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Effect of Applications of Boron and Gibberellic Acid (GA_3) on Phytochemical Contents in Different Strawberry Varieties

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Abstract

Strawberry is one of the most popular fruits in Turkey, and its production is expanding due to its resilience to a variety of climatic and soil conditions. In terms of both growth circumstances and the applicability of excellent agriculture and organic agriculture, greenhouses and traditional agriculture are favoured in strawberry farming. According to the World Health Organization, good agriculture and organic agriculture have grown in importance in generating reliable and high-quality food (WHO). Organic compound applications are expanding as a result of these factors. As a result, the number and variety of organic compounds has grown. The research was developed in a protected cultivation system in the 2020 year. The purpose of this study was to see how applying boric acid and gibberellic acid (GA_3) affected the phytochemical content (organic sugars and organic acids) in strawberry cultivars. Albion and Sabrina strawberry varieties were used in this study. The organic sugar ratios in the Sabrina cultivar were generally higher than those in the Albion variety. The highest levels of L-ascorbic acid, Malic acid, and Succinic acid were detected in Sabrina varieties when BA 500 ppm was applied. The highest level of citric acid was recorded in the Control group at 822.48. After the applications, it was determined that there was a difference in strawberry varieties among organic sugars and acids.

Key words
Strawberry, DPPH, organic sugars, organic acids, Boron, Gibberallic acids (GA_3).

Introduction

The strawberry plant (*Fragaria ananassa*) is a member of the Rosaceae family (*Rosoideae* subfamily) and is indigenous to Europe. Because of its ability to adapt to a variety of soil and climate conditions, the strawberry (*Fragaria x ananassa Duch*) is widely grown around the world (Esitken et al., 2010). Strawberries are grown in each of Turkey's nine agro-climatic zones, with Mediterranean and subtropical climate zones being the most prominent producers. Consumers may enjoy this fruit almost all year thanks to the variety of climates around the country, as well as the development of short-day and day-neutral cultivars and the use of various planting strategies (Ozdemir et al., 2013; Torun et al., 2014).
Strawberry fruits appeal to consumers because of their appealing aroma, flavor, color, structure, and nutritional qualities such as hydroxycinnamic acid, flavanols, and anthocyanins (Battino et al., 2019; Giampieri et al., 2017; Gündüz, 2016). Strawberries are high in bioactive chemicals and nutrients, and they've been used for a long time to provide health advantages like detoxification, improved blood circulation, and fatigue recovery (Kim and Shin, 2015; Naemura et al., 2005). With the increased focus on phytochemicals in recent years, many people have increased their vegetable and fruit diet in order to avoid aging and cancer. Strawberries are high in antioxidants and have been shown to reduce the formation of free radicals including superoxide radicals and peroxyl (Wang and Lin, 2000).
Sugars are the major results of photosynthesis, and they were traditionally regarded to be a key component of fruit quality, flavor, and caloric value. Sugars are now known to be essential for the synthesis of plant cell wall components and energy sources, as well as aroma compounds and signaling molecules. Embryogenesis, seed germination and seedling growth, vegetative and reproductive organ development, senescence, responses to biotic and abiotic stresses, coordinating the expression of many genes, and the synthesis of organic and amino acids, sugars, polyphenols, pigments, and aroma compounds are just a few of the processes they are involved in (Wind et al., 2010; Halford et al., 2011; Teker and Altindisli, 2021). Sugar quantification is regulated by ripeness stage, plant age, soil quality, fertilizer, location and weather conditions, agriculture, geographical origin, and genotype (Okan et al., 2018).
The goal of this study is to see how different doses of boric acid and GA_3 alter dpph and total phenolic content in the strawberry cultivars Albion and Sabrina. Five different doses of boric acid (Control, 100, 200, 300, 400, and 500 ppm) and GA3 (Control, 20, 40, 60, 80, and 100 ppm) were used for this experiment.

Material and Methods

Material

The study was conducted in a strawberry greenhouse with a low tunnel. 'Albion' strawberry variety was developed in the USA and recently carried to many regions of the world, including Turkey, where is commercially cultivated under different climatic conditions and cultivation modes (Gunnes et al., 2009). Sabrina Strawberry variety was developed in the province of Huelva by the Planasa company in Spain. Sabrina variety is currently widely used in strawberry fields of Europe (Lozano et al., 2016). Frigo seedlings were sown in a triangular configuration on the bobbins at 30x30 cm intervals. The initial blossoms and stolons of strawberry types cultivated in the strawberry garden were plucked in order to promote vigorous root development in the first year.

Method

Fruit samples were gathered from this strawberry greenhouse, which was in full production in 2020, throughout the harvest period (March to May). When the fruits were fully red, they were collected. The samples were taken to the Vocational School's laboratory in the Sivaslı district of Uşak province, where pomological analyses of the fruit samples were performed immediately and the remaining materials were preserved at -20 ^{o}C for biochemical analyzes.
In the study, several dosages of boric acid and GA_3 were utilized. The applications were created in the Vocational School's laboratory in the Sivasli district of Uşak province. A total of ten fertilizer applications and control groups were used in the study. It consists of 5 boric acid and GA_3 replications, as well as control groups. Boric acid and GA_3 were made by pouring 50 mL of pure alcohol into tiny beakers. Then, using a precision balance, Boric acid and GA_3 were weighed. Following the weighing, 50 mL of pure alcohol was added to each duplicate and stirred until it dissolved. Finally, the samples were poured into bottles containing 950 ml of pure and shaken until fully mixed for all replications. The quantities of boric acid and GA_3 in Table 1 have also been provided. Five replications and control groups of strawberry types were chosen for the experiment. For each replication, twenty plants were used.

Table 1: Boric acid and GA_3 application levels

Amounts of Boric acid application	Amounts of GA_3 application
Control	Control
100ppm= 0.1 g per 1 liter of pure water	20ppm= 0.02 g per 1 liter of pure water
200ppm= 0.2 g per 1 liter of pure water	40ppm= 0.04 g per 1 liter of pure water
300ppm= 0.3 g per 1 liter of pure water	60ppm= 0.06 g per 1 liter of pure water
400ppm= 0.4 g per 1 liter of pure water	80ppm= 0.08 g per 1 liter of pure water
500ppm= 0.5 g per 1 liter of pure water	100ppm= 0.1 g per 1 liter of pure water

Organic sugar analysis

Glucose, fructose, and sucrose content in the juice obtained from the harvested strawberries were determined by Kafkas et al. (2007). Before analysis, frozen juice samples were thawed at 25 °C 1 mL of juice was added to 4 mL of ultrapure water (Millipore Corp., Bedford, MA, USA). The reaction mixture was placed in an ultrasonic bath and sonicated at 80 °C for 15 min and then centrifuged at 5500 rpm for 15 min and it was filtered before HPLC analysis (Whatman nylon syringe filters, 0.45 µm, 13 mm, diameter). The high-performance liquid chromatographic apparatus (Shimadzu LC 20A VP, Kyoto, Japan) consisted of an in-line degasser, pump, and controller coupled to a Refractive index detector (Shimadzu RID 20A VP) equipped with an automatic injector (20 µL injection volume) interfaced to a PC running Class VP chromatography manager software (Shimadzu, Japan). Separations were performed on a 300 mm × 7.8 mm i.d., 5 µm, reverse-phase Ultrasphere Coregel-87C analytical column (Transgenomic) operating at 70 °C with a flow rate of 0.6 mL min−1. Elution was isocratic ultrapure water. Individual sugars were calculated based on their standards and expressed in % of FW.

Organic acids analysis

Strawberry juice extract was measured by HPLC analysis developed by Bozan et al. (1997). The changes in l-ascorbic, citric, malic, succinic and fumaric acid contents in strawberry juice samples were measured. 1 ml of the sample and 4 ml of 3 % metaphosphoric acid were mixed for organic acids extraction. The mixture was placed at 80 °C for 15 minutes in ultrasonic water bath and it was sonicated and centrifuged at 5500 rpm for 15 minutes. The HPLC vials were then removed after the mixture was filtered (Whatman nylon syringe filters, 0.45 m, 13 mm diameter). We employed a high-performance liquid chromatographic system HPLC (Shimadzu LC 20A vp, Kyoto, Japan) with a UV detector (Shimadzu SPD 20A vp) and an 87 H column (5 m, 300 7.8 mm, Transgenomic) to examine the extract organic acids. As a solvent, 0.05 mM sulfuric acid was utilized. Column temperature was 40°C; injection volume was 20L; detection wavelength was 210 nm and flow rate was 0.8 mL/min. The retention duration of peaks and the comparison of spectral data to standards are used to identify organic acids and determine peaks. The detected acids were assessed using the appropriate standard calibration curves.

Statistical analysis

Raw data of the experiments were summarized in Microsoft Excel and figures were prepared to better present the results. Then, the data were subjected to the analysis of variance and the mean separation was performed with Tukey's HSD test at $p < 0.05$. The R-free software was then used to perform the principal component analysis and correlations among the study parameters.

Results

The results showed that applying Boric Acid and GA_3 to various concentrations of strawberry extract had a significant effect on organic sugars (mg/100 g) (Table:2).

Table 2. The content of organic sugar (mg/100 g) in fruits of strawberry cultivars

	Sabrina			**Albion**		
Treatment	Fructose	Glucose	Sucrose	Fructose	Glucose	Sucrose
BA (100 ppm)	16.49±1.16	26.85±2.14	0.36±0.08	11.75±0.87	19.22±2.46	0.28±0.06
BA (200 ppm)	13.03±0.79	20.62±1.34	0.16±0.01	11.18±0.66	19.28±0.18	0.12±0.02
BA (300 ppm)	14.53±0.63	22.52±1.25	0.21±0.03	10.71±0.21	15.20±1.19	0.38±0.03
BA (400 ppm)	14.16±0.48	21.11±0.66	0.33±0.01	11.41±0.48	21.01±1.32	0.67±0.07
BA (500 ppm)	13.54±0.26	24.30±0.82	0.33±0.01	11.44±0.03	20.39±0.85	0.33±0.05
GA_3 (20 ppm)	10.00±0.22	17.32±0.16	0.14±0.05	11.37±0.07	17.51±0.99	0.35±0.05
GA_3 (40 ppm)	12.06±0.19	19.21±0.23	0.32±0.02	10.28±0.13	19.00±0.14	0.22±0.03
GA_3 (60 ppm)	12.39±0.06	21.77±0.44	0.30±0.04	11.44±0.66	21.10±0.28	0.31±0.08
GA_3 (80 ppm)	13.47±0.07	21.44±0.06	0.37±0.06	10.76±0.65	18.41±0.35	0.30±0.06
GA_3 (100 ppm)	11.15±0.05	18.39±0.02	0.52±0.07	11.47±0.47	20.27±0.33	0.34±0.01
Control (0 ppm)	11.64±0.29	20.19±0.01	0.29±0.02	10.12±0.80	16.48±0.73	0.23±0.03

The fructose level of the Sabrina variety ranged from 10.00 to 16.49 (mg/100 g). The maximum value was found when Boric Acid 100 ppm was applied, while the lowest value was observed when GA_3 20 ppm was applied. The glucose level of the Sabrina variety ranged from 17.32 to 26.85. The high value was found in Boric Acid 100 and the lowest level was in GA_3 100. The Sucrose levels were found between 0.14-0.52 (mg/100 g). The highest level was found in GA_3 100 ppm application and the minimum value was found in GA_3 20 ppm application. The Boric Acid application (in Sabrina variety), the fructose value ranged from 10.12 to 11.75 (mg/100 g). The maximum value was found when Boric Acid 100 ppm was applied, while the lowest value was observed when Control 0 ppm was applied. The glucose value was found between 15.20 (BA 300 ppm) – 21.10 (GA_3 60 ppm) (mg/100 g). Values of sucrose were found between 0.12 (BA 200 ppm) – 0.35 (GA_3 20 ppm) (mg/100 g).

Strawberry sugar concentration is an important taste element that is strongly linked to customer acceptability (Jouquand et al., 2008). The most abundant soluble components in strawberries are glucose, fructose, and sucrose. Sucrose levels were determined to be extremely low in the study and were not presented. Strawberry fruit has lower sucrose contents than fructose and glucose, according to Wang et al. (2000). The highest amounts of organic sugar content were achieved from both PT and OF in the first year of research, but only from PT in the second year. Organic sugar concentration was observed to be considerably lower in GH in both years. The difference in day and night temperatures in GH can explain this. Fruit matures faster and has lower sugar content when the temperature difference between day and night is small (Shiow & Camp, 2000). The quantities of fructose and glucose in the fruit varied significantly between genotypes. The highest total sugar was found in 'Ebru,"Osmanlı," and 'Kaşka.' Koşar, Paydaş, Kafkas, and Başer all reported similar outcomes (Gunduz and Ozdemir, 2014). Moreover, recent studies determined the effects of foliar-based boron treatments with various doses on fruit quality parameters. It has been concluded that boron applications can increase fruit quality parameters in some strawberry varieties (Urün et al., 2021), olive (Gündeşli and Nikpeyma, 2016), pistachio (Gündeşli et al., 2020).

The results showed that applying Boric Acid and GA_3 to various concentrations of strawberry extract had a significant effect on organic acids (mg/100 g) (Table:3).

Table 3. The content of organic acid (mg/100 g) in fruits of strawberry cultivars

	Sabrina					Albion				
Treatment	L-ascorbic acid	Citric Acid	Malic Acid	Succinic Acid	Fumaric Acid	L-ascorbic acid	Citric Acid	Malic Acid	Succinic Acid	Fumaric Acid
BA (100)	0.53±0.03	758.57±2.41	72.67±0.85	212.18±4.39	1.36±0.16	1.14±0.18	673.03±2.11	69.50±0.61	192.01±1.39	1.17±0.05
BA (200)	1.44±0.04	758.55±2.15	68.84±0.13	188.07±2.51	1.24±0.42	1.34±0.05	686.59±0.24	78.44±0.04	166.52±0.15	0.96±0.05
BA (300)	0.35±0.06	756.25±0.36	69.03±1.22	193.32±3.32	1.15±0.61	1.28±0.07	712.94±0.38	68.44±0.05	178.74±0.13	1.09±0.05
BA (400)	1.12±0.02	643.54±1.48	69.41±0.65	179.12±3.55	1.23±0.13	2.61±0.04	736.02±0.33	81.51±0.06	176.98±0.20	1.05±0.04
BA (500)	1.71±0.01	805.67±0.64	83.43±2.48	220.77±2.16	1.20±0.06	4.07±0.05	736.93±0.48	71.86±0.09	184.03±0.08	0.81±0.08
Control	0.29±0.08	822.48±0.88	75.98±1.18	216.35±1.09	1.22±0.23	2.50±0.02	574.44±0.27	62.05±0.05	156.90±0.44	0.93±0.05
GA_3 (20)	0.60±0.04	625.95±1.27	64.80±1.33	173.86±2.02	0.81±0.05	1.41±0.01	680.72±0.06	66.02±0.01	168.38±0.63	0.91±0.07
GA_3 (40)	2.10±0.09	600.10±3.29	65.72±1.26	179.62±0.86	0.94±0.01	1.24±0.03	736.31±0.04	70.46±0.02	173.46±0.09	1.03±0.04
GA_3 (60)	1.30±0.06	593.78±6.77	58.28±1.48	168.38±1.33	0.87±0.03	1.71±0.02	724.84±0.02	64.77±0.05	168.70±0.11	0.79±0.01
GA_3 (80)	1.13±0.02	567.92±5.15	67.19±0.81	168.75±1.25	1.06±0.02	4.42±0.04	630.08±0.08	61.66±0.06	163.15±0.60	0.84±0.06
GA_3 (100)	1.01±0.07	630.41±2.12	63.90±1.69	162.78±0.73	0.89±0.01	1.86±0.07	678.61±0.03	64.60±0.04	180.76±0.26	1.07±0.08

The highest levels of L-ascorbic acid, Malic acid, and Succinic acid were detected in Sabrina varieties when BA 500 ppm was applied. The highest level of citric acid was recorded in the Control group at 822.48 (mg/100 g). In a BA 100 ppm application, the maximum value of fumaric acid was found to be 1.36 (mg/100 g). In GA_3 80 ppm foliar treatments, the Albion variety had the highest L-ascorbic acid value of 4.42 (mg/100 g). Citric acid has the highest value of 736.93 (mg/100 g) in BA 500 ppm. Malic acid has the highest value of 78.44 (mg/100 g) in BA 200 ppm. BA 100 ppm foliar sprays had the highest levels of Succinic and Fumaric acid.

Organic acids are tiny components of strawberry fruit, but they are key flavor qualities that, when combined with sugars, influence strawberry fruit sensory quality. Organic acid concentration differed significantly between the genotypes studied (Wang et al., 2002). The cultivars tested in this study included two primary organic acids: malic and citric acid. Citric acid was the most abundant acid, accounting for 82.7 (mg/100 g) percent (in the growing year 2008) and 84 percent (in the growth year 2009), respectively, of the total acid content, which is consistent with earlier studies (Crespo et al., 2010). The cultivar "Osmanli" had the highest total acid content, followed by "Cigouletta," and "Sweet Charlie," which had the lowest.

The correlation among the phytochemical contents of Albion cultivars are given in Figure 1. Results suggested that some of the phytochemicals have moderate to high positive correlation, while the others have a negligible correlation. The highest concentration was observed among the citric acid and malic acid with 0.72 and was followed by the correlation between the citric acid and succinic acid with 0.71. As expected, the fructose and glucose concentrations were also noted to have a moderate positive correlation.

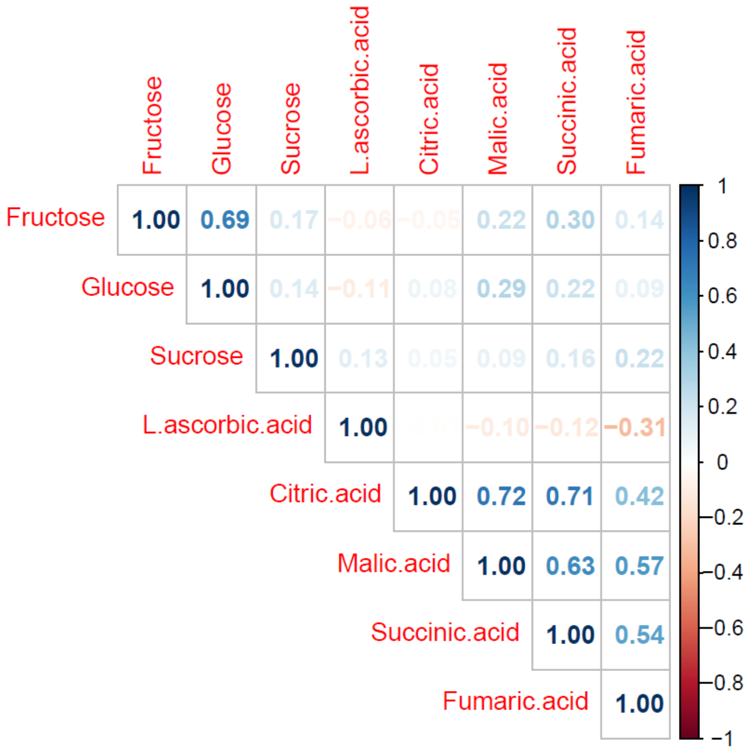

Figure 1: Correlation among the phytochemical contents of Albion cultivar

The correlation results for Sabrina were noted to be similar to the ones for Albion (Figure 2). However, the correlations for Sabrina cultivar were slightly higher than the ones for the Albion cultivar. For example, the correlation between the citric acid and malic acid was noted as 0.84 for Sabrina cultivar. Similarly, the correlation between citric acid and succinic acid was 0.82. The correlation between fructose and glucose was high, with a 0.89 correlation.

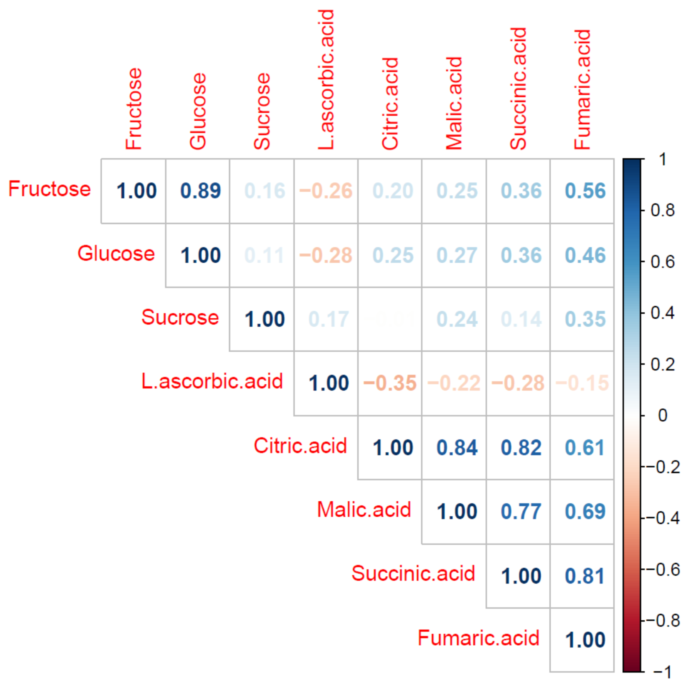

Figure 2: Correlation among the phytochemical contents of Sabrina cultivar

The PCA analysis of the data makes it easy to understand and explain the relationships among the phytochemicals of the strawberries (Figure 3). The loadings which are close to each other, forming a small angle, show that these variables are positively correlated. Results showed that the Fructose and glucose are positive correlation and these two have no correlation with the other test phytochemicals, except the L ascorbic acid, which has a negative correlation.

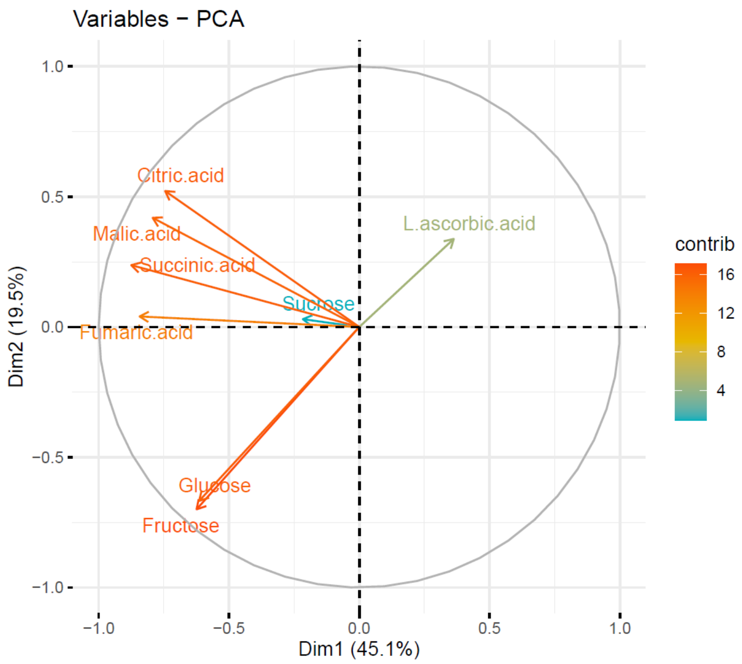

Figure 3: PCA analysis for strawberry cultivars.

N management, farming systems (organic vs. conventional), cultivar selection, planting methods (direct seeding and transplanting), and exogenous administration of plant growth regulators all have a significant impact on root and shoot growth, fruit yield, and quality (Goreta et al., 2004; Leskovar and Othman, 2018, 2021). Many aspects of plant growth and development, including seed germination, stem elongation, leaf expansion, nutrient buildup, pollen formation, and blooming, are regulated by the plant hormones (Achard and Genschik, 2009; Khan et al., 1998).

Conclusion

Field evaluation of Boron and Gibberellic acid (GA_3) applications showed positive effects on values of phytochemical content (organic sugars and organic acids) in strawberry cultivars. In general, Sabrina cultivars had higher organic sugar ratios than Albion varieties. Sabrina varieties tested with BA 500 ppm had the highest levels of L-ascorbic acid, malic acid, and succinic acid. In the Control group, the highest level of citric acid was recorded at 822.48. There was a difference among strawberry varieties among organic sugars and acids after testing. Therefore, it has been determined that Boron and Gibberellic acid applications increase the values in most organic sugars and organic acids.

Statement of Conflict of Interest

The authors are declared that they have no conflict with this research article.

Author Contribution

B.Y.: laboratory work, article writing; V.O.; Field work and article writing; A.M.Ç.: fieldwork; E.K.: laboratory work.

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