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EFFECTS OF RHIZOBACTERIA APPLICATION ON ENZYME ACTIVITY OF DIFFERENT APPLE SCION–ROOTSTOCK COMBINATIONS

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Abstract
By using biofertilizers consisting of beneficial microorganisms instead of synthetic chemicals, plant growth is increased, damage to the environment is largely prevented and soil fertility is preserved. This study was conducted using seven standard cultivars (Scarlet Spur, Red Chief, Fuji, Jeromine, Galaxy Gala, Granny Smith, and Golden Reinders), which were budded onto M9 and MM106 rootstocks commonly used in the region. During the experiment, nitrogen and phosphorus solvent rhizobacteria were applied three times over a 15-day period in the spring. The application of rhizobacteria had a positive impact on the catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD) activities observed in the leaves across all scion-rootstock combinations. This effect ranged from 4.0% to 30.0% for CAT, from 5.2% to 21.7% for SOD, and from 13.7% to 29.1% for POD. In this study, very significant results were obtained on the effects of rootstocks and rhizobacteria application on enzymatic activity. The results of the present study may provide significant leads for further studies on this subject.

Keywords: apple, biofertilizer, enzyme, rhizobacteria.

1. INTRODUCTION

Türkiye has an important position in terms of fruit growing due to its different climatic and soil conditions. A significant part of the cultivated fruit species and varieties can be grown commercially in Türkiye. Apple (*Malus* × *domestica* Borkh.), which is one of these fruit species, is a species that has spread over wide areas around the world and can easily adapt to many regions. Due to the suitability of the ecological conditions of Anatolia and being a gene center, apples have been grown in almost every part of Türkiye since ancient times (Yildiz et al., 2022; Balta et al., 2022).

Rootstocks used in fruit trees, besides forming the underground part of the plant, are also effective in holding the soil, taking water and nutrients from the soil, and transmitting them to the crown, and transporting the photosynthesis products and growth regulators made in the crown part to the roots. In addition to these, rootstocks influence the shape and size of the varieties grafted on them, early yielding, adaptation to different soil types, resistance to cold and drought, diseases and pests, as well as various fruit characteristics (Neumüller et al., 2013).

Plant Growth Promoting Bacteria (PGPR) are free-living microorganisms found in soil, and they hold significant value in agriculture. These bacteria are commonly classified into various genera, including *Pseudomonas*, *Azospirillum*, *Burkholderia*, *Bacillus*, *Enterobacter*, *Rhizobium*, *Erwinia*,

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Serratia, *Alcaligenes*, *Arthrobacter*, *Acinetobacter*, and *Flavobacterium* (Vaghela and Gohel, 2023). PGPR offer numerous beneficial effects on plant health and growth, with key advantages including the suppression of disease-causing microorganisms, enhanced nutrient availability, and accelerated nutrient assimilation. As a result, harnessing the potential of these bacteria has become a prominent strategy in agriculture, aimed at increasing soil fertility, crop yield, and mitigating the adverse impacts of chemical fertilizers on the environment in recent years.Bacteria increase the tolerance of the plant against environmental conditions such as weeds (Babalola et al., 2007), drought stress (Zahir et al., 2008), heavy metals (Kumar et al., 2009), salt stress (Kaymak et al., 2009) that adversely affect plant growth and reduce these negative effects.

When plants are stressed against adverse soil conditions, the balance between the production of reactive oxygen species and the activity of antioxidant enzymes is disturbed, and oxidative damage often occurs. These cytotoxic active oxygen species can severely disrupt normal metabolism through oxidative damage of lipids, proteins and nucleic acids (Furtana and Tipirdamaz, 2010). The objective of this study was to investigate the impact of nitrogen and phosphorus solvent bacteria application, specifically involving Azospirillum sp-245 and Bacillus megaterium M3, on the catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD) activities in the leaves of seven standard apple varieties grafted onto two different rootstocks, M9 and M106.

2. MATERIALS AND METHODS

Material and Experiment

The study was conducted in the Develi Plain, a geographical area covering approximately 1000 km², which was formed due to volcanic movements from Mount Erciyes in Turkey. The research spanned the years 2020 to 2021. The climate in this region is characterized by cold and snowy winters, along with hot and dry summers.

The experiments in this study involved seven standard apple varieties grafted onto two different rootstocks, M9 and MM106. The apple varieties used in the study included Scarlet Spur, Red Chief, Fuji, Jeromine, Galaxy Gala, Granny Smith, and Golden Reinders. The orchard was established in the year 2014, with specific planting configurations. For the M9 rootstock, the trees were spaced 75 cm apart within rows and 4.0 m between rows, and a wire tree support system was utilized. On the other hand, for the MM106 rootstock, the trees were planted with a spacing of 1.5 m within rows and 4.0 m between rows. Fertilizer application in the orchard was carried out using a drip irrigation system, known as the fertigation system.

Treatments

In the study, *Azospirillum* sp-245 and *Bacillus megaterium* M3 bacteria were utilized as rhizobacteria. The bacterial treatment, involving the application of *Azospirillum* sp-245 + *Bacillus megaterium* M3, was carried out using bacterial suspensions at a concentration of 108 CFU/mL. This treatment was applied three times, with a 15-day interval, to the canopy projectional areas of the trees after full flowering. In contrast, no bacterial application was made to the control plants. The research was organized following a randomized blocks experimental design, with three replications for each scion-rootstock combination. Within each replication, there were five trees. This experimental setup allows for rigorous and reliable data collection and analysis in the study.

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Antioxidant enzymes analysis

Leaf samples were taken in late July-early August. In each replication, 10-15 leaves in the middle parts of the annual shoots were taken. The leaves were immediately transported to laboratory in the cold chain to determine the enzymes analysis.

For the extraction of enzymes, 500 mg leaf sample was homogenized in a mortar with 5 ml of 50 mM phosphate buffer at pH 7. Homogenates were filtered through two layers of Miracloth, and the filtrate was centrifuged at 15.000g for 15 min, at 4 °C (Wang et al., 2012). The resulting supernatant was stored at –80 °C.

In the study, frozen cell samples were first finely pulverized using liquid nitrogen. Subsequently, these pulverized samples were extracted with ice-cold 0.1 mM phosphate buffer at a pH of 7.8. This extraction buffer contained 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM phenylmethanesulfonyl fluoride (PMSF), and 1% polyvinylpyrrolidone (PVP). To determine the enzymatic activities of catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) in the apoplastic fractions of the samples, spectrophotometry was employed. This technique is commonly used in biological research to measure the absorbance of specific wavelengths of light, allowing for the quantification of enzyme activities and other biochemical processes.

The catalase (CAT) activity was determined by monitoring the decrease in absorbance values at 240 nm. This measurement was conducted in a solution consisting of 50 mM phosphate buffer at a pH of 7.5, containing 20 mM H2O2. One unit of CAT activity was defined as the amount of enzyme that could consume 1 µmol of H_2O_2 per minute. Peroxidase (POD) activity was assessed by observing the increase in absorbance at 470 nm. The reaction mixture for POD activity measurement consisted of 50 mM phosphate buffer at a pH of 5.5, 1 mM guaiacol, and 0.5 mM H_2O_2. Superoxide dismutase (SOD) activity in the apoplastic fractions was estimated by monitoring the optical density decrease values using the nitro-blue tetrazolium dye. The absorbance was recorded at 560 nm. One unit of enzyme activity was defined as the amount of enzyme required to reduce the absorbance reading to 50% when compared to tubes lacking the enzyme. The results of antioxidant enzyme activities were expressed as enzyme units (EU) per gram of leaf fresh weight (fw). This method of expressing the data helps standardize the measurements and allows for meaningful comparisons between samples.

Data analysis

The study's data underwent analysis of variance (ANOVA) to check for overall differences. To pinpoint specific differences between the means, the Tukey multiple comparison test was used.

3. RESULTS AND DISCUSSIONS

The antioxidant enzyme activity (catalase, superoxide dismutase and peroxidase) in the leaves of standard varieties budded on different apple rootstocks and the effects of bacteria application on them are given in Table 1. The antioxidant enzyme activity of leaves differed statistically in control and rhizobacteria application according to scion-rootstock interactions.

In the control application, the highest catalase (CAT) activity value was obtained from Jeromine/M9 combination (154.22 EU g/plant), the lowest catalase (CAT) activity value was obtained from both Fuji/M9 (99.25 EU g/plant) and Fuji/MM106 (92.47 EU g/plant) combinations. After bacteria application, the CAT activity of the leaves was the highest in the Jeromine/M9 combination, with 208.67 EU g/plant, and the lowest in the Fuji/M9 combination was 114.58 EU g/plant. While the effect of bacteria application on the CAT activity of leaves was positive in all

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scion-rootstock combinations, this effect varied between 4.0% and 35.3% according to combinations. The increase in CAT activity of bacteria application was obtained highest in Jeromine/M9 combination and lowest in Galaxy Gala/M9 combination.
In the control application, the highest catalase (CAT) activity value was obtained from Jeromine/M9 combination (154.22 EU g/plant), the lowest catalase (CAT) activity value was obtained from both Fuji/M9 (99.25 EU g/plant) and Fuji/MM106 (92.47 EU g/plant) combinations. After bacteria application, the CAT activity of the leaves was the highest in the Jeromine/M9 combination, with 208.67 EU g/plant, and the lowest in the Fuji/M9 combination was 114.58 EU g/plant. While the effect of bacteria application on the CAT activity of leaves was positive in all scion-rootstock combinations, this effect varied between 4.0% and 35.3% according to combinations. The increase in CAT activity of bacteria application was obtained highest in Jeromine/M9 combination and lowest in Galaxy Gala/M9 combination.

Table 1. The effect of rhizobacteria application on antioxidant enzyme activity of leaves (EU/g plant)

Rootstock	Varieties	CAT		SOD		POD	
		Control	Bacteria	Control	Bacteria	Control	Bacteria
M9	Scarlet Spur	142.11b(1)	177.76b	288.42c-f	334.60ef	1590.14c-f	1840.31de
	Fuji	99.25f	114.58g	308.98b-d	375.98a	1637.02cd	2067.89b
	G.Smith	140.77b	149.00e	238.74g	273.56j	1545.01ef	1771.58e
	G.Gala	119.76de	124.50f	277.13ef	333.62e-g	1614.87c-e	1834.93de
	Golden R.	131.14c	163.19c	302.78b-e	345.31cd	1757.76a	2166.23a
	Red Chief	125.27cd	162.52c	298.45b-f	319.36h	1667.33bc	2023.50b
	Jeromine	154.22a	208.67a	281.98d-f	365.51b	1666.00bc	2010.31b
MM106	Scarlet Spur	132.29c	161.27cd	313.43bc	340.40de	1571.34d-f	1922.19c
	Fuji	92.47f	116.04fg	349.63a	377.44a	1608.74c-f	2075.95b
	G.Smith	129.67c	148.32e	270.57f	305.16i	1536.21f	1856.42cd
	G.Gala	112.35e	123.22fg	305.82b-d	332.34e-g	1606.07c-f	1916.87c
	Golden R.	124.59cd	151.83d	308.17b-d	323.95gh	1735.96ab	2048.75b
	Red Chief	112.69e	141.60e	319.69b	350.72c	1658.53bc	1928.98c
	Jeromine	145.27b	187.66b	298.45b-f	327.64f-h	1653.20c	2069.06b

(1): Differences within each treatment (control, bacteria effect) are shown with different letters.

In the control application, the superoxide dismutase (SOD) activity of the leaves was determined the highest in the Fuji/MM106 combination with 349.63 EU g/plant, and the lowest in the Granny Smith/M9 combination with 238.74 EU g/plant. After the bacteria application, the highest SOD activity of the leaves was obtained in combinations of Fuji variety with MM106 (377.44 EU g/plant) and M9 (375.98 EU g/plant) rootstock, and the lowest was obtained in Granny Smith/M9

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combination with 273.56 EU g/plant. While bacteria application had a positive effect on SOD activity in all combinations, the highest increase was achieved in Jeromine variety grafted on M9 rootstock with 29.6%. The lowest increase was obtained from the Golden Reinders/MM106 combination with 5.1%.

The peroxidase (POD) activity of the leaves was obtained the highest in the M9/Golden Reinders combination (1757.76 EU/g plant and 2166.23 EU/g plant, respectively), and the lowest in the MM106/Granny Smith combination (1536.21 EU/g plant and 1771.58 EU/g plant, respectively) after both control and bacteria application. While the effect of bacteria application on POD activity was positive, this positive effect varied between 13.6% and 29.0%. The highest increase in POD activity of bacteria application was obtained in Fuji/MM106 combination, and the lowest in Galaxy Gala/M9 combination.

Plants exposed to stress can overcome oxidative stress by activating some or all their antioxidant defense systems (Jung, 2004; Pinherio et al., 2004). The degree of increase in antioxidant enzyme activity and antioxidant content under stress varies considerably between many plant species and even between two varieties of the same species. The degree of response depends on the type, growth, and metabolic state of the plant as well as on the intensity and duration of the stress (Alexieva et al., 2003; Kalefetoglu and Ekmekci, 2005). In our study, bacteria application had a positive effect on the CAT, SOD and POD activities of the leaves in all scion-rootstock combinations and this effect increased up to 35%. It has been determined that similar positive results regarding antioxidant enzyme activity because of beneficial rhizobacteria application have also emerged in the study conducted in apple species grown under stress conditions such as salty soil conditions (Arıkan, 2017). As a matter of fact, in this study conducted with Fuji variety grafted on M9 rootstock, it was determined that 5 different rhizobacteria applications with plant growth enhancing effects increased CAT activity by an average of 26.1%, SOD activity by an average of 23.5%, and POD activity by an average of 19.6%. In studies on the effect of bacteria on antioxidant enzyme activity, it has been determined that bacteria application increased CAT, SOD and POD activities in lettuce plant (Kohler et al., 2009), cucumber plant (Kang et al., 2014), tomato and pepper plants (Hanafy et al., 2012) and okra plant (Habib et al., 2016) under salt stress. On the other hand, in a study conducted with cauliflower plant, it was determined that the combination of bacteria (*Paenibacillus polymyxa* RC14, *Bacillus subtilis* RC63 and *Pseudomonas fluorescens* RC77) caused an increase in antioxidant enzymes compared to the control, and this increase was 104% in CAT activity and 136% in POD activity (Civelek, 2017).

4. CONCLUSIONS

In this study, the effects of nitrogen+phosphorus solvent rhizobacteria (*Azospirillum* sp-245 + *Bacillus megaterium* M3) application on the enzyme activities of 7 standard apple cultivars budded on M9 and MM106 rootstock, which are widely used as rootstocks in apples, were investigated. In the study, rhizobacteria application influenced increasing the number of enzymes in general. As a result, it was concluded that plant growth promoting nitrogen + phosphorus solvent rhizobacteria applications can be used as biological fertilizer in apples. To fully reveal the effects of the use of bacteria on perennial plants such as fruit species, it is thought that different bacterial species and compositions should be tested, thus increasing the use of bacteria in agriculture.

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6. REFERENCES

Alexieva, V., Ivanov, S., Sergiev, I., & Karanov, E. (2003). Interaction between stresses. *Bulg. J. Plant Physiol*, *29*(3-4), 1-17.

Angelini, R., Manes, F., Federico, R. 1990. "Spatial an funtional correlation between daimine-oxsidase and peroxidase activities and their dependence upon deetilation and wounding in chick-pea", Planta, 182, 89-96.

Arikan, S. (2017). Effects of Beneficial Rhizobacteria Applications on Apple and Cherry in Salty Soil Conditions. Ph.D. Thesis, Selcuk University, Institute of Science and Technology, Department of Horticulture, 150 p., Konya.

Babalola, O. O., Sanni, A. I., Odhiambo, G. D., & Torto, B. (2007). Plant growth-promoting rhizobacteria do not pose any deleterious effect on cowpea and detectable amounts of ethylene are produced. *World Journal of Microbiology and Biotechnology*, *23*, 747-752.

Balta, M. F., Karakaya, O., Yaman, M., Kırkaya, H., & Yaman, İ. (2022). Sugar and Biochemical Composition of Some Apple (Malus× domestica Borkh.) Cultivar Grown in the Middle Black Sea Region. Journal of Agricultural Faculty of Gaziosmanpaşa University (JAFAG), 39(3), 129-135.

Civelek, C. (2017). The effects of bacteria and different fertilizer combinations on plant growth, yield and quality characteristics in cauliflower (Brassica oleracea L. var. botrytis).

Furtana, G. B., & Tipirdamaz, R. (2010). Physiological and antioxidant response of three cultivars of cucumber (Cucumis sativus L.) to salinity. Turkish Journal of Biology, 34(3), 287-296.

Habib, S. H., Kausar, H., Saud, H. M. (2016). "Plant growth-promoting rhizobacteria enhance salinity stress tolerance in okra through ROS-scavenging enzymes", BioMed Research International, 2016, 1-11.

Hanafy, E. A., Zaghloul, R. A., Abou-Aly, H. E., Rahal, G. H., El-Meihy, R. M. E. (2012). Effect of salt-tolerant PGPR on the activity of some microbial and plant enzymes under saline stress. The eleventh conference of agricultural development researches. Faculty of Agric., Ain Shams Univ., Egypt: 1-12.

Jung, S. (2004). Variation in antioxidant metabolism of young and mature leaves of Arabidopsis thaliana subjected to drought. Plant Sci., 166; 459-466.

Kalefetoğlu, T., Ekmekci, Y. (2005). The effects of drought on plants and tolerance mechanisms. G.U. Journal of Science, 18(4): 723-740.

Kang, S. M., Khan, A. L., Waqas, M., You, Y. H., Kim, J. H., Kim, J. G., Hamayun, M., Lee, I. J. (2014). Plant growth-promoting rhizobacteria reduce adverse effects of salinity and osmotic stress by regulating phytohormones and antioxidants in Cucumis sativus, Journal of Plant Interactions, 9 (1), 673-682.

Kaymak, H.C., Güvenç, I., Yaralı, F., Dönmez, M.F. (2009). The effects of bio-priming with PGPR on germination of radish (*Raphanus sativus* L.) seeds under saline conditions. Turkish Journal of Agriculture and Forestry, 33(2): 173-179.

Kohler, J., Hernández, J. A., Caravaca, F., Roldán, A. (2009). Induction of antioxidant enzymes is involved in the greater effectiveness of a PGPR versus AM fungi with respect to increasing the tolerance of lettuce to severe salt stress, Environmental and Experimental Botany, 65 (2), 245-252

Kumar, K.V., Srivastava, S., Singh, N., Behl, H.M. (2009). "Role of metal resistant plant growth promoting bacteria in ameliorating fly ash to the growth of *Brassica juncea*. Journal of Hazardous Materials, 170(1): 51-57.

Li, R., Kou, X., Xie, L., Cheng, F., & Geng, H. (2015). Effects of ambient PM 2.5 on pathological injury, inflammation, oxidative stress, metabolic enzyme activity, and expression of c-fos and c-jun in lungs of rats. *Environmental Science and Pollution Research*, *22*, 20167-20176.

Neumüller, M., Mühlberger, L., Siegler, H., Hartmann, W., & Treutter, D. (2013). New rootstocks with resistance to Plum pox virus for Prunus domestica and other stone fruit species: the'Docera'and'Dospina'rootstock series. In X International Symposium on Plum and Prune Genetics, Breeding and Pomology 985 (pp. 155-165).

Pinheiro, H. A., DaMatta, F. M., Chaves, A. R. M., Fontes, E. P. B., Loureiro, M. E. (2004). Drought tolerance in relation to protection against oxidative stress in clones of Coffea canephora subjected to long-term drought. Plant Sci., 167; 1307-1314.

Vaghela, N., & Gohel, S. (2023). Medicinal plant-associated rhizobacteria enhance the production of pharmaceutically important bioactive compounds under abiotic stress conditions. Journal of Basic Microbiology, 63(3-4), 308-325.

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Wang, S., Liang, D., Li, C., Hao, Y., Ma, F., Shu, H. (2012). Influence of drought stress on the cellular ultrastructure and antioxidant system in leaves of drought-tolerant and drought-sensitive apple rootstocks. Plant Physiology and Biochemistry, 51: 81-89.

Yildiz, E., Yaman, M., Ercisli, S., Sumbul, A., Sonmez, O., Gunes, A., ... & Kviklys, D. (2022). Effects of Rhizobacteria Application on Leaf and Fruit Nutrient Content of Different Apple Scion–Rootstock Combinations. Horticulturae, 8(6), 550.

Zahir, Z.A., Munir, A., Asghar, H.N., Shaharoona, B., Arshad, M. (2008). Effectiveness of rhizobacteria containing ACC deaminase for growth promotion of peas (*Pisum sativum*) under drought conditions. Journal Microbiology Biotechnology, 18(5): 958-963.

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