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ASSESSMENT OF *BACILLUS* STRAINS ON CORN GROWTH AND YIELD UNDER FIELD CONDITION

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Abstract
Plant growth promoting rhizobacteria enhance crop growth and yield by plant nutrient solubilization and mineralization, symbiotic and associative nitrogen fixation, hormone and water soluble vitamin productions. Ten strains of the genus Bacillus, characterized by their properties related to the promotion of plant growth, were evaluated to determine their effects on corm growth, development and yield under field condition. The experimental design was completely randomized plot with four repetitions. Corn seeds were treated with 10 different Bacillus strains before planting. Compared with the control, Bacillus subtilis and two unidentified Bacillus strains SZF04 and SZF07 highly increased plant height, stem diameter, ear length, seed number/ear, 1000 seed weight and seed yield/plant. Seed treatment with Bacillus strains could be an alternative option to increase corn yield without extra chemical fertilizers inputs.

Keywords: Bacillus spp, seed yield, corn, Zea mays.

1. INTRODUCTION

Corn (*Zea mays* L.), contains approximately 72% starch, 10% protein, and 4% fat, is grown throughout the world as a major food crop, producing approximately 1,144,503 million metric tons/year (PS&D Online updated 04/2023). It is major staple food for many countries of Americas, Eastern Africa, Central America and South-east Asia (Erenstein et al., 2022). Corn is now one of the most widely cultivated cereal crops from the equator to the approximately 50° Northland South, and altitude from sea level to 3000 m above sea level (Morris, et al., 2003). Compared to other grain crops, maize is a more versatile multi-purpose crop, since corn grain can be used to produce different kind of food, livestock feed and industrial products, including starch, oil, industrial alcohol, glue, beverages, sweeteners and ethanol. Thus corn has a diverse and dynamic role in global food chains and nutrient security (Grote et al., 2021; Poole et al., 2021; Shiferaw et al., 2011).

The new challenges that will be faced by agriculture in the twenty-first century impose the adoption of strategies that can be able to increase food production without further arable land increase and with low environmental impact. Increasing efforts to produce more and more economical food is required. The importance of developing new methods to increase crop yield is one of the most effective ways to save human kind from starvation. Beneficial soil microorganisms, a main

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constituent of the natural soil fertility, can promote crop growth, increase crop yield and quality, and contribute significant amount of mineral nutrition to crop plants (Weller, 1988; Joshi et al., 2006). Due to negative effect on environmental and human health of artificial fertilizers and their increasing costs, the use of beneficial soil microorganisms in sustainable crop production is increasing globally (Cakmakci et al., 2007).

Among the beneficial soil microorganisms, plant growth promoting rhizobacteria have been used in crop to supply nutrients, to promote plant growth and development, to improve or maintain soil structure and function, to control or inhibit plant pathogens; and bioaccumulation and biogeochemical cycling of inorganic compounds (Brierley, 1985; Ehrlich, 1990; Doran and Zeiss, 2000). Plant microbe interactions in the rhizosphere play a vital role in mobilization, transformation, solubilization of nutrients from the nutrient deficient soil. Currently, the use of beneficial soil microorganisms is becoming more popular as an alternative or additive to chemical fertilizers for yield increase. Therefore, the use of beneficial soil microorganisms in crop production has great potential in sustainable crop production systems (Sturz et al., 2000). 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.

A great amount of synthetic fertilizers are used to supply nitrogen and phosphorous. Most of phosphorous in insoluble compounds is unavailable to crops. Phosphorous solubilizing bacteria are important for crop nutrition by increasing phosphorous uptake of crops. These rhizobacteria secrete various types of organic acids that reduce the pH of the rhizosphere and subsequently relief the bounded phosphate like Ca3(PO4)2 in the calcareous soils. Growth improvement can be obtained by the direct application of rhizobacteria to seeds (Cakmakci et al., 2007). The objective of this research was to determine the effect of inoculation of different strains of *Bacillus* species on improving corn yield and yield components under under continentaly type of climate.

2. MATERIALS AND METHODS

Bacillus species were obtained from Erciyes University, Department of Agricultural Biotechnology. *Bacillus* species were cultivated in Tryptic Soy Broth under aerobic conditions at 32 °C with shaking at 200 rpm. The inoculation solution was prepared and the bacteria number was adjusted to 3.4 × 107 CFU/cm3.

The soil of the experimental plots was a clay silt loam with a pH of 7.57, 1.10% organic matter, 0.14% total nitrogen content, and water holding capacity of 0.36 cm^3. Based on soil analysis and local recommendations, fertilizer was applied prior to planting at a rate of 25-25-0 kg/ha NPK. Recommended practices were used for weed and insect control. Total annual precipitation at the study site was 375 mm in 2020. No rainfall occurred during the period from June to August. Uncoated seeds of corn cultivar Suerto were soaked with ten strains of *Bacillus* solution (3.4 × 107 CFU/cm3) for two second for inoculation, then the seeds were planted at a rate of 25 seeds per m row on 14 June 2020. There were three control treatments; no fertilizer applied and no bacteria inoculated control (Control 1), fertilizer applied and bacteria inoculated control (Control 2); fertilizer applied and no bacteria inoculated control (Control 3). The experimental design was a randomized complete block with three replications. Plots consisted of four 6 m rows, planted 0.75

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m apart, that were end-trimmed to final length of 5 m prior to harvest of the center two rows. The bacteria treated plots were not fertilized, but the control plots were fertilized with 165 kg/ha N as ammonium nitrate, 35 kg/P as triple super-phosphate and 25 kg/ha K as potassium chloride on 15 May. After emergence, the plant population was ajusted to 70,000 plants/ha. At physiologically maturity, plant samples were collected from the two middle two rows on 10 October 2021. At the time of plant sampling, corn ears within the two rows were separated from the plant and hand shelled then grain parameters were determined. Soil analysis in the experimental area showed that the research area had slightly alkaline loamy soils with excess lime, medium organic matter, phosphorus and high potassium content and without any salinity problems. Monthly temperature (°C), relative humidity (%) and precipitation (mm) values of the experimental year 2020 and long-term averages are provided in Table 1.

Table 1. Meteorological data in Kayseri, Turkey during the experiment

Months	Monthly average temperature (EC)		Monthly average relative humidity (%)		Monthly total precipitation (mm)	
	2020	Long term	2020	Long term	2020	Long term
January	2.0	-1.6	72.8	76.5	83.5	36.3
February	4.7	0.3	57.3	73.9	51.5	36.2
March	8.1	4.8	57.1	67.5	37.5	43.4
April	14.1	10.6	44.3	62.6	3.5	50.9
May	16.7	15.1	50.4	60.8	10.5	51.7
June	19.7	19.1	46.8	55.3	17.1	41.0
July	25.2	22.3	33.7	49.5	8.8	10.4
August	25.1	22.0	37.4	49.8	16.7	8.9

Seed germination and plant emergence were enhanced by applying light sprinkler irrigation. Drip irrigation was applied every 10 days after emergence. After emergence, weeds were controlled by hoeing or rotorcultivator.
The measured plant parameters included plant height (cm), stem diameter (mm), ear diameter (mm), ear length (cm), ear height on plant (cm), number of grains per ear, 1000 grain weight (g) and grain yield (t/ha).
Measured plant parameters were subjected to analysis of variance using the GLM procedure in the Statistical Analysis System software package (SAS Institute, 1996). Means of measured plant parameters were compared by using Fisher's protected least significance difference (LSD) at type I error of 0.05. Simple correlations were obtained with the ANOVA procedure and the MANOVA option.

3. RESULTS AND DISCUSSIONS

Treatment of different *Bacillius* ssp had significant effect on plant height (Table 2). In the current study, inoculation of SZF97 *B. subtilis* increased plant height 9.28% compared with the control 1 (No fertilizer application and no bacteria inoculation). It was reported plant growth promoting rhizobacteria inoculation increase growth up to 500% (Kloepper et al., 1980) and yield up to 57% on different crops (Asghar et al., 2004). Plant height varied between 183.35 and 202.10 cm among *Bacillius* species (Table 2). The highest plant height value was obtained from SZF97 *B. subtilis* and the lowest one was obtained from the Control 1 (No fertilizer application and no bacteria inoculation) treatments. Akinrinlola et al. (2018) reported that eleven of the twelve strains of *Bacillius* increased corn growth significantly compared to the control treatment. In a recent study,

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Li et al. (2021) tested *Bacillus* strain MGW9 at corn seeds by biopriming, they found that the *Bacillus* sp treatment significantly improved dry weight of the plant.
When stem diameter was in consideration stem diameter was not significant among the *Bacillius* ssp. The lowest and the highest stem diameters were obtained from SZF168 *Bacillus* spp and Control 1 treatments with 15.91 and 18.39 mm, respectively (Table 1).
Regarding the ear diameter of corn grains across all the treatments, there was no significant difference among *Bacillius* ssp treatments. Ear diameter values varied between 44.77 and 50.93 mm. The lowest and the highest ear diameter value were obtained from SZF97 *B. subtilis* and SZF32 *B. thuringiensis*, respectively (Table 1).
As far as the ear length is concerned, it varied between 14.99 and 16.61 cm. the lowest and the highest ear length values were obtained from SZF135 *Bacillus* spp and SZF73 *Bacillus* spp, respectively. However, it was found that ear lengths of corn were not statistically influenced by the application of different *Bacillius* ssp treatments (Table 2).

Table 2. Effects seed treatment with different Bacillus races on plant height, stem diameter, ear diameter and ear length of corn

Bacteria	Plant height (cm)	Stem diameter (mm)	Ear diameter (mm)	Ear length (cm)
SZF32 *B. thuringiensis*	200.33	16.63	50.93	15.68
SZF45 *B. cereus*	195.09	16.55	45.14	15.44
SZF73 *Bacillus* spp	198.13	18.13	48.78	16.61
SZF86 *Bacillus* spp	193.04	17.30	45.37	16.32
SZF97 *B. subtilis*	202.10	17.69	44.77	15.47
SZF120 *Bacillus* spp	189.80	17.50	45.89	15.94
SZF135 *Bacillus* spp	196.27	17.45	46.09	14.99
SZF147 *Bacillus* spp	187.08	16.78	45.03	15.12
SZF168 *Bacillus* spp	191.77	15.91	45.92	15.22
SZF194 *Bacillus* spp	191.31	16.91	44.93	17.00
Control 1	183.35	18.39	45.05	16.53
Control 2	193.40	17.26	45.32	16.34
Control 3	191.53	18.30	46.76	15.70
EGF 0.05	17.81	N.S.	N.S.	N.S.

Control 1: No fertilizer application and no bacteria inoculation. Control 2: Fertilizer application and bacteria inoculation
Control 3: Fertilizer application and no bacteria inoculation

When ear height on plant was in consideration, the ear height on plant varied between 49.53 and 58.95 cm. The lowest and the highest ear height on plant were obtained from Control 3 and SZF86 *Bacillus* spp, respectively (Table 3).
Number of grains per ear significant varied among the rhizobacteria treatments (Table 3). Control 3 had the highest number of grains per ear with 558.73 grain/ear and the lowest was obtained from SZF120 *Bacillus* spp with 465.08 ear/grain.
Among the rhizobacteria treatments, 1000 grain weight varied between 301.43 and 409.89 g. The lowest and the highest 1000 grain weight values were obtained from SZF73 *Bacillus* spp and Control 3, respectively (Table 3). The different treatments of rhizobacteria highly influenced 1000 grain weight of corn grains. In case of 1000 grain weight of our results are disagreement with Efthimiadou et al. (2020) that reported no significant differences between size and sphericity of corn grain treated by *Azotobacter chroococcum*, *Bacillus subtilis*, *Bacillus megatherium* and their mixes, respectively.

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When grain yield was in consideration, the grain yield values significantly varied among the rhizobacteria treatments (Table 3). Control 3 had the highest seed yield with 12.60 t/ha while SZF147 *Bacillus* spp treatment had the lowest grain yield with 3.47 t/ha, respectively. Dadnia (2011) reported corn grain yield increase up to 32% with *B. Subtilis* inoculation. In the present study, inoculation of SZF86 *Bacillus* spp increased grain yield 25.3% compared with the control 1 (No fertilizer application and no bacteria inoculation). Similarly, Pereira et al. (2020) reported that *B. subtilis* inoculation increased con grain yield up to 39.5% in the absence of P_2O_5 treatment. Yield increase due to *Bacillus* spp can be attributed to production of phytohormones, such as salicylic acid, gibberellins, cytokinins and indole-3-acetic acid, phosphate solubilization, nutrient availability increase and production of indolic compounds and siderophores.

Table 3. Effects seed treatment with different Bacillus races on ear height on plant, number of grains per ear, 1000 grain weight and grain yield

Bacteria	Ear height on plant (cm)	Number of grains per ear	1000 grain weight (g)	Grain yield (ton/ha)
SZF32 *B. thuringiensis*	57.70	483.87	318.68	5.44
SZF45 *B. cereus*	58.76	484.93	331.21	3.73
SZF73 *Bacillus* spp	58.11	515.24	301.43	5.04
SZF86 *Bacillus* spp	58.95	527.62	347.11	5.69
SZF97 *B. subtilis*	55.34	500.88	352.13	3.52
SZF120 *Bacillus* spp	57.58	465.08	364.59	3.96
SZF135 *Bacillus* spp	58.82	505.48	337.97	3.78
SZF147 *Bacillus* spp	52.03	490.88	335.74	3.47
SZF168 *Bacillus* spp	53.17	483.90	358.71	4.47
SZF194 *Bacillus* spp	55.69	546.07	340.88	4.60
Control 1	53.56	503.62	341.18	4.25
Control 2	52.89	508.33	367.41	11.12
Control 3	49.53	558.73	409.89	12.60
EGF 0.05	9.39	87.99	44.48	4.00

Control 1: No fertilizer application and no bacteria treatment. Control 2: Fertilizer application and no bacteria treatment
Control 3: Fertilizer application and bacteria treatment

4. CONCLUSIONS

The seed treatment of ten *Bacillus* spp. significantly affected the plant height, ear height on plant, number of grains per ear, 1000 grain weight and grain yield, while stem diameter, ear diameter and ear length were not significantly affected. Current study contributes to better understanding of which *Bacillus* strains are better suited to corn growth and yield as a supplement of seed coating agents. Further research is required to determine, the type of seed coating agent that can be combined with *Bacillus* strains.

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