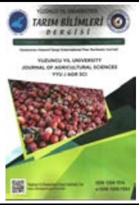
Yüzüncü Yıl Üniversitesi
Tarım Bilimleri Dergisi
(YYU Journal of Agricultural Science)

http://dergipark.gov.tr/yyutbd



Araştırma Makalesi (Research Article)

Effect Of 24- Epi Brassinolide on Some BiochemicalCharacteristics of Parus and Gaviota Strawberry Cultivars Under Heat Stress Conditions

Omid ASADI AGHDAM[1*], Jafar HAJILOU[2], Sahebali BOLANDNAZA[3], Gholamreza DEHGHAN[4]

[1]University of Tabriz, Faculty of Agriculture, Department of Horticulture, Tabriz, Iran
[4]University of Tabriz, Faculty of Natural Science, Department of Biology, Tabriz, Iran
[1]https://orcid.org/0000-0003-3430-0450 [2]https://orcid.org/0000-0001-6197-2231 [3]https://orcid.org/0000-0001-9396-7373
[4]https://orcid.org/0000-0002-7813-5226
*Corresponding author e-mail:omid.dr1365@gmail.com

Article Info

Received: 13.07.2019
Accepted: 05.05.2020
Online Published 30.06.2020
DOI:10.29133/yyutbd.590491

Keywords

Anthocyanin,
Dismutase,
Flavonoid,
Phenolics,
Superoxide.

Abstract: The study was arranged as factorial experiment in a completely randomized design with three applications of cultivar (Parus and Gaviota), 24-Epi brassinolide concentrations (0, 1 and 2 mgl^{-1}) and temperature (normal and stress) as three replications. Some of the measuring characteristics showed that there was a significant difference among the treatments. Under heat stress conditions, foliar application of 24- Epi brassinolide at 1 mgl^{-1} concentration increased the amount of catalase and superoxide dismutase activity in leaves of Parus cultivar. Both cultivars fruits showed the highest total soluble solid contents in treatment 24- Epi brassinolide spraying at 2 mgl^{-1} concentrations under normal temperature. The highest total phenolics was in Gaviota cultivar treated with 24-Epi brassinolide spraying at 2 mgl^{-1} concentrations under normal temperature. Both cultivars had the lowest flavonoid in treatments without 24- Epi brassinolide and with 24- Epi brassinolide at 2 mgl^{-1} concentrations under heat stress conditions. In Gaviota cultivar, application of high concentration of 24- Epi brassinolide (2 mgl^{-1}) prevented the reduction of total anthocyanin under heat stress conditions.

24-Epi Brassinolidin, Sıcaklık Stresi Koşullarında Parus ve Gaviota Çilek Çeşitlerinin Bazı Biyokimyasal Özellikleri Üzerine Etkisi

Makale Bilgileri

Geliş: 13.07.2019
Kabul: 05.05.2020
Online Yayınlanma 30.06.2020
DOI:10.29133/yyutbd.590491

Anahtar kelimeler

Antosiyanin,
Dismutaz,
Flavonoid,
Fenolikler,
Süperoksit.

Öz: Çalışma, çeşit (Parus ve Gaviota), 24-Epi brassinolid konsantrasyonları (0, 1 ve 2 mg/l) ve sıcaklık (normal ve stres) olmak üzere üç uygulama 3 tekerrürlü olarak tesadüf parselleri faktöriyel deneme deseninde düzenlenmiştir. Ölçüm özelliklerinden bazıları, uygulamalar arasında önemli bir fark olduğunu göstermiştir. Sıcaklık stresi koşulları altında, 24 mg-Epi brassinolidin 1 mg/l konsantrasyonunda yapraktan uygulanması, Parus çeşidinin yapraklarındaki katalaz ve süperoksit dismutaz aktivitesini arttırmıştır. Her iki çeşidin meyveleri, normal sıcaklık altında 2 mg/l konsantrasyonunda 24-Epi brassinolid uygulamasında en yüksek suda çözünür kuru madde içeriğine sahip olmuştur. En yüksek toplam fenolikler, normal sıcaklık altında 2 mg/l konsantrasyonunda 24-Epi brassinolid uygulanan Gaviota çeşidinde tespit edilmiştir. Her iki çeşit, 24-Epi brassinolid içermeyen ve 24-Epi brassinolid içeren uygulamalarda, sıcaklık stresi koşulları altında 2 mg/l konsantrasyonunda en düşük flavonoid içeriğine sahip olmuştur. Gaviota çeşidinde, yüksek konsantrasyonda 24-Epi brassinolid (2 mg/l) uygulanması, sıcaklık stresi koşulları altında toplam antosiyanin miktarının azalmasını önlemiştir.

1. Introduction

Strawberry fruit has a great flavor and rich in vitamins which has a special place in the diet of people all over the world. The strawberry is adapted to different growth of plants. Under high temperatures, in plants sexual reproduction is more sensitive than environmental conditions and is cultivated in most climatic zones.The heat stress in plants is the rise in temperature above the threshold level for a period that causes irreversible damage the vegetative processes and therefore reproductive organs of the plants will be vulnerable to short-term exposure under high temperatures at the initial stage of flowering(Reddy and Kakani, 2007). Brassinosteroids are a group of plant hormones that regulate plant growth and development.24- Epi brassinolideis one of the most important forms of brassinosteroids. They control the growth of plants from seed germination to senescence (Kaplan and Gokbayrak., 2012). Some of the components of brassinosteroid signaling pathway act as multifunctional proteins involved in other signaling networks regulating diverse physiological processes, such as photomorphogenesis, cell death control, stomatal development, flowering, plant immunity to pathogens and metabolic responses to stress conditions (Gruszka, 2013). In gossypium plant with brassinosteroids application, some of the vegetative growth characteristics such as shoot length, fresh weight, dry weight and leaf area showed high values (Johnson and Lingakumar, 2011).The use of brassinosteroid was effective in stimulating the tomato fruit ripening, increasing soluble sugars, ascorbic acid, lycopene contents, respiration rate and ethylene production (Zhu et al., 2015).In 'Tak Danehe Mashhad' plant, the fruits treated with brassinosteroid increased the color of the fruit by increasing the amount of anthocyanin, organic acids, ascorbic acid, and phenol content(Roghabadi and Pakkish, 2014). Antioxidant enzymes play the first line of defense against oxidative stress in plants. This has a significant impact on the concentration of O_2 and H_2O_2in plants(Valizadeh et al., 2013). In the *Leymus chinensis* plant, spraying of brassinolide at swollen bud stage led to increase of antioxidant enzymes system in plants under high temperature (Niu et al., 2016). The use of 24- Epi brassinolide prevents the destruction of proteins and helps to increase cell membrane stability at high temperatures (Yadava et al.,2016). The present study aimed toselect optimal concentrations of 24- Epi brassinolide to improve some biochemical characteristics in strawberry cultivars under heat stress conditions.

2. Materials and Methods

2.1. Plant Material and Experimental Design

Seedlings of strawberry (Parus and Gaviota) from The Royal Green Agricultural Company were prepared and cultivated in 10 l pots filled with perlite and cocopeat in a smart greenhouse located in the Aras Greenhouse Town in Jolfa City. Plants were fertigated by Hoagland'ssolution. At flowering stage plants were sprayed by 24- Epi brassinolide and plants were subjected to thermal stress (42 °C for 3 hours) 24 hours after 24- Epi brassinolide spraying (In an isolated environment condition and by heater has a hot air tunnel connected to the smart climate control system). The experiment was arranged as a factorial in a completely randomized design with three factors, cultivar (Parus and Gaviota), 24- Epibrassinolide concentrations(0, 1 and 2 mgl^{-1}) and temperature (normal and stress), as three replications.

2.2. Superoxide dismutase, peroxidase and catalase enzymes activityevaluation

The activity of the superoxide dismutase enzyme of leaf and fruit was evaluated by measuring its ability to control the photochemical reduction of nitro-blue tetrazolium (Beauchamp and Fridovich, 1971). The activity of theperoxidasewas performed according to (Change and Maehly, 1955). The activity of thecatalasewas performed according to (Aebi, 1984).

2.3. Titratable acidity and total soluble solids content evaluation

To measure titratable acidity, 6ml of fruit juice from each sample were mixed with 50 ml of water and used for titrating with 0.1 N NaOH to an end point of pH 8.2. The total volume of NaOH is measured and used to calculate the titratable acidity (Suarez et al., 2010). Total soluble solids content (TSS) were measured by a digital refractometer and expressed as °Brix (Adak, et al., 2018).

2.4. Total phenolics, flavonoid, antioxidant and anthocyanin evaluation

Evaluation of phenolic content was done according to Upadhyay and Maier (2016). First, 0.2 g of fruit tissues was immersed in 2 ml of acidic methanol solution for 2 hours. After extraction, centrifugation was performed at 1000 × g for 15 minutes.Supernatants (100 μl) were incubated for 5 minutes after mixing with Folin-Ciocalteu at 22˚C. Then, the reaction mixture was kept at 22 ° C for 90 minutes by adding sodium bicarbonate (0.1 M, 0.75 ml) to the reaction mixture. Absorbance values were measured in the range of 725 nm and total phenol content was calculated by p-coumaric acid standard curves and it was expressed as $\mu g \cdot g^{-1}$ fresh weight.Flavonoids were extracted from fruit tissue (0.5 g) overnight in ethanol. Flavone and flavonol levels were determined by AlCl3 colorimetric method using quercetin standard in the absorbance range of 415 nm and flavanones were determined by colorimetric method with 2,4-diphenylhydrazine using naringenin in the absorbance range of 495 nm. The sum of naringenin and quercetin equivalents was estimated as flavonoid content and it was expressedas $\mu g \cdot g^{-1}$ fresh weight (Upadhyay and Maier, 2016). Measurement of antioxidant content by DPPH (2.2-diphenyl-1-picrylhydrazyl) radical inactivation method was performed with antioxidant compounds in fruit extract in the absorbance range of 515 nm.First, 100 μM DPPH was dissolved in 80% methanol and then the fruit extract was added. After 30 minutes, the resulting solution was analyzed in the absorbance range of 515 nm. Total antioxidant activity was expressedasμM100 g–1 fresh weight (Rodrigues et al., 2011).To evaluate the anthocyanin content, 0.2 g of fruit tissue was selected and its anthocyanin was extracted using 3 ml of 1% acidic methanol overnight.After 24 hours, the formed phase was separated using chloroform (3 mL) and water (2 mL).The aqueous phase absorption was determined in the absorption bands of 530 and 657 nm.Anthocyanin content was calculated using the formula $\lambda 530 - \lambda$ 657 and expressed per g^{-1} fresh weight (Laxmi et al., 2004).

2.5. Statistical analysis

The data were analyzed using ANOVA analysis. The difference among treatment means were compared by Duncan’s test at 0.05 significant level. SPSS version 16.0 software is used for all statically analysis.

3. Results

3.1. Superoxide dismutase, peroxidase and catalase enzymes activityof leaves

Results indicated that there was significant difference in antioxidant enzymes activity (superoxide dismutase, peroxidase and catalase) in leaves between Parus and Gaviota cultivars. 24-Epi brassinolide application (at 2 and 1 mgl^{-1} concentrations) in normal temperature significantly increased the amount of antioxidant enzymes activity (superoxide dismutase, peroxidase and catalase) in leaves of Parus cultivar. Under heat stress conditions, foliar application of 24- Epi brassinolide at 1 mgl^{-1} concentration increased the amount of catalase and superoxide dismutase activityin leaves of Parus cultivar. In Gaviota cultivar under both of stress and normal temperatures, 24- Epi brassinolide had no significant effect on leaves antioxidant enzymes activity compared to control. (Figure 1- a, b and c).

3.2. Superoxide dismutase, peroxidase and catalase enzymes activityof fruits

Results indicated that there was significant difference in antioxidant enzymes activity (superoxide dismutase, peroxidase and catalase) in fruits between Parus and Gaviota cultivars. Theapplication of 24- Epi brassinolide at 2 mgl^{-1} concentrations in normal temperature significantly increased the amount of antioxidant enzymes activity (superoxide dismutase, peroxidase and catalase) in fruits of Gaviota cultivar. In Parus cultivar, application of 24- Epi brassinolide at 1 mgl^{-}

^{1}concentrationin normal temperature significantly increased the amount of catalaseactivity of fruits. In both of Gaviota and Parus cultivars, foliar application of 24- Epi brassinolideunder heat stress conditionshad no significant effect on leaves antioxidant enzymes activity compared to control. (Figure 1- d, f and e).

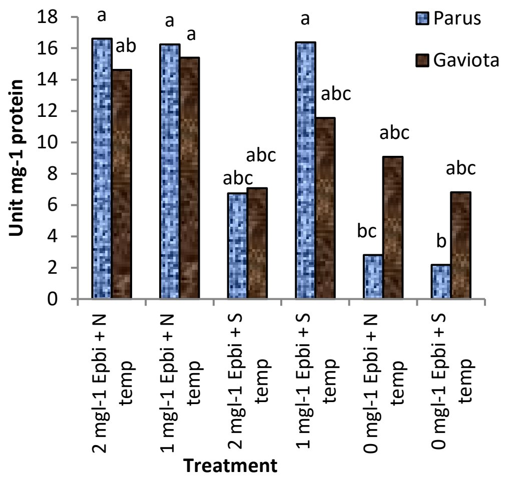
3.3. Titratable acidity and total soluble solids content

Results of data variance analysis indicate that significant differences had among treatments in titratable acidity and total soluble solid contents in fruits of Parus and Gaviota cultivars. In both of Gaviota and Parus cultivars heat stress result to reduce of fruit titratable acidity. Use of 24- Epi brassinolide had no significant effect on fruit titratable acidity compared to control. Both cultivars fruits was showed the highest total soluble solid contents in treatment of 24- Epi brassinolide spraying at 2 mgl^{-1} concentration under normal temperaturefollow by was observed in Parus cultivar in 24- Epi brassinolide sprayed at 1 mgl^{-1} concentrationunder normal temperature and at 2 mgl^{-1} concentration of 24- Epi brassinolide under heat stress condition.Control treatment was showed the lowest total soluble solid contents were observed in Parus cultivar (Table 1).

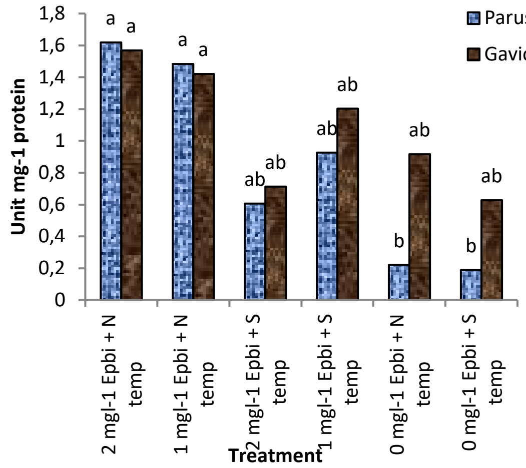
Table 1. Effect of 24- Epi brassinolide on some characteristics of Parus and Gaviota strawberry cultivars under heat stress condition

treatment	TSS (^{0}Brix)		Titratable Acidity (%)		Total Phenolics (µg·g^{-1})	
	Parus	Gaviota	Parus	Gaviota	Parus	Gaviota
2 mgl^{-1} 24- Epi brassinolide + Normal temperature	4.2^{a}	4.5^{a}	0.159^{a}	0.153^{abc}	426.5^{cd}	909.8^{a}
1 mgl^{-1} 24- Epi brassinolide + Normal temperature	3.7^{ab}	4^{a}	0.148^{abcd}	0.150^{abc}	416.5^{cd}	576.8^{bcd}
2 mgl^{-1} 24- Epi brassinolide + Stress temperature	3.8^{ab}	4.1^{a}	0.139^{bcd}	0.138^{abcd}	484.5^{bcd}	554.5^{bcd}
1 mgl^{-1} 24- Epi brassinolide + Stress temperature	3.4^{ab}	3.5^{ab}	0.135^{d}	0.139^{bcd}	365.2^{d}	595.8^{bc}
0 mgl^{-1}24- Epi brassinolide + Normal temperature	2.5^{b}	3.5^{ab}	0.155^{ab}	0.152^{abc}	470.8^{bcd}	645.2^{b}
0 mgl^{-1}24- Epi brassinolide + Stress temperature	3.4^{ab}	3.4^{ab}	0.134^{d}	0.134^{d}	419.2^{cd}	634.2^{bc}

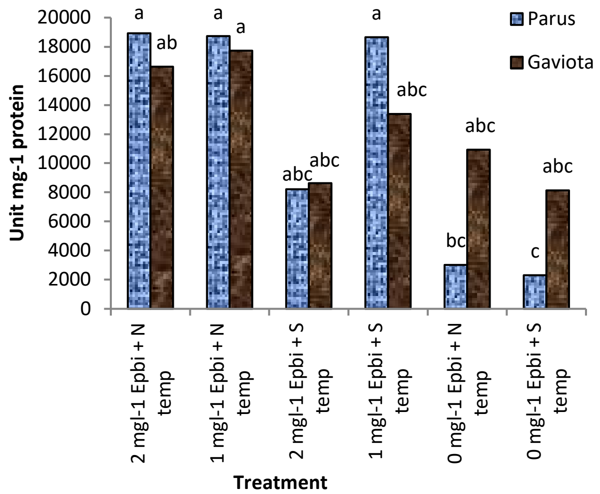
Treatments with similar letters based on Duncan test in the level of five percent have no significant difference.



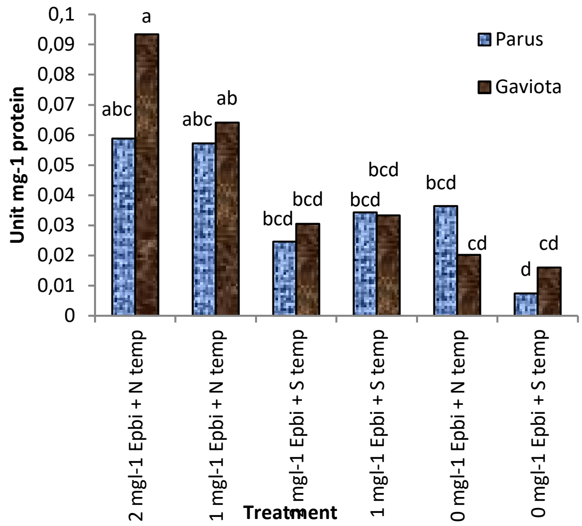
a:Superoxide dismutase activity of strawberry



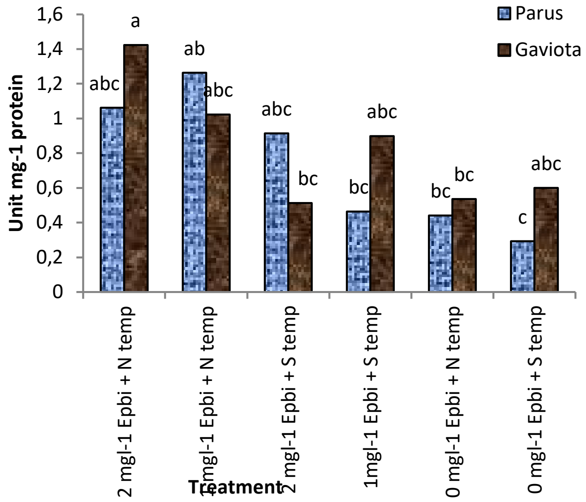
b:Peroxidase activity of strawberry leaves



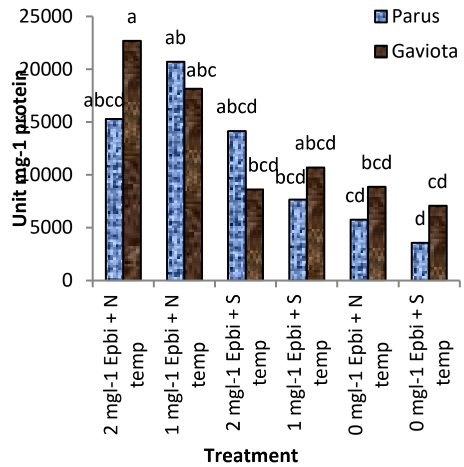
c:Catalase activity of strawberry leaves



d: Superoxide dismutase activity of strawberry



f:Peroxidase activity of strawberry fruits.



e: Catalase activity of strawberry fruits.

Figure 1. Effect of 24- Epi brassinolide on superoxide dismutase, peroxidase andcatalase activity of leaves and fruits of Paros and Gaviota strawberry cultivars under heat stress condition.

Treatments with similar letters based on Duncan test in the level of five percent have no significant difference.

3.4. Total phenolics, flavonoid, antioxidant and anthocyanin

The highesttotal phenolicswas observed in Gaviota cultivar by treatment of 2 mgl^{-1} 24- Epi brassinolide in normal temperature (Table 1). The highest flavonoid was observed in case of sprayed with 24- Epi brassinolide under normal temperature (in Parus and Gaviota cultivars respectively at 2 and 1 mgl^{-1} concentrations). Both of cultivars were showed the lowestflavonoid as no application of 24- Epi brassinolide. The highestantioxidant was in Parus cultivar in treatments of 24- Epi brassinolide spraying at 1 mgl^{-1} concentration under normal temperature and the lowestantioxidant was observed in treatments of 24- Epi brassinolide spraying at 1 mgl^{-1} concentration under heat stress conditions. In Gaviota cultivar, application of high concentration of 24- Epi brassinolide (2 mgl^{-1}) prevented the reduction of total anthocyanin under heat stress conditions. While, the lowesttotal anthocyanin in both cultivars fruits was observed with no application of 24- Epi brassinolide solution under heat stress conditions (Table 2).

Table 2. Effect of 24- Epi brassinolide on some characteristics of Parus and Gaviota strawberry cultivars under heat stress condition

treatment	Flavonoid (μg·g^{-1})		Antioxidant Content (μMol.100 g–1)		Total Anthocyanin (λ530 – λ 657 g^{-1})	
	Parus	Gaviota	Parus	Gaviota	Parus	Gaviota
2 mgl^{-1} 24- Epi brassinolide + Normal temperature	17.9^{a}	16.3^{ab}	96.13^{ab}	95.89^{ab}	10.35^{abc}	13.24^{a}
1 mgl^{-1} 24- Epi brassinolide + Normal temperature	12.3^{bcde}	18.8^{a}	96.19^{a}	95.72^{ab}	11.46^{ab}	12.35^{ab}
2 mgl^{-1} 24- Epi brassinolide + Stress temperature	9.7^{de}	8.4^{e}	96.08^{ab}	96.15^{ab}	7.5^{bc}	11.46^{ab}
1 mgl^{-1} 24- Epi brassinolide + Stress temperature	10.8^{cde}	12.1^{bcde}	96.08^{ab}	94.86^{b}	8.34^{abc}	7.12^{bc}
0 mgl^{-1}24- Epi brassinolide + Normal temperature	15.2^{abc}	14.5^{abcd}	95.87^{ab}	95.44^{ab}	11.8^{ab}	8.68^{abc}
0 mgl^{-1}24- Epi brassinolide + Stress temperature	9.1^{de}	9.6^{de}	96.02^{ab}	95.25^{ab}	5.56^{c}	5.45^{c}

4. Discussion and conclusion

Under heat stress conditions, increase of antioxidant enzyme activity as a defense mechanism is essential to reduce the negative effects of stress in plants. This can be achieved through synthesis of some plant growth regulators such as brassinosteroids within plant or sprayed. The results of present study clearly demonstrated that 24- Epi brassinolide spraying had a significant effect on increasing of antioxidant enzymes activity (superoxide dismutase, peroxidase and catalase) in Parus and Gaviota cultivars and reducing of heat stress negative effects. Our results were consistent with other studies reporting the increased antioxidant enzymes activity in response to heat stress in sunflower (Gunes et al., 2008), pea (Maecka et al., 2001) and brassica (Uprety, 2006). In general it is thought that the heat damage caused to plants is due to the excessive production of active oxygen species, reduction of antioxidant enzyme activities and membrane damage.Reactive oxygen species are destroyed by antioxidant reported an elevated production of antioxidant enzymes by the application of 24- Epi brassinolide under high temperature stress.Therefore, peroxidase has often served as a parameter of metabolism activity during growth alterations and environmental stress conditions(Gao et al., 2008). An increase in peroxidase activity under several stress conditions is associated with protection from oxidative damage (Mirzaee et al., 2013). 24- Epi brassinolide reduces the amount of respiratory activity and enzymes involved in it (Roghabadi et al., 2014). Sugars are the main ingredients ofthe total soluble solids content and There is a high correlation between the total soluble solids content and the sugars (Klunklin and Savage, 2017). Sugar is used in plant building compounds and provides the energy required for biochemical processes. Reducing photosynthetic activity under stress conditions reduces levels of sugar content in the plant.The use of 24- Epibrassinolide in conditions of stress leads to a rise in the levels of sugar in the plant(Swamy et al., 2014). Application of brassinolides in plants increases the levels of biochemical reactions associatedwith the conversion of glucose from the kelvin cycle to move towards developing fruits and subsequently increase the soluble and in soluble sugars in the fruits (Ramani, 2015).Titratable acidity is concentration of organic acids in fruit which is one of the important parameters for assessing the quality of fruits (Akhtar et al., 2010).Under high temperatures, the titratable acidity of fruits decreased significantly in both cultivars.At high temperatures, organic acids are used as substrates in the respiratory process, which reduces the titratable acidity under thermal stress conditions (Lotfi et al., 2016).In the present study, heat stress did not lead to a significant reduction in the phenolic compounds of Gaviota and Parus cultivars compared to the control treatment, which was probably due to the high stimulation of the phenylpropanoid pathway under stresscondition.Ayub et al. (2018) reported the phenylpropanoid pathway and the accumulation of phenolic compounds can be stimulated as a physiological response to a stress condition. The results of present study weresimilar tothe results of studies on grape berry (Xi et al.,2013).Plants that were treated with 24- Epi brassinolideunder heat stress condition accumulated more phenolic compounds.24- Epi brassinolide treatment helps to increase the activity of the phenylalanine ammonia-lyase enzyme, which is the enzyme necessary to start the synthesis of free phenols (Champa et al., 2015).The use of 24- Epi brassinolide solution in Parus cultivar stimulated the synthesis of antioxidant compounds. 24- Epi brassinolide has antioxidant properties and it can be used to increase the activity of enzymes and antioxidant compounds (Gomes et al., 2013). In the present study, in fruits that were affected by intense heat stresses, the number of antioxidant compounds, including flavonoids, was extracted from other fruits. Stress results in the accumulation of antioxidant compounds in vacuoles tissues of plants that can be extracted during fruit maturity.In other words, Stress increases antioxidant compounds, including flavonoids. Because most antioxidant compounds are present in the skin, flavonoids include a large group of lower molecular weight metabolites in plants that have a significant effect on biological processes, such as pigmentation of flowers. Stress produces not only cell-damaging oxidants but also allows the accumulation of a large number of flavonoids and phenolic acids in the fruits. Temperature is one of the environmental factors affecting the accumulation of anthocyanins in flowers and fruits (Dela et al., 2003). Studies have shown that high temperatures in different plants such as apple and rose reduce the anthocyanin pigmentation. In the present study, the use of 24- Epi brassinolide solution under heat stress condition increased the amount of anthocyanin synthesis in both cultivars, which seems to be due to the increased activity of phenylpropanoid in these two cultivars under heat stress condition. In strawberry, brassinosteroid application increases the expression of transcription factors and positively affects the amount of anthocyanin synthesis through interference in the phenylpropanoid pathway(Ayub et al., 2018).

References

Adak, N., Gubbuk, H., & Tetik, N. (2018). Yield, quality and biochemicalproperties of various strawberry cultivars under water stress. *Journal Of The Sci.Of Food And Agri.*,98, 304-311.

Akhtar, A., Abbasi, N. A., & Hussain, A. (2010). Effect of calcium chloride treatments on quality characteristics of loquat fruit during storage. *Pakistan* Journal Of *Botany*, 42, 181-188.

Aebi, H. (1984). Catalase in vitro. *Methods in Enzymology*, 105, 121-126.

Ayub, R. A., Reis, L., Lopes, P. Z., & Bosetto, L. (2018). Ethylene and brassinosteroid effect on strawberry ripening after field spray. *Revista Brasileira deFruticultura*, 40, 1-6.

Beauchamp, C., & Fridovich, I. (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry*, 44, 276-287.

Champa, W. H., Gill, M., Mahajan, B., Aror, N., & Bedi, S. (2015). Brassinosteroids improve quality of table grapes (*Vitis vinifera* L.) cv. flame seedless. *Tropical Agricultural Resear.*, 26, 26-31.

Change, B., & Maehly, A. (1955). Assay of catalases and peroxidase. *Methods Enzymol*, 2, 764-775 .

Dela, G., Or, E., Ovadia, R., Nissim-Levi, A., Weiss, D., & Oren-Shamir, M. (2003). Changes in anthocyanin concentration and composition in ‘Jaguar’rose flowers due to transient high-temperature conditions. *Plant Science*, 164, 333-340 .

Gao, S., Ouyang, C., Wang, S., Xu, Y., Tang, L., & Chen, F. (2008). Effects of salt stress on growth, antioxidant enzyme and phenylalanine ammonia-lyase activities in *Jatropha curcas* L. seedlings. *Plant Soil Environ*, 54, 374-381.

Gomes M, Torres A, Campostrini E, Bressan-Smith R, Zullo M, Ferraz M & Nunez M (2013). Brassinosteroid analogue affects the senescence in two papaya genotypessubmitted to drought stress. *Theoretical and Experimental Plant Physiology*, 25, 186-195 .

Gunes, A., Pilbeam, D. J., Inal, A., & Coban, S. (2008). Influence of silicon on sunflower cultivars under drought stress, I: Growth, antioxidant mechanisms, and lipid peroxidation. *Communications in Soil Science and Plant Analysis*, 39, 1885-1903.

Gruszka, D. (2013). The brassinosteroid signaling pathway new key players and interconnections with other signaling networks crucial for plant development and stress tolerance. *Molecular Science*, 14, 8740-8774 .

Hayat, S., & Ahmad, A.(2010). Brassinosteroids: a class of plant hormone. *Springer Science and Business Media*, 18-25.

Johnson, M., & Lingakumar, K. (2011). Effect of crude brassinosteroid extract on growth and biochemical changes of *Gosssypium hirsutum* L. and *Vigna mungo* L. Stress Physiology and Biochemistry, 7, 324-334.

Kaplan, U., & Gokbayrak, Z. (2012). Effect of 22 (S), 23 (S)-Homo brassinolide on adventitious root formation ingrape rootstocks. *South African Journal for Enology and Viticulture*, 33, 253 .

Klunklin, W., & Savage, G. (2017). Effect on quality characteristics of tomatoes grown under well-watered and drought stress conditions. *Foods*: 6(8), 56.

Kumar, S., Sirhindi, G., Bhardwaj, R., Kumar, M., & Arora, P. (2012). Role of 24- Epi brassinolide in amelioration of high temperature stress through antioxidant defense system in *Brassica juncea* L. *Plant Stress*, 6, 55-58 .

Laxmi, A., Paul, L. K., Peters, J. L., & Khurana, J. P. (2004). Arabidopsis constitutive photomorphogenic mutant, bls1, displays altered brassinosteroid response and sugar sensitivity. *Plant Molecular Biology*, 56, 185-201

Lotfi, H., Barzegar, T., & Ghahremani Z. (2016). Assessment of growth, yield and fruit quality of two iranian cantaloupe accessions under different irrigation levels. *Sustainagriculture and Production Science*, 26, 107-116.

Maecka, A., Jarmuszkiewicz, W. A., & Tomaszewska, B. (2001). Antioxidative defense to lead stress in subcellular compartments of pea root cells. *Acta Bioquimica Polonica*, 48, 687-698.

Mazorra, L. M., Nunez, M., Hechavarria, F. C., & Sanchez-Blanco, M. J. (2002). Influence of brassinosteroids on antioxidant enzymes activity in tomato under different temperatures. *Biologia Plantarum*, 45, 593-596.

Mirzaee, M., Moieni, A., & Ghanati, F. (2013). Effects of drought stress on the lipid peroxidation and antioxidant enzyme activities in two canola (*Brassica napus* L.) cultivars. *Journal of Agricultural Science and Technology*, 15, 593-602.

Niu, J.-H., Ahmad Anjum, S., Wang, R., Li, J.-H., Liu, M-.R., Song, J.-X., & Zong, X.-F. (2016). Exogenous application of brassinolide can alter morphological and physiological traits of *Leymus chinensis* (Trin.) Tzvelev under room and high temperatures. *Agricultural Research*, 76, 27-33 .

Ramani, M. M. (2015). Effect of shoot thining and 28- homo brassinolide sprsy on growth, flowering, yield and quality of magno cv. Fruit Science Department, *Aspee College of Horticulture and Forestry Navsari Agricultural University*, 1-140.

Reddy, K. R., & Kakani, V. (2007). Screening Capsicum species of different origins for high temperature tolerance by in vitro pollen germination and pollen tube length. *Scientia Horticulturae*, 112, 130-135 .

Rodrigues, E., Poerner, N., Rockenbach, I. I., Gonzaga, L. V., Mendes, C. R., & Fett, R. (2011). Phenolic compounds and antioxidant activity of blueberry cultivars grown in Brazil. *Food Science and Technology*, 31, 911-917.

Roghabadi, M. A., & Pakkish, Z. (2014). Role of brassinosteroid on yield, fruit quality and postharvest storage of 'Tak Danehe Mashhad'sweet cherry (*Prunus avium* L.). *Agricultural Communications*, 2, 49-56 .

Suarez, L., Zarco-Tejada, P. J., Gonzalez-Dugo, V., Berni, J., Sagardoy, R., Morales, F., & Fereres, E. (2010). Detecting water stress effects on fruit quality in orchards with time-series PRI airborne imagery. *Remote Sensing of Environment*, 114, 286-298.

Swamy, K., Vardhini, B., Ramakrishna, B., Anuradha, S., Siddulu, N., & Rao, S. (2014). Role of 28-homo brassinolide on growth biochemical parameters of *Trigonella foenugraecum* L. plants subjected to lead toxicity. *Multidisciplinary and Current Research*, 2, 317.

Upadhyay, P., & Maier, C. (2016). Effects of 17 β-estradiol on growth, primary metabolism, phenylpropanoid-flavonoid pathways and pathogen resistance in arabidopsis thaliana. *Plant Science*,7, 1693-1710.

Uprety, D. (2006). Interactive effect of moisture stress and elevated CO_2 on the oxidative stress in brassica species. *Journal of Food, Agriculture and Environmen*, 4, 298-305.

Valizadeh, M., Moharamnejad, S., Ahmadi, M., & Mohammadzadeh Jalaly, H. (2013). Changes in activity profile of some antioxidant enzymes in alfalfa half-sib families under salt stress. *Journal of Agricultural Science and Technology*, 15, 801-809.

Xi, Z., Zhang, Z., Huo, S., Luan, L., Gao, X., Ma, L., & Fang, Y. (2013). Regulating the secondary metabolism in grape berry using exogenous 24- Epi brassinolide for enhanced phenolics content and antioxidant capacity. *Food chemistry*, 141, 3056-3065.

Yadava, P., Kaushal, J., Gautam, A., Parmar, H., & Singh, I. (2016). Physiological and biochemical effects of 24- Epi brassinolide on heat-stress adaptation in maize (*Zea mays* L.). *Natural Sciences*, 8, 171.

Zhu, F., Yun, Z., Ma, Q., Gong, Q., Zeng, Y., Xu, J., & Deng, X. (2015). Effects of exogenous 24-Epibrassinolidee treatment on postharvest quality and resistance of Satsuma mandarin (*Citrus unshiu*). *Postharvest Biology and Technology*, 100, 8-15.