

## THE EFFECTS OF BACILLUS spp. ON GERMINATION AND SEEDLING GROWTH OF COMMON BEAN (*Phaseolus vulgaris*)

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### Abstract

Plant growth-promoting rhizobacteria are capable of promoting seed germination, plant growth and development by synthesizing phytohormones and enhancing plant nutrient acquisition and utilization. This study was carried out to determine the effects of *Bacillus simplex*, *Bacillus thuringiensis*, *Bacillus cereus* and *Bacillus subtilis* and 6 unidentified *Bacillus* spp. on seed germination and seedling growth of common bean (*Phaseolus vulgaris*). Surface sterilized bean seeds were inoculated with bacteria in centrifuge tubes for 30 minutes. The inoculated seeds were placed in the plates and replaced in a germination chamber at 25 °C for 21 days. At the end of germination test, germination rate, shoot length, root length, dry shoot and root weights were determined. The results showed that *Bacillus* spp. SZF135 had the highest germination rate, shoot length, root length, dry shoot and root weight. It was concluded that *Bacillus* spp. had a great potential to enhance seed germination and seedling growth. Further studies are needed to determine the effects of *Bacillus* spp. on seed yield in bean plants growing under field condition.

Keywords: Bacteria, *Bacillus* spp., bean, germination, *Phaseolus vulgaris*, seedling growth.

### 1. INTRODUCTION

Seed is the most important factor for sustainability of crop production. Germination is an important and final event in the life of a seed. Germination power and speed and high field emergence rate are the most important features that should be found in a seed to be planted. These properties are very important for commercial seeds of many crops such as wheat, barley, cotton, beans and chickpeas that were directly sown into the field. In adverse conditions such as low and high soil temperature, soil cracking, drought and soil salinity, it is vital for the seed to germinate quickly and homogeneously (Sivritepe, 2012). In addition, inaccurate practices occur during the harvesting and drying of seeds, seed viability and germination rate can decrease (Gray, 1989).

The way to obtain highest crop yield in a unit area depends on making maximum utilization of solar radiation by rapidly covering the soil surface with crop canopy. Covering quickly the soil surface with plant canopy depends on using seeds with high germination power and germination rate. The germination power and speed of seeds can be increased by the use of suitable seed coating material. The main functions of seed coating materials are to give the seeds the desired shape and size, and to protect the seeds against diseases and pests. Plant nutrients, germination and plant growth promoting hormones added to the seed coating material accelerate the germination of the seed and

increase the field emergence rate. For this purpose, many inorganic and organic materials have been used as seed coating materials, and new seed coating materials are tested day by day. The inorganic and organic materials used in the seed coating ensure that the seeds grow faster and more homogeneously, and they also form a strong root system, making the plant more quality and productive.

Excessive use of chemicals in agriculture has started to threaten human health and environment. The use of rhizobacteria that promote seed germination and plant growth is one of the best alternative methods for synthetic fertilizers. Rhizobacteria that provide plant growth and vegetables are described by Kloepper and Schroth (1993) as microorganisms that live in the plant rhizosphere and contribute positively to the crop growth. Beneficial soil microorganisms increase crop yield by mobilization, transformation, solubilization of nutrients, synthesizing plant hormones and siderophores, and nitrogen fixation (Estrada et al., 2013; Kaur and Reddy, 2014; Reis et al., 2015). The best known plant growth promoting bacteria are *Pseudomonas*, *Burkholderia*, *Bacillus*, *Azospirillum*, *Herbaspirillum*, *Enterobacter*, and *Azotobacter* (Kennedy et al., 2004).

*Bacillus* sp. are usually rod-shaped flat or nearly flat cells in the gram-positive bacteria group. Since bacteria of the *Bacillus* genus form spores, they can be easily stored for a long time and can be easily inoculated into the soil. Bacteria of this genus can survive even at very high temperatures. They have the ability to form spores in unsuitable conditions. The endospores that they form can be oval, round, cylindrical or kidney-shaped. Although their habitat is soil, they can be found widely in nature, from milk and dairy products to air, water and food (Bonwart, 1989). *Bacillus* spp. is one of the most studied bacterial genera on economically important crop species. *Bacillus* spp. produces not only auxin, but also they produce indole acetic acid (Tsavkelova, 2006; Felici et al., 2008; Rojas et al., 2011).

*Bacillus* genus can produce organic acids and phytase enzymes with increased phosphate solubility (Fernandez et al., 2005). Tenuta (2004) noted that plant growth promoting rhizobacteria can improve plant growth and health in three ways. These are a) by reducing pest/disease growth (bioprotectant): it has a direct effect on the plant against pests and b) by synthesizing phytohormones (biostimulant): indole acetic acid, cytokines, gibberellin and c) increasing the uptake of plant nutrients. In vitro, *Bacillus* bacteria strain PRBS-1 and strain AP-3 were isolated from soybean *Rhizoctonia solani*, *Colletotrichum truncatum*, *Sclerotinia sclerotiorum*, *Macrophomina phaseolina* and *Phomopsis* sp. fungi, and also selected *B. subtilis* strains increased the growth and development of soybean plant (Araújo et al., 2005). Myresiotis (2014) noted that the use of *Bacillus subtilis* GB03 and *Bacillus pumilus* SE34 strains in tomato plants increased the uptake of plant nutrients and decreased pathogen infection.

There are many symbiotic (*Rhizobium* sp.) and non-symbiotic bacteria (*Azotobacter*, *Azospirillum*, *Bacillus*, and *Klebsiella* sp., etc.) are now being used worldwide with the aim of enhancing plant productivity (Burd et al., 2000; Cocking, 2003). Among the huge number of rhizobacteria, *Azospirillum*, *Azotobacter*, *Pseudomonas*, *Acinetobacter*, *Beijerinckia*, *Derxia*, *Herbaspirillum*, *Burkholderia*, *Gluconacetobacter*, *Enterobacter*, *Bacillus*, *Rahnella*, *Alcaligenes*, *Klebsiella*, *Lysobacter* and *Paenibacillus* have great potential as biofertilizers or growth promoters.

Beans (*Phaseolus vulgaris* L), originating from South America, was brought to Europe in the 16th century and its cultivation gradually increased. In Turkey, beans are in the third place after chickpeas and lentils in terms of cultivation area and production. As in other cultivated plants, the yield increase in bean per unit area depends on germination rate, germination power, field emergence rate, number of vigorous plants per unit area, suitable environmental conditions and

applied cultural methods. The seed germination rate, speed and duration can be increased with applied chemical and natural compounds to the seed. Synthetic compounds that promote germination threaten both human health and environment. For this reason, it is of great importance to use natural compounds that do not threaten human and environmental health in crop production. Early, rapid and homogeneous seed germination and emergence are important to increase crop yield. The objective of this study was to determine the effect of different *Bacillus* strains on seed germination and seedling parameters of bean seed.

## 2. MATERIALS AND METHODS

In this study, was conducted in Erciyes University, Faculty of Agriculture, Department of Field Crops. Dry bean (*Phaseolus vulgaris* L.) cultivar Alberto was used as plant material. Plant height of Alberto is between 60 - 70 cm, and it has white flowers, thin seed coat, easy cooking, comes to harvest maturity in 115-125 days, and has a seed yield of 2800-3000 kg/ha.

The bacteria species used were *Bacillus simplex*, *Bacillus thuringiensis*, *Bacillus cereus*, *Bacillus subtilis* and six unidentified *Bacillus* spp. (Table 1).

**Table 1. Different *Bacillus* species used in the study**

Treatment	Bacteria species and code
A	SZF32 <i>Bacillus thuringiensis</i>
B	SZF45 <i>Bacillus cereus</i>
C	SZF73 <i>Bacillus</i> spp
D	SZF86 <i>Bacillus</i> spp
E	SZF97 <i>Bacillus subtilis</i>
F	SZF120 <i>Bacillus</i> spp
G	SZF135 <i>Bacillus</i> spp
H	SZF147 <i>Bacillus</i> spp
I	SZF168 <i>Bacillus</i> spp
J	SZF194 <i>Bacillus</i> spp
K	Control

Bean seeds were surface sterilized for 5 minutes in 1000 ml sterile jars filled with 50% commercial bleach (5 - 6% NaOCl, Ace). After surface sterilization, the seeds were thoroughly rinsed three times with sterile distilled water. After sterilization, the seeds were placed in tubes containing different *Bacillus* spp. and treated with  $3.4 \times 10^7$  CFU/cm<sup>3</sup> concentrations of bacteria in a shaker for 30 minutes at 25 °C. Control group seeds were placed in tubes containing only distilled water and kept in a shaker at 25 °C for 30 minutes. After incubation, the seeds were filtered and dried on sterile filter paper. Ten bean seeds were placed in each germination pot. Then, seeds were placed in the germination jar (Magenta vessels GA7TM) with three layers of filter paper and 30 ml of sterile distilled water was added. Seeds placed in germination pots were placed in a climate chamber at 25 ± 1 °C under white fluorescent light (42 µmol photons m<sup>-2</sup> s<sup>-1</sup>) in a photoperiod of 16 hours of light and 8 hours of dark. The experimental design was completely randomized design with 4 repetitions.

Germination rates of inoculated seeds were checked every day and 20 ml of sterile distilled water was added when necessary. Germination pots were checked two days after the bacteria inoculation. Seeds with a rootlet length of 2 mm were considered as germinated. The germination experiment was continued for 8 days, and the germination percentage was determined by counting the total germinated seeds on the 8th day. Germination rate was calculated using the following formula.

Germinated seed number

$$\text{Germination rate (\%)} = \frac{\text{Germinated seed number}}{\text{Total seed number}} \times 100$$

In addition, at the end of the germination test, root and shoot lengths were measured with a ruler and determined in cm. In order to find the dry root and shoot weight, the roots and shoots were cut with scissors and separated from each other. Roots and shoots were placed in glass petri dishes and kept at 70 °C for 48 hours and then weighed on sensitive scales and root and shoot dry weights were found in mg/plant.

### 3. RESULTS AND DISCUSSIONS

*Bacillus* species caused the roots to come out of the seed coat and germination start 2 days after the inoculation, compared to the control. Especially the treatment G (SZF135 *Bacillus* spp) had the earliest germination. The seeds in the control treatment started to germinate after the 3rd day. The germination rate, shoot length, root length, and shoot dry weight of bean seeds inoculated with different *Bacillus* species are given in Figures 1.

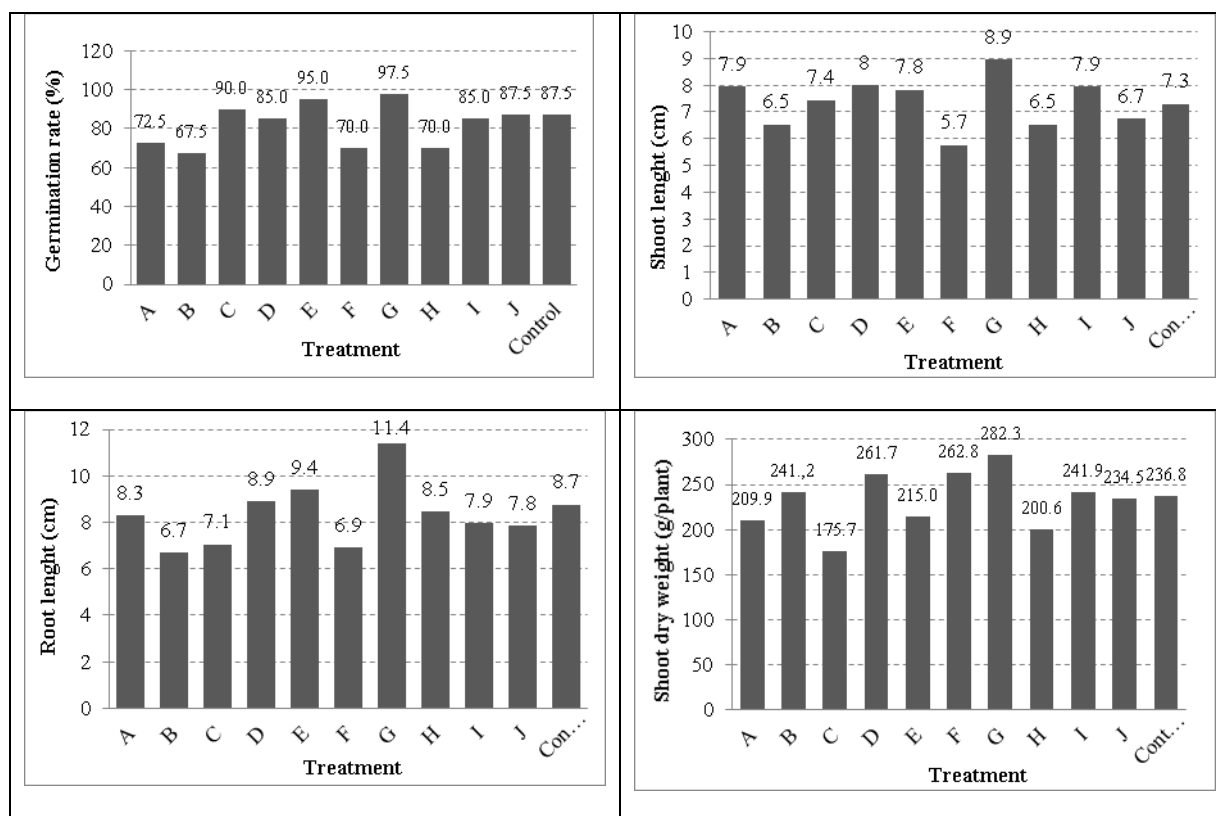


Figure 1. Effect of different *Bacillus* species on seed germination rate, shoot length, root length and shoot dry weight

As can be seen from the Figure 1, different *Bacillus* species had different effects on the seed germination. The germination rate varied between 67.5% and 97.5%. The highest germination rate

was obtained from *treatment G* (SZF135 *Bacillus* spp), and the lowest germination rate was obtained from B (*Bacillus thuringiensis*). The control treatment had a higher germination rate than the treatments A, B, D, F, H and I. In the current study, the germination results obtained from the control treatment were above 85%. In some bacteria treatments, the germination rate was lower than the control. This showed that some *Bacillus* species can synthesize compounds that can inhibit seed germination. On the contrary, Izzeddin and Medina (2011); Luna-Martinez et al. (2013) incubated tomato seeds with some *Bacillus* species and noted that it increased seed germination by 5-6% more than the control.

When root length was in consideration, the root length values varied between 5.7 and 8.9 cm (Figure 1). The highest root length was obtained from the treatment G (SZF135 *Bacillus* spp), and the lowest root length value was obtained from treatment F (SZF120 *Bacillus* spp) with 5.7 cm. Control treatment had higher shoot length than treatments B, F, H and J.

When the effects of different *Bacillus* species on root length were considered, the highest shoot length was obtained from the treatment G with 11.4 cm, and the lowest shoot length was obtained from treatment B with 6.7 cm (Figure 1). Control treatment had higher root length with 8.7 cm than treatments B, C, F, H, I and J.

When shoot dry weight was in consideration, shoot dry weight varied between 282.3 and 175.7 g/plant. Treatment G had the highest shoot dry weight, followed by treatments D and F. Control treatments had higher values than treatments C, E, E, F and H in terms of shoot dry weight.

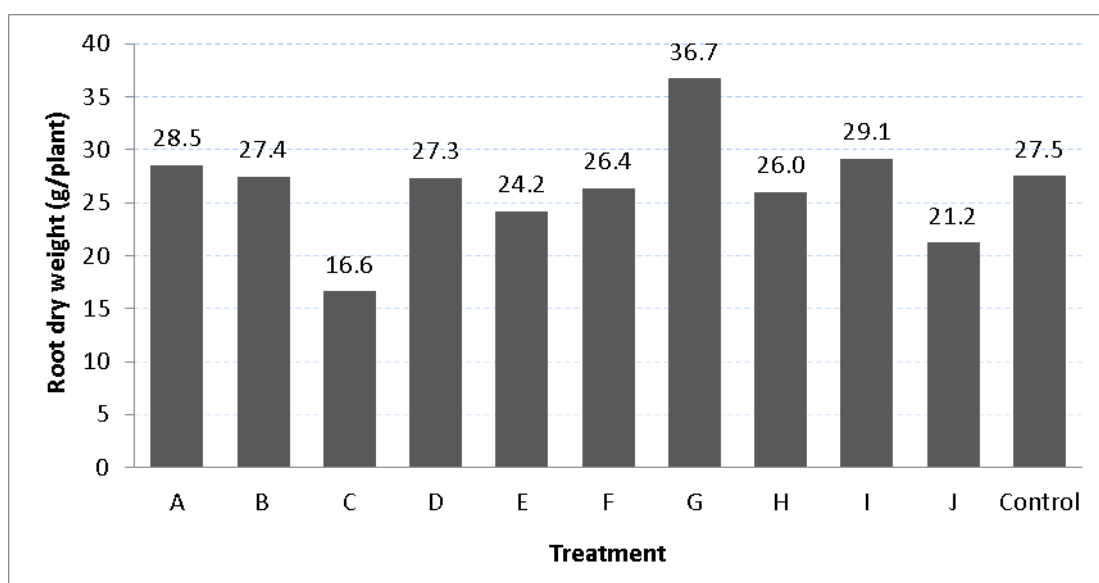


Figure 2. Effect of different *Bacillus* species on root dry weight

When the seedling root dry weight values were considered, it is seen that the root dry weight varied between 16.59 and 36.66 mg/plant (Figure 9). In terms of root dry weight, the highest value was obtained from treatment G, and the lowest value was obtained from treatment C. Control treatment had higher root dry weight value than treatments C, E, F, H and I. It is thought that the increase in seed germination, shoot length, root length, shoot dry weight and root dry weight due to plant hormones synthesized by *Bacillus* species Tsavkelova (2006), Felici et al. (2008) and Rojas et al. (2011). On the other hand, some bacterial species used in this study caused lower shoot length, root

length, shoot and root dry weights compared to the control. Likewise, it shows that some *Bacillus* species synthesize toxic substances that prevent seedling growth and development, as well as inhibit seed germination. By promoting cell division and tissue differentiation in plants, indole acetic acid (IAA) causes an increase in plant production (Roberts et al., 1988; Santillana et al., 2005).

#### 4. CONCLUSIONS

Ten *Bacillus* bacteria species used in this study had significantly different effects on bean seed germination, shoot growth and development. As in treatment G (SZF135 *Bacillus* spp.), bacteria inoculation promoted seed germination and shoot growth, while some bacterial inoculations adversely affected seed germination and shoot growth compared to the control.

The increase in germination, shoot and root length, shoot and root dry weight showed that *Bacillus* spp. coded SZF135 can be used as seed coating agent. Future studies are needed to test SZF135 *Bacillus* spp. under field conditions how much it will contribute to seed yield increase.

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