in plants.

Keywords: Auxin, plant growth promoting rhizobacteria, salt stress, soybean

1. Introduction

Inadequate management of the irrigation and other practices, together with unfavorable natural conditions, has accelerated soil salinity and leading to the abandonment of agricultural lands (Munns and Tester, 2008). Reduction in germination, plant growth and development by increasing salinity levels has been described by many authors (Lambers, 2003; Jamil et al., 2006; Ghazizade et al., 2012). Soybean (*Glycine max* (L.) Merr.) is a major food legume crop and an important source of protein in many countries and its production is limited by soil salinization as well. Soybean is known as salt sensitive plant, while differences in salt tolerance exist within varieties (Han and Lee, 2005). Several reports indicated that germination and seedling growth of soybean is reduced under saline condition (Singleton and Bohlool, 1984; Serraz et al., 1998). According Debez et al (2001) the suppressive effect of salinity on seed germination and plant growth could be related to a decline in endogenous levels of phytohormones. It has been suggested that plants might benefit from external supply of plant growth regulators under stressed conditions (Li et al., 2005). The application of plant growth regulators

such as gibberellins (Afzal et al., 2005), auxins (Khan et al., 2004), cytokinins (Gul et al., 2000), and kinetins (Khan and Unga,r 1998) have been shown to alleviate salinity stress in plants. Thus bacterial inoculation that colonize plant root and produce phytohormones is one of the option to mitigate salt stress in plants and improve their growth and development under saline conditions (Lugtenberg and Kamilova, 2009). Under salt stress, PGPR bacteria *Bacillus subtilis* and *Serratia proteamaculans* induced soil salinity tolerance and growth promotion in soybean seedlings (Han and Lee, 2005). Since salt stress reduces the supply of phytohormones in plants, it is important to study response of soybean seedlings to bacterial IAA which could mitigate salt stress and improve plant growth. The present study was aimed to evaluate the comparative effect of auxin (IAA) and IAA producing bacteria on the amelioration of salt stress on seedling growth of soybean under saline condition.

2. Materials and Methods

Soybean (*Glycine max* L.) cultivar Orzu used in this study was provided by the Tashkent State University of Agriculture, Uzbekistan. 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. Seed germination was carried out in 85mm x 15 mm tight fitting plastic Petri dishes with 5 ml of solution consisted of 0, 50, 75 and 100 mM NaCl. Thirty healthy and uniform seeds were sown in each petri plate with three replications. Filter paper (Whatman No.2) was soaked in a solution of the respective salt concentrations. To determine the effects of plant growth regulators on seed germination, and seedling growth auxin (IAA) were used at 1, 0.1, 0.01, 0.001 µM concentrations under non saline and saline (100 mM NaCl) conditions.

Strain *Pseudomonas putida* TSAU1, which is able to produce phytohormone IAA (7.4 µg/ml) was obtained from the culture collection of National University of Uzbekistan and used for inoculation of sterile soybean seeds. Bacterial suspension was adjusted to an optical density at 620 nm of 0.1 (OD_{620}=0.1). The seeds are placed in the bacterial suspension with sterile forceps and shaken gently for 10 minutes. The inoculated seeds were then aseptically placed in the Petri plates moistened with water, with 100 mM NaCl solution. All germinations were carried out in a plant growth chamber at 28°C. The lengths of roots and shoots of the germinated seeds which were more than 0.2 mm in length were measured and recorded after 5 days.

The effect of IAA concentrations and IAA producing bacteria on the growth of soybean seedlings exposed to salt stress (100 mM NaCl) was also studied under gnotobiotic conditions with 6 replicate tubes (25 mm in diameter, 200 mm in length) as described by Simons et al. (1996). The tubes contained 60 g of a sterilized mixture of washed sand and vermiculite (1:1) soaked with 6 ml of diluted nitrogen-free Jensen nutrient solution (Vincent, 1970). Salinity condition was

established by adding 100 mM NaCl into the Jensen nutrient solution. The treatments were as follows: i) seeds treated with IAA (0.01 and 0.001 µM) ii) seeds inoculated with IAA producing *P. putida* TSAU1 strain. Bacterial strain was grown overnight in KB broth and prepared as described above. Inoculated seedlings with bacteria were planted into sterile glass tubes, one seed per tube with ten replicates. The seedlings were grown in a growth cabinet with a 16-h light period at 22°C and an 8-h dark period at 16°C. At harvest after 21 days, the length of shoots and roots, and the dry weight of whole plants were measured. Data were tested for statistical significance using the analysis of variance package included in Microsoft Excel 2007. Mean comparisons were conducted using a least significant difference (LSD) test ($P < 0.05$). Standard error and a LSD result were recorded.

3. Results and Discussion

The salinity had negative effect on seed germination of soybean. One hundred percent of soybean seeds germinated in the non-saline control, whereas a lower germination rate was observed at 50mM (80%), 75mM NaCl (60%) and 100mM NaCl (45%). Considering the length of root 3.4 cm, shoot 4.2 cm (control, no salt) as 100%, analysis of data shows that salt stress of 100 mM reduced the length of root to 1.5 cm (56%) and shoot to 2.8 cm (by 34%) (Data not shown). The inhibitory effect of salinity on germination and seedling growth of various leguminous plants were reported in many studies (Wignarajah, 1990; Rabie et al., 2005). The decrease of phytohormone synthesis in root system of plants under salt stress resulted in reduction in plant growth and development (Sakhabutdinova et al., 2003). Pre-sowing seeds with plant growth regulators like IAA, gibberellins alleviated the growth inhibiting effect of salt stress in crops (Afzal et al., 2005; Javid et al., 2011). In our observation, IAA did reverse the growth inhibiting effect of salt stress in both shoot and root growth (Fig. 1 and 2).

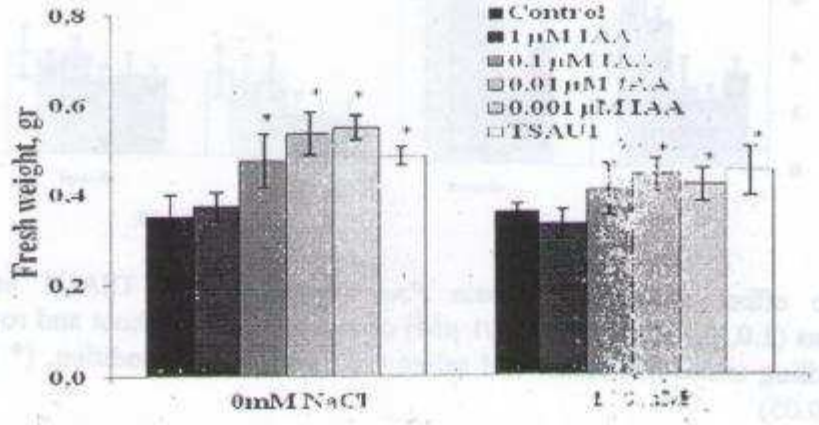

Fig. 1. The effect of bacterial strain *Pseudomonas putida* TSAU1 and different concentrations (1.0, 0.1, 0.01, and 0.001 µM) of auxin (IAA) on fresh weight of soybean seedling under non saline and saline (100 mM NaCl) conditions (* significantly different $P<0.05$)

The low concentration of IAA (0.01, 0.001 µM) showed high stimulatory effect on the root (up to 2.8 cm) and shoot growth (up to 3.6 cm) of soybean seedling, compared to root (1.5 cm) and shoot (2.8 cm) length of control plant in saline condition. The fresh weight of soybean seedling was also stimulated (Figure 1). In contrast, higher concentrations (1.0, 0.1 µM) inhibited root and shoot growth of soybean seedling. Remans et al. (2008) reported that high concentration of pure IAA inhibited root growth, whereas low concentration stimulated root and shoot growth of common bean. Similar results were observed in our previous study where low concentration IAA did reverse the growth inhibiting effect of salt-stress to a certain extent in both shoot and root of wheat (Egamberdieva, 2009). Hayat et al. (2008) also observed where IAA significantly affected the length of mung bean, fresh and dry mass of roots and shoots, the number of nodules and the nitrogenase activity. According Bianco and Defez (2009) the phytohormone auxins (IAA) enhance different cellular defense systems for protection plants from external adverse conditions.

We have also observed that IAA producing bacterial strain *P. putida* TSAU1 significantly increased seedling root growth up to 29% in non-salinated conditions and up to 86% at 100 mM NaCl, compared to control plants. This strain was able to stimulate seedling fresh weight up to 37% in non-saline condition and up to 25% at 100 mM NaCl compared to control plants (Figure 2). The IAA that is secreted by the bacteria, together with the endogenous plant IAA will be taken by plant cells which can stimulate plant cell proliferation (Glick et al., 2007).

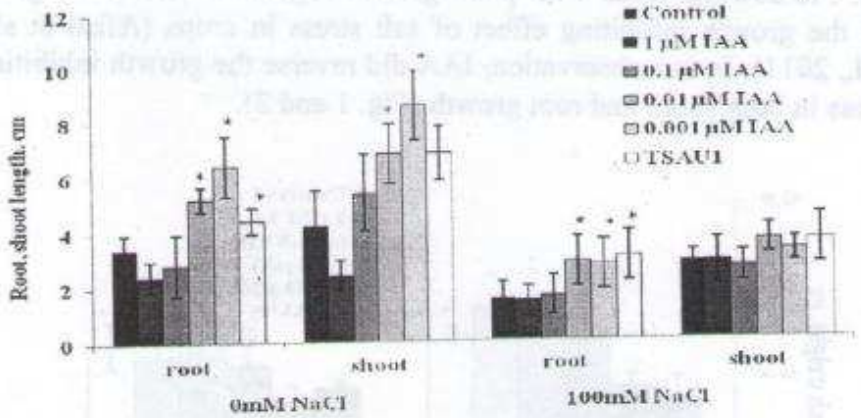

Fig. 2. The effect of bacterial strain *Pseudomonas putida* TSAU1 and different concentrations (1.0, 0.1, 0.01, and 0.001 µM) of auxin (IAA) on shoot and root growth of soybean seedling under non saline and saline (100 mM NaCl) condition. (* significantly different $P<0.05$)

An effect of IAA concentrations and *P. putida* TSAU1 on root and shoot growth was also tested under gnotobiotic sand conditions. The presowing treatment of seeds with IAA and bacterial strain overcame the inhibitory effect of salt stress

on the root and shoot growth of soybean seedling. The data on root and shoot length presented in Table indicated significant differences between the treatments and control plant. The maximum root length was recorded in seedling inoculated with *P. putida* TSAU1 (9.7cm) and treated with 0.01 µM IAA (9.1cm) and was found significantly superior over 0.001 µM IAA and control. Among the treatments, strain TSAU1 and 0.001 µM IAA recorded significantly higher shoot length (11.6 cm) and dry weight of seedling (0.061 g/plant) over the control.

Table 1. The length of roots and shoots and biomass of whole soybean plants when seedlings were treated with IAA concentrations and inoculated with IAA producing *P. putida* TSAU1 strain. Plants were grown in the gnotobiotic sand system under salt stress for three weeks. Values represent means for ten plants (N = 10).

Treatments	Root length (cm)	Shoot length (cm)	Dry weight (g/plant)
Control	8.0	9.3	0.040
TSAU1	9.7*	11.6*	0.061*
0.01 µM IAA	9.1*	10.0*	0.050
0.001 µM IAA	8.9	11.4*	0.059*

*Significantly different from control plants at $P<0.05$

In early studies the inhibition of root and shoot growth explained by decline in endogenous levels of hormones in the rhizosphere (Zholkevich and Pustovoytova, 1993) and releasing phytohormones by rhizobacteria effect positively to seedling development (Afzal et al., 2005). The ameliorative effects of PGPR on plant growth under saline conditions have been shown on various plant species, such as bean, canola, eggplant, lettuce, maize, pepper and tomato (Zahir et al., 2008; Egamberdieva 2011; Rojas-Tapias et al., 2012). In our previous study the production of IAA by *Pseudomonas* strains represents a beneficial mechanism that stimulated root system and growth of leguminous plant goat's rue grown in a salt amended gnotobiotic sand system (Egamberdieva et al. 2013). A similar result was observed by Han and Lee (2005) where PGPR strains induced soil salinity tolerance and growth promotion in soybean seedlings under saline conditions. It was concluded from the present study that the application of the low concentration of auxin (IAA) or IAA producing bacteria improved the growth of root and shoot of soybean seedling which would ultimately leads to induce soil salinity tolerance in plants.

Seedling growth of soybean was inhibited at 100 mM NaCl condition and salt stress reduced the length of root up to 56%. The inhibitory effect of salinity on germination and seedling growth of various leguminous plants were reported in many studies (Zahran and Sprent, 1986; Wignarajah, 1990). The decrease of phytohormone synthesis in root system of plants resulted in reduction in plant growth and development (Werner and Finkelstein, 1995; Sakhabutdinova et al., 2003).

Pre-sowing seeds with plant growth regulators like IAA, gibberellins alleviated the growth inhibiting effect of salt stress in crops (Datta et al., 1998; Sastry and Shekhawa, 2001; Afzal et al., 2005; Javid et al., 2011). We have also observed that low concentration of IAA (0.01, 0.001 µM) alleviated the inhibitory effects of salinity on seedling growth. In contrast higher concentrations (1.0, 0.1 µM) inhibited root and shoot growth of soybean seedling. Remans et al (2008) reported that high concentration of pure IAA inhibited root growth, whereas low concentration stimulated root and shoot growth of common bean. Similar results where observed in our previous study where low concentration IAA did reverse the growth inhibiting effect of salt-stress to a certain extent in both shoot and root of wheat (Egamberdieva, 2009). In our study the inoculation of soybean with IAA producing bacterial strain *P. putida* TSAU1 significantly increased seedling root growth and shoot growth at 100 mM NaCl compared to control plant.

These bacteria able to colonize plant root, produce IAA and enhance the growth and development of seedlings, especially under stressed conditions. The ameliorative effects of PGPR on plant growth under saline conditions have been shown on various plant species, such as bean, canola, eggplant, lettuce, maize, pepper and tomato (Mayak et al., 2004; Zahir et al., 2008; Egamberdieva, 2011; Rojas-Tapias et al., 2012). In our previous study the production of IAA by *Pseudomonas* strains represents a beneficial mechanism that stimulated root system and growth of leguminous plant goat's rue grown in a salt amended gnotobiotic sand system (Egamberdieva et al., 2013). A similar result was observed by Han and Lee, (2005) where PGPR strains induced soil salinity tolerance and growth promotion in soybean seedlings under saline conditions. It was concluded from the present study that the application of the low concentration of auxin (IAA) or IAA producing bacteria improved the growth of root and shoot of soybean seedling which would ultimately leads to induce soil salinity tolerance in plants.

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