Bulgarian Journal of Agricultural Science, 26 (No 4) 2020, 744–747

Effect of *Bacillus subtilis* 1 strain on the growth and development of wheat (*Triticum aestivum* L.) under saline condition

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Abstract

Jabborova, D. P., Narimanov, A. A., Enakiev, Y. I. & Kakhramon, D. D. (2020). Effect of *Bacillus subtilis* 1 strain on the growth and development of wheat (*Triticum aestivum* L.*)* under saline condition. *Bulg. J. Agric. Sci., 26 (4)*, 744–747

Salt-tolerant microorganisms may improve soil fertility through decomposition of organic matter and nutrient cycling, by fixation of atmospheric nitrogen or through production of growth hormones. Plant growth in promoting rhizobacteria shows the positive effect in plants on such parameters as germination, plant growth and plant development under salt stress. The present investigation aims at application of salinity tolerant *Bacillus subtilis* 1 strain on plant growth of wheat (*Triticum aestivum* L.) under saline soil conditions. 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. Phosphate-solubilization test was conducted qualitatively by plating the bacteria on agar containing precipitated tricalcium phosphate. Salinity was obtained by adding 150 and 200 mM NaCl. Plants were grown for 4 weeks in a greenhouse. The results of this study showed that *B. subtilis* 1 strain tolerated 8% NaCl in medium. *B. subtilis* 1 strain produced P-solubilization in medium contained 1 to 8% NaCl. Salinity tolerant *B. subtilis* 1 strain was used for inoculation studies on wheat crop using saline soil under pot conditions. The *B. subtilis* 1 strain significantly increased the biomass, root and shoot growth of soybean at 150 and 200 mM NaCl condition compared to control uninoculated plants.

Keywords: wheat (*Triticum aestivum* L.); inoculation; *Bacillus subtils;* NaCl; salinity

Introduction

Salinity is a major problem of soil degradation, limiting crop productivity in the world (Flowers & Yeo, 1995; Parida & Das, 2005; Rengasamy, 2006). Total land around 20% the world and almost half of all irrigated land are adversely affected by salinity stress (Silva & Gerós, 2009). Salinity negative effects are the morphological, physiological and biochemical processes including seed germination, plant growth and development, grain yield and nutrient uptake (Qiu et al., 2003; Lee et al., 2008; Agarwal et al., 2015). Mittler (2002) reported that salinity causes ion toxicity and osmotic stress.

Wheat (*Triticum aestivum* L.*)* is the major foods for more than 35% of world population (Jing and Chang, 2003). Wheat grain yield is depressed, among other factors, by environmental stresses such as drought, heat, low temperatures, low fertility (especially nitrogen) and soil salinity (Mehmet et al., 2006). Several plants are sensitive to high salinity during germination and the seedling stage (Ghoulam & Fares, 2001). There are numerous reports on germination, growth and development of wheat under saline conditions (Fethi et al., 2011; Abdoli et al., 2013).

PGPR (Plant Growth Promoting Rhizobacteria) plays an important role in improving plant growth through a variety of mechanisms. PGPR can contribute to plant growth including abiotic stress tolerance in plants, nitrogen uptake, synthesis of phytohormones, minerals solubilization, siderophore production, inhibition of plant ethylene synthesis, production of antibiotics and the production of enzymes (Mohamed & Gomaa, 2012; Glick, 2014; Egamberdieva & Jabborova, 2015; Egamberdieva et al., 2015; Jabborova et al., 2018). Plant growth promoting rhizobacteria have been found to improve growth of soybean (*Glycine max* L.), mungbean (*Vigna radiata* L.), liquorice (*Glycyrrhiza glabra* L.) and fodder galega (*Galega orientalis* Lam.) under saline conditions (Egamberdieva et al., 2013; Jabborova et al., 2013; Egamberdieva et al 2015; Jabborova & Qodirova 2016; Jabborova et al., 2016; Egamberdieva et al., 2016, Egamberdieva et al., 2017). Patel et al. (2007) reported that *Bacillus* sp. increased the plant resistance to stress and produces various plant hormones for growth enhancement.

The aim of the present study is to determine the effect of plant growth promoting *Bacillus subtilis* on plant growth and salinity tolerance of wheat grown under salt stress.

Materials and Methods

Wheat (*Triticum aestivum* L.) cultivar Elomon was obtained from Genetics and Plant Experimental Biology Institute, Kibray, Uzbekistan. *Bacillus subtilis* 1 strain was obtained from the culture collection of the Department of Microbiology and Biotechnology, National University of Uzbekistan. Bacteria was grown on nutrient – agar and nutrient-broth and incubated at 30°C for 24 h. NaCl was added into nutrient agar medium and various concentrations of NaCl (1 to 10%) and the test bacterial strain was streaked.

Phosphate-solubilization test was conducted qualitatively by plating the bacteria onagar containing precipitated tricalcium phosphate. The medium was a modification of Pikovskaya medium (Subba Rao, 1999) 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 $MnSO_4$, $FeSO_4$, 0.5 g yeast extract, 15 g agar and 1–10% NaCl in 1000 ml distilled water. Bacterial culture was streaked on the surface of replicated agar plates and incubated at 30°C for 5–7 days. The presence of clear zone diameters around bacterial colonies was measured.

Siderophore production was measured as described by Alexander & Zuberer (1991). Briefly, 10 µL of the bacterial suspension in triplicate was inoculated on chrome azurol S (CAS) medium and the plates were incubated at 28°C for 24 h. Change of colour from blue to orange indicated production of siderophore and the diameter of the orange halo around colonies were measured. Siderophore production was assessed by measuring a ratio of halo diameter to colony diameter on CAS medium (Sung et al., 2001).

The experiment was conducted at Genetics and Plant Experimental Biology Institute. Seeds were sorted to eliminate broken, small, infected seeds and sterilized with 10% sodium hypochlorite solution for five minutes and washed three times with sterile, distilled water. *B. subtilis* 1 strain was used for inoculation of sterile wheat seeds. For the seed inoculation, *B. subtilis* 1 strain was grown in nutrient broth. Uniform seeds were first placed with sterile forceps into bacterial suspension for 10 min and were then cultivated into plastic pots (9 cm diameter, 15 cm) containing 300 g of soil. Two concentrations of salt (150 and 200 mM) in the form of NaCl were used for pot experiment. Each pot was watered every 3 days.

The experiment was a completely randomized block design with four replicates per treatment. At harvest, after 4 weeks, the length of shoots and roots, the fresh weight of whole plants were measured. Experimental data were analysed with the Stat View Software using ANOVA. Significance of the effect of treatment was determined by the magnitude of the P value ($P < 0.05$).

Results and Discussion

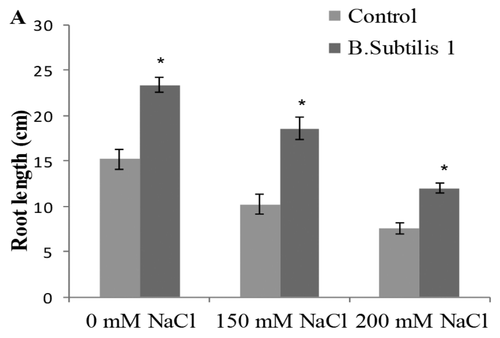
The results of this study showed that *B. subtilis* 1 strain tolerated 8% NaCl in medium. The results illustrated in Table 1 plant growth promoting characteristics of *B. subtilis* 1 strain. Further *B. subtilis* 1 produced siderophore and P-solubilization in medium containing1 to 8 % NaCl (Table 1). In the present investigation *B. subtilis* 1 has shown positive results for plant growth promoting characteristics such as salt tolerance, phosphate solubilization and siderophore production. Several *Bacillis* sp. strains that can solubilize P, salt tolerance, produce siderophore and IAA have widely been reported (Mohamed & Gomaa, 2012; Damodaran et al., 2013) and have been shown to promote the growth of cucumber, wheat and soybean (Garcia-Lopez & Delgado, 2016; Khande et al., 2017).

Table 1. Salt tolerance and plant growth promoting characteristics of *B. subtilis* 1 strain

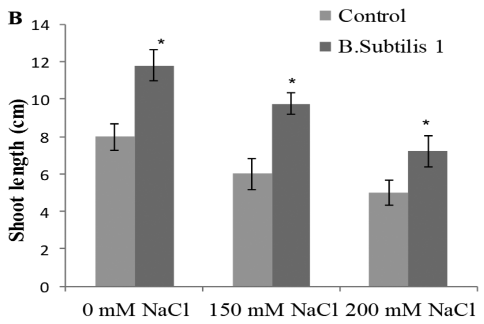
Strain	Salt tolerance (1–8 % NaCl)	Phosphate solubilization Clear zone, cm	Siderophore production Clear zone, cm
B. subtilis 1	+	0.5	1.58

*+ Positive for the test; – Negative for the test

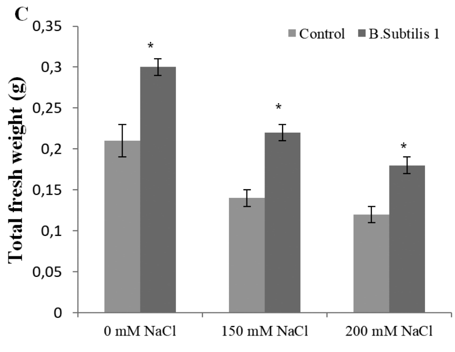
Salinity had also a negative effect on plant growth. The biomass, root length and shoot length of control plants steadily declined by 33%, 25% and 39% when the level of 150 mM NaCl. The root length, shoot length and fresh biomass of wheat plants significantly decreased with increasing salt concentrations. The concentration of 200 mM NaCl significantly decreased biomass by 43%, root length by 50% and shoot length by 37.2% (Table 1). Han & Lee (2005) found that NaCl-stressed (5.0 dS m^{-1}) soybean plants showed decrease in the plant height, the shoot and root dry weight.



A) Root length, cm



B) Shoot length, cm



C) Total fresh weight, g

Fig. 1 a-c. Root and shoot lengths and total fresh weight of wheat inoculated with *B. subtilis* 1 strain under levels of NaCl (150 mM and 200 mM)
***Significantly different from the control at P < 0.05**

Inoculation with the *B. subtilis* 1 strain significantly increased the biomass, root and shoot lengths of wheat compared to the uninoculated control under both non-saline and saline conditions. Root length significantly increased by 82% and 57.8% in 150 mM and 200 mM when inoculated with *B. subtilis* 1 compared to uninoculated control. In 150 mM and 200 mM NaCl, bacterial inoculated wheat had significantly shoot lengths by 62% and 17%. The root length and shoot length by inoculation with *B. subtilis* 1 increased 53.9% and 47.5% compared to uninoculated control. The fresh biomass of seed inoculated with *B. subtilis* 1 was significantly increased under both non-saline and saline conditions. Inoculation of *Bacillus* sp. increased the shoot and lengths of chilli (*Capsicum annuum*) compared to control under saline conditions (Patel et al., 2017).

Conclusions

The fresh matter, root and shoot lengths of wheat was negatively affected by increasing dosages of sodium chloride. Inoculation with *B. subtilis* 1 demonstrated positive results for plant growth promoting traits such as salt tolerance, phosphate solubilization and siderophore production. Significant potential *B. subtilis* 1 strain could play role in promoted plant growth under both saline andnon-saline conditions. Inoculation with *B. subtilis* 1 strain 3 significantly increased the fresh matter, root and shoot lengths of wheat compared to control under both saline and un-saline conditions. The B. *subtilis* 1 could be an effective strain to promote the growth of wheat in saline soils.

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Received: August, 20, 2019; *Accepted:* September, 4, 2019; *Published:* August, 31, 2020