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Research Article

Effects of Plant Growth Promoting Rhizobacteria (PGPR) Applications on Biochemical Activity and Enzyme Activity in Strawberries

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Abstract

Plant growth promoting rhizobacteria (PGPR), which have environmentally friendly properties, are important for their use as biofertilizers and biocontrol agents. The aim of this study was to determine the effects of single and combined applications of PGPR bacteria on maloninaldehyde (MDA), proline, superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) activities of leaves in Albion and Monterey strawberry cultivars. *Bacillus subtilis* OSU-142 (*B. subtilis* OSU-142), *Bacillus megaterium* M3 (*B. megaterium* M3) and *Paenibacillus polymyx* (*P. polymyx*) were used as PGPR in the study. The effect of rhizobacteria varied according to strawberry cultivars. Rhizobacteria applications showed positive effects on MDA, SOD and POD. However, rhizobacteria treatments did not have a significant effect on proline, while they showed a varying effect on CAT according to the cultivars. In this study, significant results were obtained on the effects of strawberry and rhizobacteria application on biochemical activity and enzymatic activity. The results of this study may provide important clues for future studies on similar subjects.

Keywords: Strawberry, enzyme activity, antioxidant enzyme, rhizobacteria, PGPR

Bitki Büyümesini Teşvik Eden Rizobakteri (PGPR) Uygulamalarının Çileklerde Biyokimyasal Aktivite ve Enzim Aktivitesi Üzerine Etkileri

Öz

Çevre dostu özelliğe sahip olan bitki büyümesini teşvik eden rizobakteriler (PGPR), biyogübre ve biyokontrol ajanı olarak kullanımıyla önem arz etmektedir. Bu çalışmanın amacı, Albion ve Monterey çilek çeşitlerinde PGPR bakterilerinin tekli ve kombine uygulamalarının yaprakların maloninaldehit (MDA), prolin, süperoksit dismutaz (SOD), peroksidaz (POD) ve katalaz (CAT) aktiviteleri üzerine etkilerini belirlemektir. Çalışmada *Bacillus subtilis* OSU-142 (*B. subtilis* OSU-142), *Bacillus megaterium* M3 (*B. megaterium* M3) ve *Paenibacillus polymyx* (*P. polymyx*) PGPR olarak kullanılmıştır. Rizobakterilerin etkisi çeşitlere göre değişiklik göstemiştir. Rizobakteri uygulamaları MDA, SOD ve POD üzerinde olumlu etki göstermiştir. Ancak rizobakteri uygulamalarının prolin üzerinde önemli derecede bir etkisi görülmez iken CAT üzerinde ise çeşitlere göre değişen etki göstermiştir. Bu çalışmada, çilekte rizobakteri uygulamasının biyokimyasal ve enzimatik aktivite üzerindeki etkileri konusunda çok önemli sonuçlar elde edilmiştir. Bu çalışmanın sonuçları gelecekte bu konuda yapılacak çalışmalar için önemli ipuçları sağlayabilir.

Anahtar Kelimeler: Çilek, enzim aktivitesi, antioksidan enzim, rizobakteri, PGPR

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1. Introduction

Türkiye is among the important countries in the world in terms of fruit growing due to its different climate and soil conditions. Many of the fruit species grown in Türkiye are of commercial importance. One of these fruit species is strawberry (*Fragaria x ananassa* Duch). Strawberry, which is one of the temperate climate fruit species, is grown almost everywhere in the world within wide ecological boundaries from Ecuador to Siberia thanks to its high adaptability. Strawberry is an important berry fruit that is consumed with pleasure by people and has a great market advantage as fresh and industrial [1].

Strawberries are one of the most important sources of bioactive substances that are important for healthy nutrition, such as vitamins, minerals, sugars, anthocyanins, phenols, flavonoids and antioxidants. Thanks to its high biochemical content and antioxidant potential, strawberries provide a direct effect against diseases such as cardiovascular and cardiometabolic diseases [2]. Strawberry fruits are an important fruit due to their antioxidant, anti-inflammatory, antihyperlipidemic and blood pressure lowering effects [3].

With the understanding of the importance of strawberries in human nutrition, the demand for strawberry fruits is increasing day by day. In response to the increasing demand for strawberries, the use of chemical fertilizers and chemical pesticides is becoming widespread to increase yield and quality. Indiscriminate use of chemical fertilizers causes air, water and soil pollution and is dangerous for human health. Indiscriminate use of chemical fertilizers causes air, water and soil pollution and is dangerous for human health. Alternative production systems that are more environmentally friendly and conducive to soil health are urgently needed [4]. Plant growth-promoting microorganisms are generally classified as biofertilisers, which increase nutrient levels in the plant; phyto-stimulants, which promote plant growth by producing plant hormones; root cleaners, which break down resistant toxic pollutants; and biopesticides, which control disease by producing antibiotic and antifungal metabolites. The application of environmentally friendly and soil-friendly biofertilisers and biocontrol agents in agriculture has increased in recent years [5].

Soil microorganisms improve nutrient uptake by plants and have great potential when used as biofertilizers. Free-living microorganisms used as biological control agents or biofertilizers are known as PGPR [6]. PGPR are free-living organisms in the soil and are very beneficial in plant production. These rhizobacteria generally belong to the species Pseudomonas spp., Azospirillum spp., Burkholderia spp., Bacillus spp., Enterobacter spp., Rhizobium spp., Erwinia spp., Serratia spp., Alcaligenes spp., Arthrobacter spp., Acinetobacter spp. and Flavobacterium spp. [7]. Rhizobacteria have many benefits on plant growth and productivity. Rhizobacteria increase plant growth by increasing nutrients in plants. They especially increase nitrogen fixation [8] and phosphorus solubility [9]. In recent years, the use of rhizobacteria in sustainable agriculture has increased to increase soil fertility, improve agricultural products and reduce the negative effects of chemical fertilizers on the environment [10]. In addition, rhizobacteria increase the resistance of plants against biotic and abiotic stress conditions such as water stress [11], high temperature [12], low temperature [13], salinity [14], lime [15], heavy metal [16] and biotic factors [17].

Plants are exposed to abiotic (frost, salinity, high temperature, drought, etc.) and biotic (pathogens, competition between organisms) stress factors. Abiotic and biotic stress factors reduce the biosynthetic capacity of plants and cause plant death as the effect of stress increase [18]. When plants are exposed to stress, they try to protect themselves from the negative effects of stress by activating various metabolic, physiological and biochemical mechanisms [19, 20]. Plant cells produce oxygen radicals and their derivatives, reactive oxygen species (ROS), during various processes associated with abiotic stress. ROS production, one of the most important consequences of abiotic stress, disrupts the balance between both enzymatic and non-enzymatic antioxidant defence systems and causes oxidative stress in plants [19]. ROS are highly reactive and damage biomolecules such as lipids, proteins and nucleic acids [21]. Plants have developed an effective antioxidant system to protect against the effects of oxidative stress [22].

The aim of this study was to investigate the effects of single and combined applications of PGPR bacteria (*B. subtilis* OSU-142, *B. megaterium* M3, *P. polymyx*) on MDA, proline, SOD, POD and CAT activities in the leaves of Albion and Monterey strawberry cultivars.

2. Material and Methods

Plant Material

Albion and Monterey strawberry cultivars (*Fragaria x ananassa* Duch), which constitute the study material, were selected from the open growing area in Develi district of Kayseri province in 2023. The study was carried out in three replications and ten plants were used for each replication. The leaf samples were transported to the laboratory in cold chain and stored at -80 °C until analysis.

Methods

In the study, *B. megaterium* M3, *B. subtilis* OSU-142, *P. polymyxa* species were used as bacteria. All bacterial strains were obtained from Dr. Metin Turan (Istanbul Yeditepe University, Faculty of Engineering and Architecture, Department of Genetics and Bioengineering culture collection). Bacteria were grown on nutrient agar by line plate inoculation method and kept at 27 °C for 48 h. At the end of this period, a single colony was taken from the cultures that completed growth and transferred to bottles containing 250 ml nutrient broth. Bacteria were grown aerobically in flasks on a rotating shaker (150 rpm) for 48 h at 27 °C. Then, bacterial suspensions were diluted in sterile distilled water to a final concentration of 108 CFU mL^{-1}. The bacterial suspensions obtained were applied to soil as 10 mL for each bacterial species in Albion and Monterey varieties. Bacterial species *B. megaterium* M3, *B. subtilis* OSU-142 and *P. polymyxa* PGPR were applied as single applications. Also, a mixture of equal amounts of these three bacterial species was applied as a mixed application. Bacterial applications were applied to the root areas of the plants from the soil. This study was carried out as four bacterial treatments and one control group application.

Lipid peroxidation (MDA) analysis: 0.5 g of fresh leaf sample was homogenized with 10 mL of 0.1% trichloroacetic acid (TCA) solution and then centrifuged at 10000 rpm for 10 min. 1 ml of the obtained supernatant was transferred to the tubes and 4 mL of 20% TCA and 0.5% thiobarbituric acid (TBA) were added to the tubes. The obtained mixture was kept in a water

bath at 95℃ for 30 minutes and then the samples were quickly cooled in ice. The cooled samples were centrifuged at 10000 rpm for 10 minutes. The absorbance of the supernatant formed in the tubes after centrifugation was read at 532 and 600 nm wavelengths on a spectrophotometer. Lipid peroxidation was calculated as nmol/g Maloninaldehyde (MDA) with the formula MDA= (A532- A600) x Extract volume (ml) / (155mM/cm x Sample amount (mg)) (Stresty and Rao, 1999).

Proline analysis: Proline analysis was determined by modifying the method specified by [23]. For the analysis, 0.5 g of leaf sample was pulverized in liquid nitrogen. The pulverized samples were taken into tubes and then mixed with 3 ml of 3% sulfosalicic acid solution. The mixture obtained was centrifuged at 15000 rpm for 10 minutes at room temperature. After transferring 2 ml of the supernatant to each tube, two parallels were performed for each tube. 2 ml of glacial acetic acid and 2 ml of acetic acid with phosphoric acid and ninhydrin solution were added to the tubes. The tubes were boiled for one hour and then subjected to a rapid cooling process in ice water. 4 ml of toluene was added to the cooled tubes and vortexed for 30 seconds. Then it was kept for 5 min and the absorbance of the pink phase formed at the top was read at 520 nm wavelength in a spectrophotometer. Proline contents were determined using the equation of the standard graph.

Preparation of extracts for measurement of SOD, POD and CAT analysis activities: 0.5 g of fresh leaf samples were homogenized in 5 ml of 50 mM phosphoric buffer (pH: 7.0). Homogenates were centrifuged at 15000 rpm for 15 min at 4℃. Obtained supernatants were stored at -80℃ for determination of enzyme activities [24].

SOD, POD & CAT analysis: Frozen samples were pulverized using liquid nitrogen. 1 mM ethylenediaminetetraacetic acid (EDTA) was extracted with cold 0.1 mM phosphate buffer (pH 7.8) containing 1 mM phenylmethanesulfonylfluoride (PMSF) and 0.5% polyvinylpyrrolidone (PVP). Spectrophotometry was used to determine CAT, POD and SOD enzymatic activities in apoplastic fractions of samples. CAT activity was measured by monitoring the decrease in absorbance at 240 nm in a solution of 50 mM phosphate buffer (pH 7.5) containing 20 mM H_2O_2. One unit of CAT activity is defined as the amount of enzyme using 1 μmol of H_2O_2 per minute. POD activity was evaluated by observing the increase in absorbance at 470 nm. The reaction mixture for POD activity measurement consisted of 50 mM phosphate buffer (pH: 5.5) containing 1 mM guaiacol and 0.5 mM H_2O_2. One unit of POD activity was defined as the amount of enzyme causing an absorbance increase of 0.01 per minute. SOD activity in apoplastic fractions was estimated by recording the decrease in optical density of the nitro-blue tetrazolium dye by the enzyme. Absorbance values were determined at 560 nm wavelength. One unit of enzyme activity was defined as the amount of enzyme required to reduce the absorbance reading by 50% compared to tubes without enzyme. Antioxidant enzyme activity results were expressed as enzyme units (EU) per gram of leaf fresh weight (fw).

Data Analysis

In order to determine the differences between the parameters examined in the study, the data were subjected to analysis of variance (ANOVA). Differences between means were compared

with Tukey's multiple comparison test. Heatmap and principal component analysis of the data were performed using the JMP PRO 17 statistical package program.

3. Results and Disscussion

The effects of bacterial applications on biochemical (MDA and proline) and antioxidant enzyme activities (SOD, POD and CAT) in the leaves of Albion and Monterey strawberry cultivars are given in Table 1. Biochemical and antioxidant enzyme activities of leaves differed according to strawberry cultivars. The effect of bacterial applications on the examined parameters was found to be statistically significant ($p<0.05$).

In the control application, the MDA content of the leaves was determined as 14.49 nmol/g in the Albion variety, while it was determined as 8.41 nmol/g in the Monterey variety. The effect of bacterial applications varied according to varieties. The highest MDA content was detected in *P. polymyxa* bacterial application in both varieties. The highest MDA content was determined as 19.27 nmol/g in the Albion variety. The lowest MDA content was observed in *B. megaterium* M3 (6.65 nmol/g) treatment in Albion variety and in *B. subtilis* OSU-142 (13.98 nmol/g) treatment in Monterey variety. The lowest MDA content was observed in application of *B. megaterium* M3 (6.65 nmol/g) in Albion variety and in application of *B. subtilis* OSU-142 (13.98 nmol/g) in Monterey variety. MDA, which is an important marker for determining the degree of membrane damage due to oxidative stress in plants under abiotic stresses is a product of lipid peroxidation accumulated in plant tissues under stress conditions [25]. High MDA concentration in plant tissues is associated with oxidative damage of plant cell membranes [26]. High MDA content in plants indicates that the plant is sensitive to stress, while low MDA content in plants indicates that the plant is resistant to stress [27]. Decreasing MDA content due to increased antioxidant enzyme activities in plant tissues helps the plant survive under stress conditions [28, 29]. Rhizobacteria applications were reported to reduce MDA content in rice by [30], in wheat by [31], in strawberry by [32] and [15]. In general, the effect of bacterial applications on MDA content varied according to strawberry varieties. In the Albion variety, bacterial applications (except for the *P. polymyxa* bacterial application) showed lower values than control application, while in the Monterey variety, the control application showed lower values than bacterial applications.

The proline contents of the leaves were similar in both varieties in the control application. The effect of bacterial applications varied according to varieties. *B. megaterium* M3 (0.11 mg/g) application in Albion variety and *P. polymyxa* (0.11 mg/g) application in Monterey variety showed the highest proline content. Other bacterial applications generally showed similar results to the control application in varieties. Plants provide tolerance to stress factors with the help of osmotic regulators [33]. Plants minimize damage to cells by accumulating osmolytes in cells and thus aim to adapt to stress. Osmotic regulation mechanisms protect plants from stress by osmotic adjustments through detoxifying reactive oxygen species (ROS), stabilizing membranes, and natural structures of enzymes and proteins [34]. Proline, one of the important osmolytes for plants, provides protection of the cell against ROS accumulation in the cell and regulates the redox homeostasis of the cell [35]. The cellular level of proline varies between species and depends on the severity and duration of the stress situation [36]. Some studies

reported that rhizobacteria applications increased proline content [37, 38] and some studies reported that rhizobacteria applications decreased proline content [31, 39]. It has also been reported that rhizobacteria application decreases proline concentration and increases defence enzymes against stress [40]. As a result, our study findings are consistent with the literature.

SOD activity of leaves was determined between 117.01 EU/g plant (Albion) and 148.10 EU/g plant (Monterey) in control applications. In Albion variety, *P. polymyxa* (162.73 EU/g plant) and *B. megaterium* M3 (159.87 EU/g plant) bacteria applications showed the highest values, while in Monterey variety, *B. megaterium* M3 (187.77 EU/g plant) and *P. polymyxa* (186.53 EU/g plant) bacteria applications showed the highest values. Mix application showed the lowest SOD activity values in both varieties. The lowest SOD activity was determined as 98.28 EU/g plant in Albion variety and 104.91 EU/g plant in Monterey variety. In general, SOD activity showed lower values in Albion variety. POD activity of leaves was determined between 242.00 EU/g plant (Monterey) and 2266.67 EU/g plant (Albion) in control applications. In general, POD activity in bacterial applications showed higher values in Albion cultivar compared to Monterey cultivar. The highest POD activity was detected in Mix bacteria application (7071.10 EU/g plant) in Albion variety, while it was detected in *P. polymyxa* bacteria application (2033.33 EU/g plant) in Monterey variety. In the Albion variety, lower POD activity was detected in the *P polymyxa* and B subtilis OSU-142 bacterial applications compared to the control, while in the Monterey variety, lower POD activity was detected in the control application compared to the bacterial applications. In control applications, the CAT activity of leaves was found to be highest in the Albion variety (5806.00 EU/g plant) and lowest in the Monterey variety (2966.00 EU/g plant). The effects of bacterial applications on CAT activity differed according to the varieties. In Albion variety, the highest CAT activity was determined in *B. megaterium* M3 treatment (5402.00 EU/g plant) and the lowest CAT activity was determined in *P. polymyxa* treatment (1598.00 EU/g plant), while in Monterey variety, the highest CAT activity was determined in *P. polymyxa* treatment (6190.00 EU/g plant) and the lowest CAT activity was determined in *B. subtilis* OSU-142 treatment (140.00 EU/g plant). Plants exposed to stress can overcome oxidative stress by activating antioxidant defense systems [41, 42]. Antioxidant enzymes such as SOD, POD and CAT protect plants against oxidative stress (Khan et al. 2019a). Although some researchers reported that rhizobacteria applications increased antioxidant enzyme activities [15, 28, 31, 32, 43], some researchers reported that rhizobacteria applications decreased antioxidant enzyme activities [30]. The degree of increase in antioxidant enzyme activity and antioxidant content under stress varies significantly among many plant species and even between two varieties of the same species. The degree of response of plants depends on the plant species, growth, metabolic status, intensity and duration of stress [18, 44]. According to our study results, antioxidant enzymes varied according to varieties and rhizobacteria applications. In general, rhizobacteria applications showed positive effects on SOD and POD activities, but showed varying effects on CAT activities. This situation can be explained by the fact that high SOD activity can inactivate CAT activity [45].

Table 1. Effects of rhizobacteria applications on leaf biochemical and antioxidant enzyme activities

		MDA (nmol/g)	Prolin (mg/g)	SOD (EU/g plant)	POD (EU/g plant)	CAT (EU/g plant)
Albion	Control	14.49±0.10 b	0.09±0.00 b	117.01±1.49 c	2266.67±41.63 c	5806.00±223.72 a
	P. polymyxa	19.27±0.13 a	0.09±0.00 b	162.73±1.17 a	1486.67±30.55 e	1598.00±90.27 e
	B. subtilis OSU-142	9.33±0.16 c	0.07±0.00 c	151.35±3.41 b	1826.67±30.55 d	4662.00±89.60 c
	B. megaterium M3	6.65±0.17 d	0.11±0.00 a	159.87±0.81 a	4333.33±128.58 b	5402.00±12.00 b
	Mix	9.31±0.21 c	0.07±0.00 c	98.28±1.00 d	7071.10±3.81 a	3188.00±104.84 d
Monterey	Control	8.41±0.10 c	0.08±0.00 b	148.10±0.20 c	242.00±3.46 e	2966.00±96.99 bc
	P. polymyxa	16.43±0.04 a	0.15±0.00 a	186.63±5.14 a	2033.33±80.83 a	6190.00±90.27 a
	B. subtilis OSU-142	13.98±0.15 b	0.07±0.00 c	156.88±0.85 b	473.33±64.29 d	140.00±17.32 d
	B. megaterium M3	14.30±0.39 b	0.07±0.00 c	187.77±0.49 a	1493.33±80.83 b	3054.00±41.57 b
	Mix	14.39±0.23 b	0.07±0.00 c	104.91±0.63 d	693.33±11.55 c	2626.00±289.14 c

The heatmap analysis and principal component analysis graphs of the parameters examined in the study are presented in Figure 1. Heatmap and principal component analysis graphs were made to reveal the effectiveness of bacterial applications according to the examined parameters. In the heatmap analysis, the color intensity changing from blue to red indicates that the values of the bacteria related to the examined parameters have increased. According to the hetmap analysis, rhizobacteria applications were divided into two groups. *P. polymyxa* was grouped separately from other rhizobacteria applications. *P. polymyxa* showed high values on MDA, proline, SOD, while it showed partially high values on CAT. *B. megaterium* M3 rhizobacteria showed high effect on SOD and CAT activitiy, control application showed high effect on CAT activity and Mix application showed high effect on POD activity. In general, the effect of B. subtilis OSU-142 rhizobacteria on the examined parameters was not high.

The principal component analysis plot shows positive correlation between narrow-angle features and negative correlation between wide-angle features [46]. According to the principal component analysis graph, it can be said that POD activity and other parameters show a negative correlation among the examined parameters.

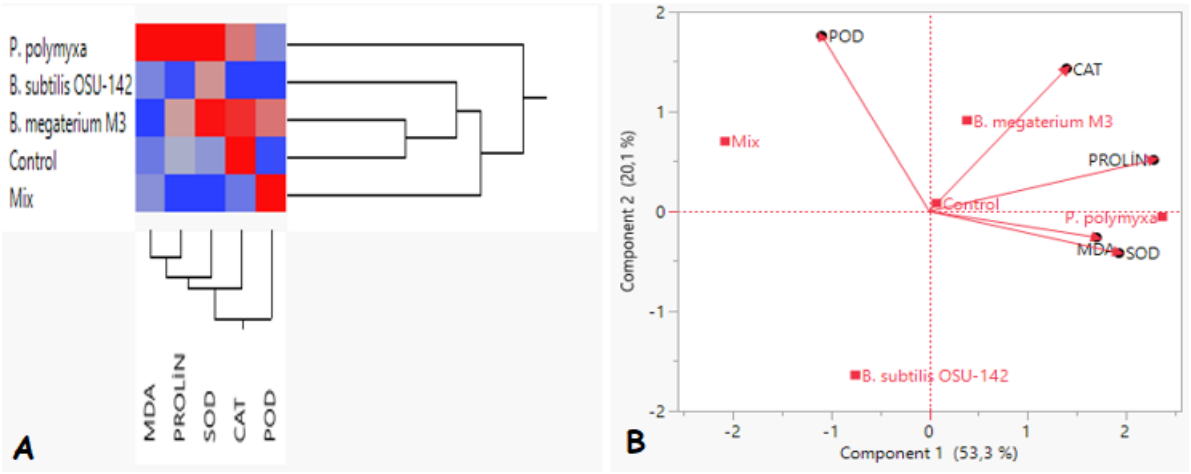

Figure 1. Heatmap and PCA analysis graph of the investigated parameters of bacteria applications (A: Heatmap analysis, B: PCA analysis graph of two components)

4. Conclusion

The effects of rhizobacteria applications in the study vary according to the varieties. However, rhizobacteria applications showed different levels of positive effects on MDA, proline and

antioxidant enzymes (SOD, POD and CAT). As a result of the study, it was concluded that plant growth-promoting rhizobacteria applications can be used as biofertilizers and biocontrol agents in strawberries. In order to fully reveal the effects of using rhizobacteria on strawberries, different bacterial species and mixtures need to be tested on different varieties. The information obtained as a result of the study will shed light on future studies on similar subjects.

Ethics in Publishing

There are no ethical issues regarding the publication of this study

Author Contributions

All authors contributed to the design of the study, collection of data, evaluation of the results, and writing of the article.

References

[1] Amil-Ruiz, F., Blanco-Portales, R., Munoz-Blanco, J., Caballero, J.L., (2011) The strawberry plant defense mechanism: A molecular review, Plant and cell physiology, 52(11), 1873-1903.

[2] Koraqi, H., Petkoska, A. T., Khalid, W., Sehrish, A., Ambreen, S., Lorenzo, J. M., (2023) Optimization of the extraction conditions of antioxidant phenolic compounds from strawberry fruits (*Fragaria x ananassa* Duch.) using response surface methodology. Food Analytical Methods, 16(6), 1030-1042.

[3] Basu, A., Nguyen, A., Betts, N.M. Lyons, T.J. 2014. Strawberry as a functional food: an evidence-based review. Critical Reviews in Food Science and Nutrition, 54, 790-806

[4] Ali, S., Moon, Y. S., Hamayun, M., Khan, M. A., Bibi, K., & Lee, I. J. (2022). Pragmatic role of microbial plant biostimulants in abiotic stress relief in crop plants. Journal of Plant Interactions, 17(1), 705-718.

[5] Arikan, S., Ipek, M., Pirlak, L., (2016). Effect of some plant growth promoting rhizobacteria (PGPR) on growth, leaf water content and membrane permeability of two citrus rootstock under salt stress condition. In Proceedings of the VII International Scientific Agriculture Symposium, Jahorina, Bosnia and Herzegovina, 6–9 October 2016; 845–850.

[6] Verma, P. P., Shelake, R. M., Das, S., Sharma, P., Kim, J. Y., (2019). Plant growth-promoting rhizobacteria (PGPR) and fungi (PGPF): potential biological control agents of diseases and pests. Microbial Interventions in Agriculture and Environment: Volume 1: Research Trends, Priorities and Prospects, 281-311.

[7] Adeyemi, N. O., Atayese, M. O., Olubode, A. A., Akan, M. E., (2020). Effect of commercial arbuscular mycorrhizal fungi inoculant on growth and yield of soybean under controlled and natural field conditions. Journal of Plant Nutrition, 43(4), 487-499.

[8] Fukami, J., de la Osa, C., Ollero, F. J., Megías, M., Hungria, M., (2017). Co-inoculation of maize with *Azospirillum brasilense* and *Rhizobium tropici* as a strategy to mitigate salinity stress. Functional Plant Biology, 45(3), 328-339.

[9] Soumare, A., Boubekri, K., Lyamlouli, K., Hafidi, M., Ouhdouch, Y., Kouisni, L., (2020). From isolation of phosphate solubilizing microbes to their formulation and use as biofertilizers: status and needs. Frontiers in bioengineering and biotechnology, 7, 425.

[10] Yaman, M., Yildiz, E., Sumbul, A., Ercisli, S., Sonmez, O., Gunes, A., Say, A., Kece, Y.M., Unsal, H. T. (2023). The Effect of PGPR Applications on Bioactive Content and Fruit Characteristics of Different Apple Scion–Rootstock Combinations. Erwerbs-Obstbau, 65(5), 1267-1273.

[11] Paliwoda, D., Mikiciuk, G., Mikiciuk, M., Kisiel, A., Sas-Paszt, L., Miller, T., (2022). Effects of rhizosphere bacteria on strawberry plants (*Fragaria× ananassa* Duch.) under water deficit. International Journal of Molecular Sciences, 23(18), 10449.

[12] Meena, H., Ahmed, M. A., Prakash, P., (2015). Amelioration of heat stress in wheat, *Triticum aestivum* by PGPR (*Pseudomonas aeruginosa* strain 2CpS1). Biosci Biotechno Res, 8(2), 171-174.

[13] Zubair, M., Hanif, A., Farzand, A., Sheikh, T. M. M., Khan, A. R., Suleman, M., Gao, X. (2019). Genetic screening and expression analysis of psychrophilic Bacillus spp. reveal their potential to alleviate cold stress and modulate phytohormones in wheat. Microorganisms, 7(9), 337.

[14] Sangiorgio, D., Cellini, A., Spinelli, F., Donati, I., (2023). Promoting strawberry (*Fragaria× ananassa*) stress resistance, growth, and yield using native bacterial biostimulants. Agronomy, 13(2), 529.

[15] Arıkan, Ş., İpek, M., Eşitken, A., Pırlak, L., Dönmez, M. F., Turan, M., (2020). Plant growth promoting rhizobacteria mitigate deleterious combined effects of salinity and lime in soil in strawberry plants. Journal of Plant Nutrition, 43(13), 2028-2039.

[16] Kang, S. M., Asaf, S., Khan, A. L., Lubna, Khan, A., Mun, B. G., Lee, I. J., (2020). Complete genome sequence of *Pseudomonas psychrotolerans* CS51, a plant growth-promoting bacterium, under heavy metal stress conditions. Microorganisms, 8(3), 382.

[17] Lee, B. D., Dutta, S., Ryu, H., Yoo, S. J., Suh, D. S., Park, K., (2015). Induction of systemic resistance in Panax ginseng against *Phytophthora cactorum* by native *Bacillus amyloliquefaciens* HK34. Journal of Ginseng Research, 39(3), 213-220.

[18] Kalefetoğlu, T., Ekmekci, Y., (2005). The effects of drought on plant sand tolerance mechanisms. Gazi University Journal of Science, 18(4), 723-740.

[19] Hasanuzzaman, M., Bhuyan, M. H. M. B., Zulfiqar, F., Raza, A., Mohsin, S.M., Mahmud, J.A., Fotopoulos, V., (2020). Reactive Oxygen Species and Antioxidant Defense in Plants under Abiotic Stress: Revisiting the Crucial Role of a Universal Defense Regulator. Antioxidants, 9(8), 681.

[20] Giordano, M., Petropoulos, S.A., Rouphael, Y., (2021). Response and defence mechanisms of vegetable crops against drought, heat and salinity stress. Agriculture 11(5):463.

[21] Apel, K., Hirt, H., (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu. Rev. Plant Biol., 55(1), 373-399.

[22] Ahmad, P., Kumar, A., Ashraf, M., Akram, N. A., (2012). Salt-induced changes in photosynthetic activity and oxidative defense system of three cultivars of mustard (*Brassica juncea* L.). African Journal of Biotechnology, 11(11), 2694.

[23] Karabal, E., Yücel, M., Öktem, H.A., (2003). Antioxidant responses of tolerant and sensitive barley cultivars to boron toxicity. Plant Sci 164:925–933

[24] 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.

[25] Kar, M., Öztürk, N., (2020). The evulation of stress related gene expression level and relationship to cellular H_2O_2 in chickpea (*Cicer arietinum* L.) under copper stress. Bursa Uludağ Üniversitesi Ziraat Fakültesi Dergisi, 34(2), 303-315.

[26] Assaha, D. V. M., Liu Li Yun, L. L., Mekawy, A. M. M., Ueda, A., Nagaoka, T., Saneoka, H., (2015). Effect of salt stress on Na accumulation, antioxidant enzyme activities and activity of cell wall peroxidase of huckleberry (*Solanum scabrum*) and eggplant (*Solanum melongena*).

[27] Koca, H., Bor, M., Özdemir, F., & Türkan, İ. (2007). The effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. Environmental and experimental Botany, 60(3), 344-351.

[28] Karagöz, H., Çakmakçi, R., Hosseinpour, A., Kodaz, S., (2018). Alleviation of water stress and promotion of the growth of sugar beet (*Beta vulgaris* L.) plants by multi-traits rhizobacteria. Applied Ecology and Environmental Res. 16(5):6801-6813.

[29] Yassin, M., El Sabagh, A., Mekawy, A. M. M., Islam, M. S., Hossain, A., Barutcular, C., Saneoka, H., (2019). Comparative performance of two bread wheat (*Triticum aestivum* L.) genotypes under salinity stress. Applied Ecology & Environmental Research, 17(2).

[30] Jha, Y., Subramanian, R. B., (2014). PGPR regulate caspase-like activity, programmed cell death, and antioxidant enzyme activity in paddy under salinity. Physiology and Molecular Biology of Plants, 20, 201-207.

[31] Khan, N., Bano, A., Babar, M. A. (2019). The stimulatory effects of plant growth promoting rhizobacteria and plant growth regulators on wheat physiology grown in sandy soil. Archives of microbiology, 201(6), 769-785.

[32] Erdogan, U., Çakmakçi, R., Varmazyarı, A., Turan, M., Erdogan, Y., Kıtır, N., (2016). Role of inoculation with multi-trait rhizobacteria on strawberries under water deficit stress. Zemdirbyste-Agriculture, 103(1), 67-76.

[33] Ahmed, C. B., Rouina, B. B., Boukhris, M., (2008). Changes in water relations, photosynthetic activity and proline accumulation in one-year-old olive trees (*Olea europaea* L. cv. Chemlali) in response to NaCl salinity. Acta Physiologiae Plantarum, 30, 553-560.

[34] Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., Basra, S., (2009). Plant drought stress: effects, mechanisms and management. In: Lichtfouse E, Navarrete M, Debaeke P, Souchère V, Alberola C (eds) Sustainable agriculture. Springer, Berlin, pp 153–188

[35] Sharma, S., Villamor, J.G., Verslues, P.E., (2011). Essential role of tissue specific proline synthesis and catabolism in growth and redox balance at low water potential. Plant Physiol 157(1):292–304.

[36] Delauney, A.J., Verma, D.P.S., (1993). Cloning of ornithine δ-aminotransferase cDNA from *Vigna aconitifolia* by trans-complementation in Escherichia coli and regulation of proline biosynthe sis. Plant J 4:215–223

[37] Matysik, J., Alia, Bhalu, B., Mohanty, P., (2002). Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. Current Science, 525-532.

[38] Nawaz, S., Bano, A., (2020). Effects of PGPR (*Pseudomonas* sp.) and Ag-nanoparticles on enzymatic activity and physiology of cucumber. Recent patents on food, nutrition & agriculture, 11(2), 124-136.

[39] Khan, N., Bano, A., Rahman, M.A., Guo, J., Kang, Z., Babar, M.A., (2019). Comparative physiological and metabolic analysis reveals a complex mechanism involved in drought tolerance in chickpea (*Cicer arietinum* L.) induced by PGPR and PGRs. Sci Rep 9(1):2097

[40] Singh, R.P., Jha, P.N., (2017). The PGPR *Stenotrophomonas maltophilia* SBP-9 augments resistance against biotic and abiotic stress in wheat plants. Front Microbiol 8:1945

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

[42] 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.

[43] Yıldız, E., Yaman, M., Say, A., (2023). Effects of Rhizobacteria Application on Enzyme Activity of Different Apple Scion–Rootstock Combinations. Current Trends in Natural Sciences, 12(23), 42-48.

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

[45] Azarmi, F., Mozafari, V., Abbaszadeh Dahaji, P., Hamidpour, M., (2016). Biochemical, physiological and antioxidant enzymatic activity responses of pistachio seedlings treated with plant growth promoting rhizobacteria and Zn to salinity stress. Acta physiologiae plantarum, 38, 1-16.

[46] Garazhian, M., Gharaghani, A., Eshghi, S., (2020). Genetic diversity and inter-relationships of fruit bio-chemicals and antioxidant activity in Iranian wild blackberry species. Scientific Reports, 10(1), 18983.