

The Effects of Biofertilizers on Some Physiological Responses in Heritage Raspberries

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ABSTRACT

Raspberry, which belongs to the genus *Rubus* of the Rosacea family, is an important berry fruit with economical potential due to its benefits in terms of human health and wide usage area in industry. In this study, the effects of biological fertilizers (PGPR and mycorrhiza) on some chemical properties of Heritage raspberries were investigated. For this purpose, chlorophyll, anthocyanin, total phenolic content, proline, total carbohydrate levels of plant leaves were evaluated. The highest chlorophyll and anthocyanin contents were obtained from BF5 and BF4 bacterial inoculations (37.24 and 37.85 and 5.58 and 5.14, respectively). The effect of applications on total carbohydrate content was not significant. The highest phenolic and proline contents (2.94 GAE mg g⁻¹ and 0.091 proline g⁻¹) were obtained from BF5 treatment. The results indicated that bacterial applications were the first application in terms of the parameters examined for Heritage raspberry cultivation in the region.

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Biyolojik Gübrelerin Heritage Ahududu Çeşidinde Bazı Fizyolojik Tepkiler Üzerine Etkileri

ÖZET

Rosacea familyasının *Rubus* cinsine ait olan ahududu, hem insan sağlığı açısından faydaları hem de sanayi ve sofralık olarak geniş kullanım alanına sahip olması sebebiyle ekonomik potansiyeli yüksek üzümü meyvelerden birisidir. Bu çalışmada bitki gelişimini olumlu etkileyen biyolojik gübrelerinin (PGPR ve mikoriza) Heritage ahududu çeşidinin bazı kimyasal özellikleri üzerine etkileri incelenmiştir. Bu amaçla bitki yapraklarının klorofil, antosiyanin, toplam fenolik madde, prolin, toplam karbonhidrat miktarları değerlendirilmiştir. En yüksek klorofil ve antosiyanin içerikleri BF5 ve BF4 bakteri formülleri aşlamalarından (sırasıyla 37.24 ve 37.85 ve 5.58 ve 5.14) elde edilmiştir. Toplam karbonhidrat miktarları üzerine uygulamaların etkisi önemsiz bulunmuştur. Toplam fenolik ve prolin içerikleri bakımından en yüksek değerler (2.94 GAE mg g⁻¹ ve 0.091 prolin g⁻¹) BF5 aşlamasından elde edilmiştir. Araştırma sonucuna göre bakteri uygulamalarının bölgemizde Heritage ahududu yetiştiriciliği için incelenen parametreler açısından önce çıkan uygulama olduğu tespit edilmiştir.

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INTRODUCTION

Intensive farming is a system aimed at achieving high product yield and quality. The inputs used in this system are expensive and negatively affect the environment. Chemical fertilizers are one of the most used inputs of this system. These are used to replace the missing elements in the soil such as nitrogen and phosphorus. However, the use of these fertilizers can pollute the environment and force the producer

economically. It has been reported that efficiency of chemical fertilizers was lower than expected. For instance, their effect that replaces missing elements is 50 % or less for N, less than 10 % for P and less than 40 % for K (Baligar et al., 2001). In spite of their harmful effects, the use of inorganic fertilizers seems to increase in order to obtain higher yields per unit area.

Materials such as organic wastes, animal manure,

green manure, biological manure can be used as organic fertilizer in order to develop the physical, biological and chemical characteristics of soils (Dursun et al., 2019). In recent years, there has been a significant increase in the number of studies on the use of some organic materials to reduce the use of inorganic fertilizers and biological fertilizers are the subject of the majority of these studies.

Plant growth-promoting rhizobacteria (PGPR) and mycorrhiza are the most common biological fertilizers known to be beneficial to plant and soil properties. PGPR can fix the atmosphere nitrogen and convert elements into a form that can be taken by plants, and they can also contribute to plant development by synthesizing growth-promoting substances (Garcia-Fraile et al., 2017; Arshad and Frankenberger, 1998). Mycorrhiza, with its hyphae, enlarges the surface areas of the roots, increases the absorption of immobilized elements by reaching the depths of the soil, and supports development by increasing phytohormone production (Miller et al., 2010; Koç et al., 2016). In the previous studies, it was determined that applications of PGPR and mycorrhiza increase the yield and growth in many species (Kutlu et al., 2019; Çiylez and Eşitken 2018; Dursun et al., 2019; Altuntaş et al., 2016; Koç et al., 2016; Arıkan et al., 2018; İpek et al., 2017).

Raspberry (*Rubus Ideabotus* L.), with its unique aroma and wide range of use in the industry, has an important place among the berry fruits worldwide (Anonymous, 2019). Purpose of the study was to determine the effect of the combination of 2 bacterial formulations and mycorrhiza application on some leaf

properties of raspberry plants grown in semi-control greenhouse conditions.

MATERIAL and METHOD

The study was carried out in 2014 in the semi-control greenhouse in Gedikhasanlı Research Station of Bozok University Agricultural Research and Application Center.

Material

Plant material

One-year-old 'Heritage' raspberry variety, which was suggested in the studies carried out in regions with ecologies similar to Yozgat ecology, was used as plant material (Eyduran et al., 2006; Demirsoy et al., 2006; Eroğlu and Gerçekçioğlu, 2006). The shoots of 'Heritage' can grow up to 1.5-2.0 meters with a good fruit size and look (Balci and Keles, 2019).

Bacterial combinations

Bacterial combinations used in the experiment were isolated from wild raspberry root rhizosphere soil in 56 different agroclimatic locations from Rize and Trabzon region. The phosphate dissolving and nitrogen-fixing capacities of the bacteria were determined as reported by Çakmakçı. et al. (2009). Bacterial isolates were diluted to a final concentration of 10⁹ cells ml⁻¹ from distilled water suspensions of fresh cultures grown on Nutrient Broth (NB) medium at 25 ° C for 24 hours. Bacterial strains and some properties of the bacterial combinations used in the experiment are given in Table 1.

Table 1. Bacterial strains and some properties of the bacterial combinations used in the experiment

Çizelge 1. Bakteri izolatları ve çalışmada kullanılan bakteri kombinasyonlarının bazı özellikleri

Bio-formulations	Bacterial strains	N-free media development	NBRIP-BPB media Development	Catalase	Amylase	Sucrose	Oxidase
BF4	<i>Pseudomonas putida</i> 625	S+	+	+	-	+	+
	<i>Bacillus atropheus</i> RIA1	-	+	+	+	-	-
	<i>Rhodococcus erythropolis</i> RHA3	S+	S+	+	-	-	-
BF5	<i>Pseudomonas fluorescens</i> 583	+	W+	+	-	+	S+
	<i>Pseudomonas putida</i> R2B1	S+	+	S+	-	+	+
	<i>Bacillus pumilus</i> R4A1	S+	S+	+	-	-	-
	<i>Bacillus licheniformis</i> R7B1	S+	+	+	-	-	-

S+ = Strong positive; W+ = weak positive; + = positive; - = negative

Mycorrhizal fungi

Mycorrhiza containing 23% mycorrhiza and 9 different glomus fungi (*Glomus intraradices* 21 %, *Glomus aggregatum* 20 %, *Glomus mosseage* 20 %, *Glomus clarum* 1 %, *Glomus monosporus* 1 %, *Glomus deserticola* 1 %, *Glomus brasilianum* 1 %, *Glomus etunicatum* 1 % ve *Gigaspora margarita* 1 %) was used as preparation.

Glomus clarum 1 %, *Glomus monosporus* 1 %, *Glomus deserticola* 1 %, *Glomus brasilianum* 1 %, *Glomus etunicatum* 1 % ve *Gigaspora margarita* 1 %) was used as preparation.

Method

The experiment was established as a randomized complete plot design with four applications (BF4, BF5, mycorrhiza, and control) which contain 4 replications with 4 plants each. Seedlings were planted on 18.02.2014 in pots with a volume of 10 liters containing mixture of perlite: peat (1:3).

At the beginning of the vegetative growth period (03.05.2014), each pot was irrigated with ½ liter bacteria (prepared to be 10⁹ cell ml⁻¹) and mycorrhiza (prepared by mixing 250 g of powder in 10 liters of water) solutions. At the end of the experiment some physiological response of the leaves was evaluated.

Relative chlorophyll (SPAD) value was measured with a Minolta SPAD-502 chlorophyll meter (Minolta Camera Co, Ltd, Osaka, Japan) and relative anthocyanin content of the leaves was determined with an anthocyanin content meter (ACM-200 plus). For each plant measurements were taken at four locations on each leaf by taking two on each side of the mid rib of fully expanded leaves (Khan et al., 2003).

Total phenols were determined using Folin-Ciocalteu reagent (Singleton and Rossi, 1965). Each leaf sample (0.5 mg) was treated with methanol and then mixed with Folin-Ciocalteu reagent (0.5 mL, diluted with 8 mL distilled water). After 1 h, the level of total phenols was determined by spectrophotometrically at a absorbance of 765 nm. Total phenol values were expressed in terms of mg gallic acid.

Table 2. The effect of PGPR and mycorrhiza applications on the chlorophyll and anthocyanin contents

Çizelge 2. PGPR ve mikoriza uygulamalarının klorofil ve antosiyanin içeriklerine etkisi

	<i>BF4</i>	<i>BF5</i>	<i>Mycorrhiza</i>	<i>Kontrol</i>
<i>Chlorophyll (SPAD)</i>	37.24 a	37.85 a	33.18 b	29.22 c
<i>Anthocyanin (ACI)</i>	5.14 a	5.58 a	4.24 b	3.67 b

Values within the same letter are not significantly different at $P < 0.05$ by Duncan

Significant differences were found between the applications in terms of chlorophyll amounts. While the highest chlorophyll amount was obtained from BF4 and BF5 bacterial combinations within the same statistical group, overall, the application with the lowest amount of chlorophyll was the control. It was found that bacterial applications had a more positive effect on chlorophyll content which is one of the most important factors determining photosynthesis capacity and these results were in agreement with many similar studies (Aydın et al., 2012; Güllüce et al., 2012; Heidari et al., 2011). SPAD value reflects chlorophyll content decreases under stress conditions (Aras and Eşitken, 2019b; Aras and Keles, 2019). Akay and Kararaslan (2012) reported that mycorrhiza applications, especially in poor soils, contribute significantly to chlorophyll content in plants. In the present study, it was determined that also the application of mycorrhiza had a positive effect on the

The proline content was estimated by the method of Bates et al. (1973). The plant material was homogenized in 3% aqueous sulfosalicylic acid and the homogenate was centrifuged at 10.000 rpm. The supernatant was used for estimation of the proline content. The reaction mixture consisted of 2ml supernatant, 2ml acid ninhydrin and 2ml of glacial acetic acid, which was boiled at 100 °C for 1h. After termination of the reaction in ice bath, the reaction mixture was extracted with 4ml of toluene and the absorbance was read at 520 nm.

Total carbohydrate of the leaves was determined according to Ebell (1970) with some modifications. 1 g leaf samples were extracted with 25 ml 72% sulfuric acid and 2 ml of anthron were added to the extract. Finally, the samples were heated at 95°C for 10 min. Sample tubes were transferred into an ice bath and cooled to room temperature. Absorption of the extracts was recorded at 540 nm.

Statistical analysis

All results were analyzed by SPSS 20.0 and the differences between the means were compared using the Duncan test ($p < 0.05\%$).

RESULTS and DISCUSSION

Chlorophyll and Anthocyanin Content

The effects of bacterial combinations and mycorrhiza applications on the anthocyanin and chlorophyll contents of the plants are given in Table 2.

chlorophyll content compared to the control group. When the amount of anthocyanin in the leaves was examined, it was seen that the applications were divided into two different statistical groups. The highest anthocyanin values were obtained from BF5 and BF4 bacterial applications (5.58 and 5.14) as well as chlorophyll contents. Plants accumulate anthocyanins for capturing more light (Aras and Eşitken, 2019a). In accordance with Rodriguez et al. (2014), it has been found that PGPR applications have positive effects on the amount of anthocyanin in the leaves. And the lowest results were obtained from the mycorrhiza and control applications (4.24 and 3.67) which were in the same statistical group. Baslam and Goicoechea (2012) reported that mycorrhiza applications in lettuce exposed to drought stress increase anthocyanin content in leaves. Similarly, in the study, it was determined that mycorrhiza application increased the content of

anthocyanin compared to the control group however, the results were not found to be statistically significant. It has been reported that anthocyanins contribute to the preservation of chlorophyll structure (Farrant, 2000; Johnston et al., 2007). Also in the present study, the changes in the chlorophyll content were similar to anthocyanin. These results can be considered as an indicator of a positive relationship between the two features.

Total phenolic, total carbohydrate and proline content

The effect of applications on total phenolic (TP) and proline (PR) content was found significant. When plants are exposed to environmental stress factors, they tend to survive by synthesizing some chemicals (Koç et al., 2016). Phenolic compounds are important in regulating plant growth, development and interaction with other organisms and they also behave as a defense in stress conditions and help plants to eliminate stress factors (Harborne, 1980; Parida et al., 2004; Aras et al., 2019). Similarly, proline is synthesized by plants and contributes to the balancing of osmotic pressure in cells and to plant growth (Edreva, 1998). In the present study, the highest TP value was determined from the BF5 application (2.94 mg g⁻¹ GAE) while the lowest TP

content was identified from mycorrhiza (1.46 mg g⁻¹ GAE). When the amount of proline was examined, the highest result was obtained from the application of BF5 (0.091 proline g⁻¹) (Table 3). It is known that reserves, especially carbohydrates, play important roles in all plants (Loescher et al., 1990). Changes in the amount of carbohydrates in the plant are directly related to physiological processes such as photosynthesis, translocation, and respiration (Kameli and Lösel, 1993). Although the effects of the applications were found to have a positive effect on carbohydrate accumulation compared to the control group, the results were not found to be statistically significant. In terms of carbohydrate accumulation, the BF4 application had the highest value (34.28 % g anthrone), while the lowest value (30.85 % g anthrone) was observed in the control (Table 3). Some studies revealed that mycorrhiza applications possess positive effects on plants (Rouphael et al., 2010; Naghashzadeh, 2014). In the current experiment we found that PGPR applications had beneficial influences on raspberry, while mycorrhiza couldn't exhibit promoting effects on raspberry. That may be due to unavailable soil conditions in the experimental area (not determined for mycorrhiza survival in our study).

Table 3. Effects of PGPR and mycorrhiza applications on some biochemical properties of Heritage raspberry cultivar

Çizelge 3. Heritage ahududu çeşidinin bazı biyokimyasal özellikleri üzerine PGPR ve mikoriza uygulamalarının etkisi

	<i>BF4</i>	<i>BF5</i>	<i>Mycorrhiza</i>	<i>Control</i>
<i>Total phenolics (GAE mg g⁻¹)</i>	2.40 b	2.94 a	1.46 c	2.44 b
<i>Total carbohydrate (% g anthron)</i>	34.28 ^{NS}	33.94	33.17	30.85
<i>Proline (prolin g⁻¹)</i>	0.080 ab	0.091 a	0.034 b	0.066 b

Values within by the same letter are not significantly different at $P < 0.05$ by Duncan, NS: non-significant

CONCLUSION

The results of the research indicated that the application of BF5 bacteria formulations was the best in terms of all the properties examined. While BF4 inoculations showed a similar result with the control group in terms of effect on total phenolic, it was the best application after BF5 inoculations in all other parameters. It was determined that application of mycorrhiza did not make a significant difference compared to the control. The characteristics examined in this study may contribute to plant growth and increase plant tolerance under stress conditions and we determined that BF4 and BF5 inoculations may be useful for these purposes in Haritege raspberry plants.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

Author's Contributions

The contribution of the authors is equal.

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