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ORIGINAL ARTICLE

Biological Control of Crown Gall in GF677 Rootstock and Changes in Antioxidant Enzyme Activities

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Abstract
Biological control agents are bacteria that are used to control plant diseases by providing direct biocontrol of pathogens and/or by stimulating the plant's defense system. The aim of this study was to determine the ability of the five most effective bioagent bacteria to control crown gall on GF677 rootstock, to characterize their biocontrol and plant growth-promoting properties, and to assess the defense-related antioxidant enzyme activities. Bioefficacy tests of bioagent bacteria were carried out for root and stem treatments on GF677 rootstock according to a randomized plot design with five replications and one rootstock in each replication. Three different groups were created as controls. After ~6 months, the number of tumors formed on the roots and the diameter of the tumors formed on the stem were examined. According to the results, the commercial product Nogall had the highest effect in root treatments, followed by strain RK 1 B + 1978. In stem treatments, the lowest tumor diameter was observed with strain 1 B + RK1978. Strain RK 1978 synthesized chitinase, phytase, amylase, protease, and cellulase enzymes but could not synthesize hydrogen cyanide, salicylic acid, and siderophores. The amount of antioxidant enzymes with root and stem treatments in rootstocks increased compared to the control group without any treatment. Molecular analysis identified strain RK 1978 as *Bacillus subtilis*. Thus, biological control with *B. subtilis* strain RK 1978 is recommended as a new ecofriendly candidate for the management of crown gall disease.

Keywords Antagonism · Bioagents · Crown gall · Microbial control · Plant bacterial diseases · Plant growth promoting bacteria

Introduction

Crown gall is a disease caused by *Rhizobium radiobacter* (formerly *Agrobacterium tumefaciens*) and is characterized by the formation of tumor-like galls on plant tissues near the soil surface (Liang et al. 2019). *R. radiobacter* affects a broad range of plant species (~140 genera and more than 100 different plant families), is subject to quarantine worldwide, and causes significant economic losses (Rhouma et al. 2004; Pulawska 2010; Mansfield et al. 2012). The following control strategies are implemented to manage crown gall disease: (1) using disease-free propagation material, (2) selecting resistant rootstocks, (3) soil solarization, and (4) using copper or bleach-based bactericides (Escobar and Dandekar 2003). (5) The non-pathogenic *A. radiobacter* strain K-84 and the genetically derived K 1026 that produces the antibiotic Agrocin 84 are widely used in the biological control of crown gall disease around the world (Kerr 1980; Jones and Kerr 1989; Vicedo et al. 1993). However, it has been reported by different researchers that this cannot effectively control all *Agrobacterium* species or biovars after a certain time (Ophel and Kerr 1990; Utkhede and Smith 1993; Khmel et al. 1998; Raio et al. 2009). For this reason, the identification of new biological control agents (BCAs) such as bacteria that can control crown gall disease has become more necessary than ever (Khmel et al. 1998; Kahla et al. 2017).

Globally, BCAs have widespread use as plant protectants in sustainable agricultural systems (Tripathi et al. 2020). In recent years, laboratory, greenhouse, or field studies have been conducted to find new potential BCAs for the biolog-

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ical control of crown gall disease. It has been determined by different researchers that species belonging to the genera *Bacillus, Curtobacterium, Lactobacillus, Pseudomonas, Pantoea, Serratia,* and *Paenibacillus* as BCAs suppress the formation of tumors (Khmel et al. 1998; Otten et al. 2008; Kawaguchi 2009; Tolba and Soliman 2013; Limanska et al. 2015; Abdallah et al. 2018; Bozkurt and Soylu 2019).

It has been shown that BCAs can suppress phytopathogens simultaneously through one or more mechanisms (antagonism, nutrient and space competition, ability to produce antibiotics, lytic enzymes and toxins, promoting plant growth, and inducing systemic resistance [ISR]) in plants (Tripathi et al. 2020).

Inducing systemic resistance is one of the important mechanisms used by various BCAs to protect the plant against a wide range of plant pathogens (Vallad and Goodman 2004; Shafi et al. 2017). When a non-pathogenic microorganism acts as a BCA or a weakly virulent pathogen acts as a real pathogen, ISR activates the host plant's defense mechanism and makes the plant susceptible to any subsequent attack (Bora and Özaktan 1998). This situation depends on the accumulation of salicylic acid (SA) in the intercellular space. It is also characterized by activation of genes encoding pathogenicity-related (PR) proteins (Cook and Baker 1983). Although the ISR of beneficial microorganisms is often regulated through SA-independent mechanisms, some of the microorganisms used as BCAs have been reported to trigger an SA-dependent type of ISR similar to pathogen-induced systemic acquired resistance (SAR; Pieterse et al. 2014).

Furthermore, ISR is also associated with the synthesis of defense-related enzymes, PR proteins, and oxidative enzymes. Oxidative enzymes such as phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), and superoxide dismutase (SOD) play a key role in catalyzing the synthesis of lignin and other phenolic compounds. Antioxidant enzymes have been reported as potential elicitors of ISR because their roles in plants are highly related to disease suppression. Additionally, antioxidant enzymes help reduce the level of reactive oxygen species (ROS), which are a source of oxidative stress during pathogen infection of plants (Shi et al. 2006; Liu et al. 2011; Yasmin et al. 2016; Rais et al. 2017). Some studies have reported that BCAs have the ability to induce antioxidant enzymes that play a role in plant defense mechanisms (Rais et al. 2017).

This study aimed to (1) test five BCA bacteria (FDG 48, RK 570 A, RK 1977, RK 1978, RK 1986), which were found to be effective in a previous study (Tekiner Aydın and Kotan 2025) against crown gall disease under in vivo conditions; (2) determine the mechanisms that the five bioagent bacteria use to prevent the disease; and (3) to determine the changes in some defense enzyme activities as a result of bioagent bacterial treatments.

Material and Methods

Plant Material and Pathogen-Bioagent Bacterial Strains

For the in vivo experiments, we used ~ 30-cm-high GF677 (*Prunus persica* × *P. amygdalus*) rootstock, which is sensitive to crown gall disease (Horuz et al. 2018). Treatments were carried out in five (23 × 17 cm) cultivation pots containing sterile peat and perlite mixture (3:1) in a greenhouse at 20–24 °C.

R. radiobacter 1 B, which was isolated from the gall peach plant as a pathogen (Tekiner Aydın and Kotan 2022). Five antagonistic bacterial strains (FDG 48, RK 570 A, RK 1977, RK 1978, RK 1986), which were previously found to be effective against crown gall disease in in vitro and semi-in vivo tests, were used as BCAs (Tekiner Aydın and Kotan 2025). The diagnosis of bacterial strains was made using microbial identification systems (MISs) and conventional methods (biochemical and cultural diagnostic tests). Bacterial strains that were not diagnosed in the MIS were diagnosed with molecular methods. Bacterial strains were stored at –80 °C in Luria Bertani (LB) + Glycerol (20%) in the Microorganism Culture Collection of Atatürk University Faculty of Agriculture, Plant Clinical Laboratory.

Characterization of Biocontrol and Plant Growth-Promoting Features of Bioagent Bacteria

The biocontrol and plant growth-promoting properties (hydrogen cyanide (HCN), indole acetic acid [IAA], salicylic acid [SA], siderophore and lytic enzyme [chitinase, phytase, amylase, cellulase, protease]) of the bacterial strains to be used in the in vivo study were measured by growing them in the relevant medium as described in the following sections (Rais et al. 2016):

HCN Production

According to the method of Albert et al. (1987) for qualitative testing, bioagent bacteria were grown in King B medium (Merck) supplemented with 4.4 g/L glycine at 25–28 °C for 48 h. Sterile filter paper moistened with picric acid solution was placed on the lid of a Petri dish (90 mm), covered with parafilm, and incubated at 28 °C for 48 h. The color of the filter paper changed from yellow to light brown (weak: +), brown (medium: ++), and reddish

brown (strong: +++). No color change was evaluated as negative (–).

Siderophore Production

In this test using chrome azurol S (CAS) agar (Schwyn and Neilands 1987) medium, Petri dishes (90 mm) were divided into four equal parts and different bioagent bacterial strains were inoculated into four separate points and incubated for 24 h at 28 °C. Since the color change from blue to orange is an indicator of siderophore production, the diameter of the orange zone formed around the colonies was measured.

Chitinase Enzyme Production

Enzyme activity was determined according to the Senol (2014) method. Briefly, bioagent bacteria were grown in Nutrient Broth (NB, BD Difco) at 27 °C for 48 h, and then the pellet obtained by centrifugation was used for activity determination. A total of 200 µL enzyme solution +500 µL colloidal chitin was mixed and kept in a water bath at 37 °C for 30 min. Then, 750 µL of 0.01 M dinitrosalicylic acid (DNS, Sigma Aldrich) was added to the mixture and kept in a water bath at 80 °C for 10 min. Finally, 1300 µL of pure water was added to each sample removed from the water bath, and the reading values of the blank samples were analyzed on a spectrophotometer (Shimadzu) at a wavelength of 540 nm.

Phytase Enzyme Production

Following the method of Zou et al. (2008), the bioagent bacteria were developed in NB for 24 h; 100 µL enzyme solution and 250 µL substrate were mixed and incubated in a hot water bath at 37 °C for 10 min. To stop the reaction, 500 µL of 10% (w/v) trichloroacetic acid (TCA) was added and then 500 µL of coloring solution was added to the tubes and incubated for 15 min and centrifuged at 4000 rpm for 5 min. After centrifugation, the samples were analyzed on a spectrophotometer (Shimadzu) at a wavelength of 700 nm.

Amylase Enzyme Production

Bioagent bacteria grown in 1% starch medium were incubated at 28 °C for 2–3 days and the production of amylase enzyme was determined by adding iodine to the medium. A change in purple color was considered positive, and the purple color remaining the same was considered negative (Sung et al. 1993).

Cellulase Enzyme Production

Bioagent bacteria were grown in a medium containing 1% carboxymethyl cellulose (CMC, Sigma Aldrich) and the amount of zone formed by the addition of 0.1% Congo red at the end of 48 h was measured in millimeters (Duman et al. 2016).

Protease Enzyme Production

Discs impregnated with bioagent bacteria were placed in the middle part of Petri dishes (90 mm) containing skim milk agar (SMA, Sigma Aldrich) and incubated at 27 °C for 48 h. The zone formed around the bacterial colonies was measured in millimeters (Atlas 1995).

IAA Production

Production of IAA was determined colorimetrically in the presence of L-tryptophan (L-trp). First, 5 mL L-trp (passed through a 0.2-µm filter) was added to 20 mL NB. Then, 100 µL 10^8 colony-forming units/mL (cfu/mL) of bioagent bacterial strains were inoculated into NB + L-trp medium and incubated in a horizontal shaker at 27 °C. At the end of the incubation period, liquid inoculums were transferred to centrifuge tubes and centrifuged at 6000 rpm for 15 min, and the supernatant was filtered through Whatman (no. 2) filter paper. The resulting filtrate was placed in Eppendorf tubes and centrifuged at 10,000 rpm for 5 min. Subsequently, 3 mL of the supernatant was placed in a sterile tube, and 2 mL of Salkowski reagent (2 mL 0.5 M $FeCl_3$ + 98 mL 35% $HClO_4$) was added for 30 min for color formation. Then, absorbance values were recorded at a wavelength of 535 nm in a spectrophotometer (Shimadzu; Gorden and Paleg 1957; Asghar et al. 2002).

SA Production

According to the method of Meyer and Abdallah (1978), bioagent bacteria strains were incubated in succinate medium for 48 h at 28 °C. Then, the inoculums were centrifuged at 8000 rpm for 5 min and mixed with 1 mL of 0.1 M phosphate buffer solution. The resulting 4-mL filtrate was adjusted to pH 2 with 1 N HCl and SA was extracted in $CHCl_3$. In the next step, 4 mL of water and 5 µL of 2 M $FeCl_2$ were added. The absorbance of the purple iron SA complex developed in the aqueous phase was read at 527 nm, and the quality of the SA produced was calculated by obtaining the standard curve with SA dissolved in succinate medium.

Bioefficacy of Bioagent Bacteria Under Greenhouse Conditions

The effectiveness of the bioagent bacterial strains against *R. radiobacter* was tested on stem and root treatments using GF677 rootstocks in pots under greenhouse conditions. Pathogen and bioagent bacterial suspensions were prepared at 10^8 cfu/mL (Eastwell et al. 2006). For the stem treatment, first, 100 µL of bioagent bacteria and a few hours later, 100 µL of pathogenic bacteria were administered to the wounds made on the rootstock stems (three wounds were made) using a sterile scalpel. The wounds were wrapped with cotton and parafilm, and the wound areas were moistened with water for 10 days. The root treatment was made to the injured rootstock roots by mixing equal volumes of pathogen + bioagent bacterial solution (50 mL pathogen + 50 mL bioagent) and pouring it into the potting soil. Three different control groups were created: control 1, treated with sterile distilled water only; control 2, rootstock with pathogen only; control 3, rootstock with Nogall (K-1026 strain). The study was conducted according to the randomized plot design with five replicates and one rootstock in each replication. After ~6 months, the rootstocks were removed from the pots and the number of tumors formed on the stem and root was examined (Sharma et al. 2016).

Determination of Antioxidant Enzyme Activities

For the extraction of enzymes, wounds opened from the root collar of the rootstocks and fresh root samples (~200 mg) were used. First, 2 mL of cold homogenate buffer (0.1 M KH_2PO_4, pH 7.0, containing 1% PVP and 1 mM EDTA) was added to the ground samples and centrifuged at 15,000 rpm and +4 °C for 15 min. The supernatant obtained as a result of the centrifugation process was used as a stock culture for activity measurements of antioxidant enzymes (Wang et al. 2012).

Catalase activity was measured on the rate of H_2O_2 decomposition at 240 nm according to the method of Tejera García et al. (2007). Peroxidase activity was measured according to its capability to turn guaiacol to tetraguaiacol at 470 nm (Rao et al. 1996); SOD activity was analyzed by determining inhibition in the photochemical reduction of nitro blue tetrazolium at 560 nm according to the method of Abedi and Pakniyat (2010). We calculated APX activity on the basis of H_2O_2-dependent oxidation of ascorbate at 290 nm (Liu et al. 2014).

Molecular Analysis

According to Lazo et al. (1987), genomic DNA of the pathogen and bioagent bacteria used in the study was obtained and PCR was performed using 27 F (5′-AGAGTTTG-ATCCTGGCTCAG-3′) and 1492 R (5′-TACGGYTAC-CTTGTTACGACTT-3′) primers designed according to the 16s rRNA gene region (Heuer et al. 1997). The PCR products were then sequenced by the Refgen Biotechnology Company (Ankara, Turkey) for molecular diagnosis. Sequence data of bacterial strains were combined with the BioEdit program and deposited in the NCBI sequence database.

Data Analysis

Bioagent bacterial strain suppression of the crown gall rate was analyzed and evaluated using JMP 5.0.1. statistical program. The data obtained from the antioxidant enzyme activity tests were analyzed using the statistical package SPSS 20. Data were subjected to analysis of variance (ANOVA), and differences in means were determined by Duncan's multiple comparison test ($p < 0.05$).

Results

The results of the biocontrol and plant growth-promoting properties of bioagent bacteria are given in Table 1. All bioagent bacteria were found to produce metabolites in the tests, except for salicylic acid in the siderophore test. Strain RK 570A produced the most HCN, followed by strains FDG 48 and RK 1977. The IAA test results indicated that strain RK 1978 produced the highest amount of IAA, while strain RK 570A strain produced the least. It was determined

Table 1 Biocontrol and plant growth-promoting properties of bioagent bacteria

Strains	HCN	IAA[3] (µg/ml)	SA[4]	S[5]	Chitin. [6]	Phyt. [7]	Amyl. [8]	Prt. [9] (mm)	Cellul. [10] (mm)
FDG 48	+[1]	0.140	–	–	0.183	0.298	+	43.5	20.0
RK 570A	++[2]	0.031	–	–	0.266	0.387	–	33.5	8.0
RK 1977	+	0.102	–	–	0.462	0.168	+	0.00	5.0
RK 1978	–	0.286	–	–	0.250	0.294	+	37.0	17.0
RK 1986	–	0.128	–	–	0.250	0.284	+	49.5	21.0

[1]HCN + weak, [2]HCN ++ medium, [3]indole acetic acid, [4]salicylic acid, [5]siderophore, [6]chitinase, [7]phytase, [8]amylase, [9]protease, [10]cellulase
[+]Positive reaction
[–]Negative reaction

Table 2 Bioefficacy test results of bioagent bacteria against pathogens

Treatments	Root		Stem	
	Tumor (*n*)/Plant (*n*)		Tumor diameter (cm)	
Unwounded plant (Control 1)	0 ± 00	d	0 ± 0.00	e
R. radiobacter 1 B (Control 2)	5.4 ± 1.62	a	0.49 ± 0.08	a
R. radiobacter 1 B + Nogall (Control 3)	1.8 ± 0.9	cd	0.48 ± 0.13	b
R. radiobacter 1 B + RK 1986	3.8 ± 1.9	a–c	0.47 ± 0.06	ab
R. radiobacter 1 B + FDG 48	3.0 ± 1.7	a–c	0.47 ± 0.14	ab
R. radiobacter 1 B + RK 570A	4.4 ± 1.8	ab	0.46 ± 0.08	a–c
R. radiobacter 1 B + RK 1977	3.8 ± 1.3	a–c	0.45 ± 0.08	a–c
R. radiobacter 1 B + RK 1978	2.4 ± 1.5	b–d	0.24 ± 0.08	a–e
CV	*0.52*	–	*0.62*	–
LSD	*2.48*	–	*0.25*	–

CV coefficient of variation, *LSD* least significant difference
*Differences between treatments marked with the same letter in the same column were not found to be statistically significant, $p < 0.01$

that strains FDG 48, RK 1978, and RK 1986 synthesized to a greater or lesser extent all the lytic enzymes tested.

The bioefficacy of bioagent bacteria under greenhouse conditions was tested on GF677 rootstock in root and stem treatments, and the results are given in Table 2.

According to the results, differences were observed between root treatments and the three control treatments. The treatment with the highest impact was the commercial biopesticide 1 B + Nogall, followed by 1 B + RK 1978. The 1 B + RK 570A bioagent treatment was the least effective treatment. In stem treatments, the lowest tumor diameter was observed in the 1 B + RK 1978 treatment. No statistical difference was observed between other treatments.

The antioxidant enzyme activity test results from the in vivo root and stem treatment of GF677 rootstock are given in Fig. 1.

Increases were observed in root and stem antioxidant enzyme amounts compared to control 1 (no wound treatment). The highest root CAT enzyme amount was seen in the 1 B + RK 570A treatment (0.145 ± 0.02) and the lowest in the control 1 treatment (0.072 ± 0.01). In stem treatments, the highest CAT enzyme activity was observed in 1 B + RK 1986 (0.110 ± 0.03) and the lowest in control 1 (0.037 ± 0.01).

The highest root POD enzyme activity was observed in the control 3 treatment (2.895 ± 0.43), followed by the 1 B + RK 570A treatment (2.100 ± 0.06). In stem treatments, the highest POD enzyme activity was observed in 1 B + FDG 48 (1.665 ± 0.03), followed by 1 B + RK 570A treatment (1.181 ± 0.18).

Root SOD enzyme activity was highest in RK 1 B + RK 1977 (751.4 ± 60.5) and lowest in control 1 (367.1 ± 17.8). The highest stem SOD enzyme activity was observed in the control 3 treatment (1218.2 ± 8), followed by the 1 B + FDG 48 treatment (1072.4 ± 86).

Root APX enzyme activity was highest in control 3 (1.046 ± 0.07) and 1 B + RK 1977 (0.969 ± 0.11) treatments, and lowest in 1 B + RK 570A (0.647 ± 0.04). The highest stem APX enzyme activity was observed in the 1 B + RK 1977 treatment (1.50 ± 0.05) and the lowest was observed in the 1 B + RK 570A treatment (0.57 ± 0.05). It was observed that the antioxidant enzyme activities supported each other in root and stem treatments.

Sequence results of bioagent and pathogen bacteria were analyzed and GenBank numbers were obtained. Accordingly, the following was determined: *Bacillus amyloliquefaciens* FDG 48 (MN967174; 98.84%); *B. cereus* RK 570A (MN967299; 92.45%); *Aneurinibacillus migulanus* RK 1977 (MN967173; 99.42%); *B. subtilis* RK 1978 (MN967135; 97.49%); and *B. mojavensis* RK 1986 (MN967303, 99.54%); *R. radiobacter* 1B (MN967438; 85.38%).

Discussion

Bacillus, Curtobacterium, Pseudomonas, Lactobacillus, and *Serratia* species were found to be effective in the biological control of *A. tumefaciens* (Dandurishvili et al. 2010; Tolba and Soliman 2013; Bozkurt and Soylu 2019). Strains of similar genera were effective according to the results of a previous in vitro and semi-in vivo test study (Tekiner Aydın and Kotan 2025), and the results of this study showed that *Bacillus* genus strains were effective at different levels in the in vivo tests. Verschuere et al. (2000) reported that the most important BCA group used against plant pathogens is the *Bacillus* genus.

Researchers have determined that *Bacillus* spp. have high competitiveness, can be found everywhere, can colonize quickly, can maintain their viability in the soil for a long time, can form endospores, and can produce lytic enzymes–lipopeptides–antibiotics (Hardoim et al. 2008; Kotan et al. 2009; Arrebola et al. 2010; Tiwari and Thakur 2014).

The use of BCA bacteria can prevent plant disease mechanisms such as production of SA, IAA, HCN, siderophores, antimicrobial compounds, and lytic enzymes (Tariq et al. 2014). In this study, the production of lytic enzymes and secondary metabolites (HCN, IAA, SA, and siderophores) of five bioagent bacteria was investigated. We found that all bioagent bacterial strains produced chitinase, phytase, protease, and cellulase enzymes, and amylase was produced by the other four strains except RK 570A. Five bioa-

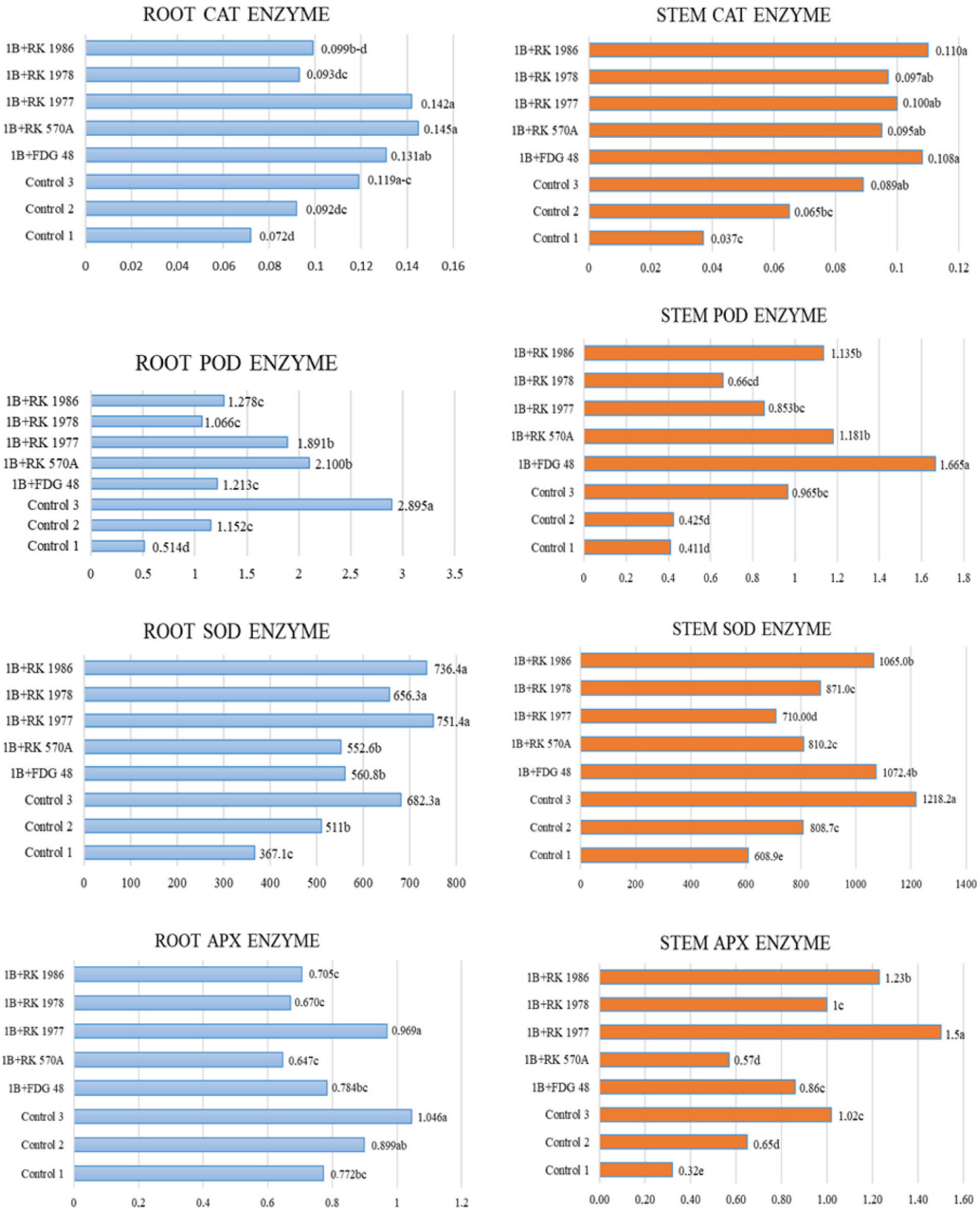

Fig. 1 Antioxidant enzyme activity test results in GF677 rootstock treated with bioagent bacteria against crown gall disease. Asterisk:The difference between the treatments indicated by the same letters in the column is not significant (Duncan multiple comparison test, $p < 0.05$)

gent bacteria strains produce IAA, while HCN was produced by strains FDG 48 (*B. amyloliquefaciens*), RK 570A (*B. cereus*), and RK 1977 (*A. migulanus*). It was determined that no strain could produce SA and siderophore. Thus, it is thought that bioagent bacteria producing IAA and enzymes can suppress tumor formation. Nandhini et al. (2012) reported that strains belonging to the genus *Bacillus* produce high amounts of IAA. The results of this study revealed that the most effective strain, RK 1978 (*B. subtilis*), produced the highest amount of IAA. Hassanein and El-Goorani (1991) used the *B. subtilis* strain for in vitro and in vivo biological control of crown gall and found that the development of the disease was suppressed. Thus, the results of our study are in line with the findings reported in different studies.

Low levels of ROS serve as a signal in development, in response mechanisms to abiotic and biotic stress factors, and in cell death (Miller et al. 2010). Even under optimum conditions, ROS are produced in many metabolic situations. Plants have systems that protect them from the negative effects of oxidative reactions and are effective in scavenging ROS. Antioxidant enzymes, which are part of this system, are a key part of the defense mechanisms (Zhu et al. 2007). Antioxidant enzymes serve as defense against many oxidative stresses in plants (Saed-Moucheshi et al. 2014). This difference in enzyme amounts may depend on whether the bioagent bacterium used activates the defense system weakly or strongly, on the success of in vitro and in vivo treatments, and on the fact that enzyme production in various parts of the plant may not be the same. Researchers reported that there are differences in the amounts of enzymes that play a role in the defense system of plants under stress conditions caused by abiotic and biotic factors (Dashtipour et al. 2017; Shams 2019).

Generally, SOD activity increases in root and stem treatments compared to control treatments. This can be considered a response of the plant to stress. It has been reported that POD activity generally increases in plants under stress conditions. The content of H_2O_2 was not investigated in this study, but it is thought that the increase in POD activity also causes an increase in the internal change of H_2O_2 levels in the cells. It is thought that POD activity increased in tumor tissue due to their role in growth and cell wall formation. Jang et al. (2004) reported increased POD activity and induction of several POD genes after infection with *Pectobacterium chrysanthemi*. Catalase is one of the other antioxidant enzymes responsible for scavenging H_2O_2 resulting from stress in the plant (Antunes et al. 2002), and it plays a key role with other antioxidant enzymes in oxidative stress tolerance and also uses H_2O_2 as a substrate (Apel and Hirt 2004). There are studies showing that there may be increases and decreases in CAT activity under biological stress conditions (Malolepsza and Urbanek 2000; Kuzniak and Sklodowska 2005; Patykowski 2006). In this study, increases in CAT activity were observed compared to the control treatments. Mohammadi (2018) investigated *Clavibacter michiganensis* subsp. *michiganensis* biological control of POD, polyphenol oxidase (PPO), SOD, and CAT enzyme activities and reported that the enzyme amounts increased after the treatments. In this study, APX activity increased significantly in pathogen and pathogen + bioagent treatments compared to the control. Differences were also observed when comparing pathogen-only treatment with pathogen + bioagent treatment. The reason for this may be that there was no need to increase the activity of APX due to the excessive activation of CAT and POD enzymes, which function similarly to APX (Scebba et al. 1999). Furthermore, APX activity may vary depending on the plant species and even the different varieties of the same species, the intensity and duration of stress, the plant development period, the different organs, and the metabolic status of the plant under stress (Karakuş 2016). Rais et al. (2017) determined that the amount of antioxidant enzymes in young leaves was higher than in plant roots. In this study, differences were observed in enzyme amounts and levels between root and stem treatments.

Conclusion

The results of the study showed that strain RK 1978 was the most effective strain against crown gall, which was identified as *B. subtilis*, and differences were observed in the amounts and levels of antioxidant enzymes in the root and stem.

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Data Availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest N. Tekiner Aydın and R. Kotan declare that they have no competing interests.

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