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Original article

Impact of soil salinity on the plant-growth – promoting and biological control abilities of root associated bacteria

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ABSTRACT

The effectiveness of plant growth – promoting bacteria is variable under different biotic and abiotic conditions. Abiotic factors may negatively affect the beneficial properties and efficiency of the introduced PGPR inoculants. The aim of this study was to evaluate the effect of plant growth – promoting rhizobacteria on plant growth and on the control of foot and root rot of tomatoes caused by *Fusarium solani* under different soil salinity conditions. Among the five tested strains, only *Pseudomonas chlororaphis* TSAU13, and *Pseudomonas extremorientalis* TSAU20 were able to stimulate plant growth and act as biological controls of foot and root rot disease of tomato. The soil salinity did not negatively affect the beneficial impacts of these strains, as they were able to colonize and survive on the roots of tomato plants under both saline and non-saline soil conditions. The improved plant height and fruit yield of tomato was also observed for plants inoculated with *P. extremorientalis* TSAU20. Our results indicated that, saline condition is not crucial factor in obtaining good performance with respect to the plant growth stimulating and biocontrol abilities of PGPR strains. The bacterial inoculant also enhanced antioxidant enzymes activities thereby preventing ROS induced oxidative damage in plants, and the proline concentrations in plant tissue that play an important role in plant stress tolerance.

1. Introduction

The increase in harsh abiotic stresses such as drought, salinity and abrupt changes in temperature are part of the main consequences of climate change. These stresses have led to loss of soil organic matter and other forms of soil degradation that negatively affect agricultural productivity (Ahmad et al., 2015). Another important consequence of climate change and abiotic stresses is the increased infection/infestation of plants by pathogens and pests (Chakraborty, 2013). Intensive research attempts are underway to improve plant growth, and tolerance to various abiotic stresses, and to protect plants from soil borne pathogens using plant growth promoting rhizobacteria (PGPR) which have great potential for sustainable crop production (Lugtenberg and Kamilova, 2009; Berg and Martinez, 2015; Egamberdieva et al., 2015a, 2015b, 2016).

Root associated microbes, including endophytes, closely cooperate with each other and can mediate important physiological processes, especially nutrient acquisition and plant fitness to abiotic stresses (Berg et al., 2013; Abd_Allah et al., 2015). Plants inoculated with PGPR produce more root hairs and take up mineral and microelements more efficiently from the soil. The growth of several plants is enhanced by PGPR treatment, e.g. lentil (*Lens esculenta*) (Faisal, 2013), pea (*Pisum sativum* L.) (Meena et al., 2015), cucumber (*Cucumis sativus*), (Egamberdieva et al., 2011), rice (*Oryza sativa*) (Yadav et al., 2014) and soybean (*Glycine* max) (Egamberdieva et al., 2015b). PGPR also induces systemic tolerance

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to various abiotic stresses in plants such as salinity, drought and heavy metals through alteration of plant physiology (Wang et al., 2012). The beneficial traits of plant growth promoting bacteria include the ability to synthesize biological active compounds such as plant growth stimulators (Parray et al., 2016; Egamberdieva et al., 2017), osmolytes (Berg et al., 2013), antifungal compounds (Landa et al., 2004), and 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme (Ali et al., 2014). The strain *Pseudomonas* was able to suppress soybean root disease caused by fungal pathogens (Susilowati et al., 2010) and showed antagonistic activity against several fungal pathogens, such as *Fusarium oxysporum*, and *Rhizoctonia solani*. In another study, *P. agglomerans* and *Bacillus* sp. reduced the charcoal root rot of soybean caused by *Macrophomina phaseolina* under greenhouse conditions (Vasebi et al., 2013). An induced systemic resistance in plants against foliar pathogens by PGPR was also reported (Choudhary et al., 2007a, 2007b). In addition some reports have suggested that some PGPR induces systemic tolerance (IST) in plants through elevated antioxidant responses at the levels of enzyme activity and metabolite accumulation (Hashem et al., 2015, 2016; Jha et al., 2011). The antioxidant defence system plays a major role in plant adaptation to salinity stress that allow the scavenging of reactive oxygen species (ROS) (Ahanger et al., 2014).

The variability in the effectiveness of biologicals is of concern when used under different conditions or cropping systems. Hostile environmental conditions are deleterious for the root associated microbiome and effective functioning of the introduced PGPR inoculants (Landa et al., 2004). In earlier studies Landa et al. (2001) observed an effect of temperature on plant growth and biological control ability of PGPR *Pseudomonas fluorescens*. In their study fusarium wilt of chickpea was suppressed by these rhizobacteria at 30 °C, but not at 25 °C at which temperature disease potential was high. Interestingly this suppression was related to the production of extracellular metabolites that inhibit *F. oxysporum*, and the root colonization and plant growth stimulating abilities of rhizobacteria, which was higher at 30 °C (Landa et al., 2004). In this study, we evaluated the effect of plant growth promoting rhizobacteria on plant growth and on the control foot and root rot disease of tomato caused by *Fusarium solani* under different conditions of soil salinity.

2. Materials and methods

2.1. Soil, seeds and bacterial strains

The soil used for pot experiments was selected from deep tillage (0–40 cm) irrigated agricultural fields in the Tashkent (non-saline, EC 1.3 dS m^{-1}) and Syr-Darya Provinces (affected by salinity, EC 7.1 dS m^{-1}) of Uzbekistan. Soils with an EC of greater than 4.0 dS m^{-1} soil were considered saline. The characterisation of the experimental soil used in the current study are described in Table 1. Tomato seeds (*Lycopersicon esculentum* cv. Bella) were obtained from Enza Zaden, the Netherlands. The bacterial strains *Pseudomonas putida* TSAU1, *P. extremorientalis* TSAU6, *P. chlororaphis* TSAU13, *P. extremorientalis* TSAU20, and *P. aurantiaca* TSAU22 were obtained from the culture collection of the Faculty of Biology, National University of Uzbekistan. These strains were previously isolated from the rhizosphere of wheat grown in salinated soil (Egamberdieva and Kucharova, 2009). All *Pseudomonas* strains were grown on King's B agar medium (KB; Difco Laboratories, Detroit, MI, USA) at 28 °C. The fungal pathogen *Fusarium solani* was obtained from the National University of Uzbekistan and was grown on potato dextrose agar plates (PDA; Difco Laboratories, Detroit, MI, USA).

2.2. Fungal isolate

The fungal pathogen *Fusarium solani* was previously isolated from diseased tomato plants grown in salinated Uzbek soil that showed typical *Fusarium* foot and root rot symptoms. The procedure for the isolation and identification of pathogen from tomato root was described in previous work by Egamberdieva et al. (2011). Briefly, a small piece of tissue from the diseased plant was plated on potato dextrose agar (PDA) and incubated at 28 °C in the dark for 5 days. A single microconidial culture was prepared from isolate. The pathogenicity test was carried out on tomato under controlled growth chamber conditions. A randomized complete block design experiment with five replicates was carried out to examine the pathogenicity of the isolated phytopathogen (*F. solani*) to fulfil Koch's postulates. Healthy tomato seedlings that had developed their second set of true leaves were transplanted into plastic pot (500 ml capacity) containing sand:peat-moss: vermiculite mixture (1:1:1, w/w/w) which infested with the phytopathogen (*F. solani*) as 2 ml of spore suspension (4×10^7 spore/ml). Control pots (without fungal pathogen) were used as references. The pots were incubated in growth chamber for five weeks at 27 ± 2 °C, then symptoms of root rot disease were evaluated using the following formula.

$$\text{Disease incidence}(\%) = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \text{ X } 100$$

After 5 weeks, a piece of diseased root from a sick plant was removed and plated on a PDA plate followed by incubation for 5 days to isolate the pathogen. Prior to identification, fungus was grown on sterile filter paper placed on PDA agar. The filter paper containing the fungal hyphae was collected and ground in liquid nitrogen. DNA was isolated from pulverized fungal biomass using the Nucleon Phytopure kit (Amersham Biosciences GmbH, Freiburg, Germany). To identify the fungal isolates, the mtSSU rDNA sequences of two strains were analyzed. The mtSSU rDNA fragments were amplified using MS1 and MS2 primers (Zeng et al., 2003) and sequenced by ServiceXS (Leiden, the Netherlands). The sequences of the fragments were compared with those in GenBank using the BLAST program. The sequences of the two analyzed strains showed 99% similarity with the mtSSU rDNA sequence from *Fusarium solani* f. sp. *glycines* isolate 1-potato (GenBank Acc. N. AF125026). Therefore the isolates can be referred to as *Fusarium solani* on the basis of their mtSSU rDNA fragment sequences.

Table 1
Soil characterization.

Soil[a]	E_C dS m^{-1}	K^+	Ca^{+2}	Mg^{+2}	CO_3^{2-}	N	P	C_{Org}	Na^+	Cl^-
	(mg g^{-1} soil)								(μg g^{-1} soil)	
None-saline	2.3	5.92	53.4	23.7	16.1	1.06	1.30	8.69	600.2	52.0
Saline	7.1	6.58	67.4	24.6	17.6	0.95	1.23	7.19	813.1	94.2

EC = Electrical Conductivity. K^+ = exchangeable potassium. Ca^{+2} = exchangeable calcium. Mg^{+2} = exchangeable magnesium. Na^+ = exchangeable sodium. CO_3^{2-} = carbonate and Cl^- = chloride. C_T = total C. C_{Org} = total organic C. N = total nitrogen. and P = total phosphorus.

[a] Non-saline soil was collected from Tashkent province. Saline soil from Syrdarya province of Uzbekistan.