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EFFECTS OF SELENIUM BIOFORTIFICATION ON PHYTOCHEMICAL CHARACTERISTICS OF SOME TABLE GRAPE CULTIVARS

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Abstract: Table grapes, rich in vitamins and minerals, play an important role in human nutrition, thus largely used in daily diets. Selenium (Se) with positive impacts on human health and anticancerogenic effects, has recently become prominent in human nutrition and animal feeding. In this study, selenium fortifications were made at different doses (control, 4 ppm and 8 ppm) to 9 different table grape cultivars (Alphonse Lavallée, Bilecik İrikarası, Cardinal, Sultani Seedless, Tekirdağ Seedless, Italia, Lival, Victoria, Royal) and total phenolics, anthocyanins and flavonoids of the cultivars were determined. While total phenolics of the whole berry was presented, skin and pulp total anthocyanins and total flavonoids were presented separately. The greatest total phenolic amount was obtained from 4 ppm selenium treatment in Bilecik İrikara (157.31 mg/g) cultivar. The greatest total anthocyanin contents were obtained from the skin of with 8 ppm selenium treatment in Alphonse Lavallée (11.22 mg/g). Selenium treatments increased total flavonoids of Bilecik İrikarası, Lival, Royal and Sultani Seedless cultivars. It was concluded based on findings that Se treatments influenced phytochemical characteristics of the table grapes.

Keywords: Selenium, Total phenolic, Total anthocyanin, Italia, Alphonse Lavallée

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1. Introduction

Table grapes with a high allure, a wide range of nutrients and pharmacological characteristics are mostly available for fresh consumptions (Yadav et al., 2009). Compared to several other fruit species, grapes are quite rich in phenolic compounds (Xia et al., 2010).

Grape quality largely depends on vineyard management, cultivar and harvest time (Rizzuti et al., 2015). Phenolic compounds, responsible for color, taste and aroma of grapes, are the most important quality components and have supplementary effects on human nutrition and health (Kunter et al., 2013). Until recently, phenolic substances of wine grapes have been analyzed, but such compounds are also important quality traits in table grapes, especially in colored cultivars. Table grapes were reported as an important source of phenolics (catechin, flavonols, phenolic acids, anthocyanins) (Rolle et al., 2010). Following sugars and organic acids, phenolic compounds constitute the third greatest compound group in grapes. Existence and relative ratios of certain phenolic substances in a grape berry are genetically controlled species and cultivar characteristics. However, the quantity of these substances is mainly dependent on climate and soil conditions, maturity stage and cultural practices (Ribéreau-Gayon et al., 2000). In terms of total phenolics, black grape cultivars were reported to be richer than white cultivars (Yang and Xiao, 2013). Anthocyanins constitute the largest sub-group of phenolic substances. Anthocyanins exist in berry skin and are defined as natural pigments giving specific red, blue and purple tones of the grapes (Ho et al., 2001). Anthocyanins begin to form at veraison stage, accumulate in berry skin throughout the maturity and reach maximum levels at the end of maturity.

Potential market value of different grape cultivars has gradually been discovered. Exploring grape quality and special medicinal effects will play significant theoretical and practical roles in the future of table grape cultivation (Zhu et al., 2017).

Selenium is a trace element. In human nutrition,

selenium (Se) reduces the risk of cancer (Kiskova et al., 2014), scavenges free radicals (Bors and Saran, 1987), exhibits resistance against membrane lipid peroxidation and slows down the aging process (Rice-Evans, 2001) and boosts the immune system (Keskinen et al., 2009). Plants are the primary source of selenium. However, fundamental of Se is still ambiguous. Plants play a vital role in Se deficiency and toxicity. Therefore, there is always a need for detailed studies about selenium mechanism (Gupta and Gupta, 2017). Previous studies on selenium biofortification revealed that selenium treatments influenced phytochemical contents of the plants differently. It was reported that total phenolics of broccoli increased with selenium treatments (Bachiega et al., 2016), total phenolics of onion and tomato decreased with increasing selenium doses (Pöldma et al., 2013; Schiavan et al., 2013), total phenolics of apples generally decreased with selenium treatments (Groth et al., 2020).

In this study, effects of different doses of selenium biofortifications on phytochemicals contents of 9 different table grape cultivars were investigated.

2. Materials and Methods

2.1. Experimental Design

Experiment was conducted in 2017 in adaptation vineyard of Middle Black Sea Transitional Zone Agricultural Research Institute in Tokat province of Türkiye. Experimental grapevines are 7 years old and the plot was established at 3.00 x 1.75 m (row spacing x on-row vine spacing) spacing. The trunks are 70 cm high and double-arm ed training system was used in the vineyard. The cultivars used in the present experiment included Alphonse Lavallée, Italia, Lival, Victoria, Royal, Bilecik İrikarası, Cardinal, Prima, Trakya İlkeren, Flame Seedless, Sultani Seedless, Tekirdağ Seedless. All the cultivars were grafted on 1103 Paulsen rootstocks. Soil samples were taken from 0-30 cm soil profile to determine physical and chemical properties of vineyard soils. The characteristics of the vineyard siol are as follows: Sand ratio was 54.02%, clay ratio was 31.58%, silt ratio was 14.39%, salt content was 0.02%, organic matter content was 1.18%, degree of saturation was 56%, soil texture was CL, EC was 0.57, pH was 7.78, P ratio was 5.72 and K ratio was 102.4. Selenium content of experimental soil was around 1.15 μg kg^{-1}.

2.2. Measurement Methods of Grape Samples

Pruning, chemical treatments, irrigation, soil tillage, cluster thinning, removal and cluster tipping were practiced in accordance with the relevant standards (Anonymous, 1992; Ateş and Kısmalı, 2007). Fertilization was practiced to have 12 kg N/da, 8 kg P2O5/da and 8 kg K2O/da at two different periods. Besides, sufficient quantity of micro elements was applied half to soil and half to leaves. Selenium fertilization was practiced 3 times to spray the entire canopy in 10-20-30 day intervals from the berry set period according to Zhu et al. (2017). Treatment doses were selected as 0 (control), 4, 8 mg kg^{-1} of Selenium (Sodium Selenate fertilizer). Cluster samples were taken at harvest maturity. Samples were brought to laboratory and cold-stored until total phenolics, total anthocyanin and total flavonoid analyses.

Total Phenolics contents; About 5 g sample was taken from each replicate of each cultivar. Berry samples were divided into small pieces using a bistoury. Samples were extracted with 50% ethanol, completely homogenized, filtered through Whatman filter papers and final volume was completed to 25 ml with 50% ethanol. Samples were kept in a fridge until the time of analysis. Total phenolics of the samples was determined according to Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965). Spectrophotometer readings were performed at 765 nm wavelength. Results were expressed as gallic acid equivalent mg/g with the use of a standard curve prepared by using standard as gallic acid solutions (Harmankaya, 2003).

Total Anthocyanin and Flavonoids Contents; Samples prepared according to Bino et al. (2005) and analysis was conducted in accordance with Di Stefano and Cravero (1991). Results were expressed in mg/g. For 10 berry skins of present cultivars (about 10 g), 40 ml solution was prepared (Skin:solution=1:4), berry skins were placed into the solution, kept at 30 °C for 72 hours, then preserved at -20 °C until the time of analysis. Before spectrophotometer reading, samples were diluted with hydrochloric ethanol at 1/10 ratio and readings were performed at 280 nm and 520 nm wavelengths.

2.3. Statistical Analysis

Experiment was conducted in randomized blocks – split plots experimental design with 3 replications and 3 grapevines in each replicate. A total of 243 grapevines (9 cultivars x 3 doses x 3 replicates x 3 grapevines in each replicate) were used in the experiment. Experimental data were subjected to analysis of variance and means were compared with the LSD (0.05) test.

3. Results and Discussion

Effects of 0, 4 and 8 ppm selenium (Se) biofortifications on total phenolics, skin-pulp anthocyanins and skin-pulp flavonoids of 9 different grape cultivars were investigated in this study. Selenium treatments influenced total phenolics of the cultivars (Table 1) (Figure 1). The greatest total phenolics was obtained from 4 ppm Se treatment of Lival cultivar (214.67 mg/g) and the lowest from 4 ppm Se treatment of Victoria cultivar (68.01 mg/g). In Tekirdağ Seedless cultivar, increasing total phenolics were observed with increasing treatment doses, the total phenolics of 80.29 mg/g in control treatment increase to 82.03 mg/g at 4 ppm Se treatment and 108.90 mg/g at 8 ppm Se treatment. In Bilecik İrikarası cultivar, Se treatments positively influenced total phenolics, the value of 98.81 mg/g in control treatment increased to 157.31 mg/g at 4 mg/g Se and to 134.81 mg/g 8 ppm Se treatments. In Royal cultivars, decreasing total phenolics were observed with increasing treatment doses, the total phenolics of 170.61 mg/g in control treatment decreased to 157.05 mg/g 4

mg/g Se and to 91.99 mg/g at 8 ppm treatments. In Victoria cultivar, selenium biofortifications reduced total phenolics. Effects of selenium treatments on total phenolics of Alphonse Lavaellee, Cardinal, Lival and Sultani Seedless cultivars were not found to be significant. Skin anthocyanin contents of the cultivars are shown in Table 2. The greatest value was observed in control treatment of Lival cultivar (12.20 mg/g) and the lowest in 4 ppm Se treatment of Tekirdağ Seedless cultivar (2.05 mg/g). In Alphonse Lavallée, Bilecik İrikarası and Cardinal cultivars, skin anthocyanin contents increased with increasing treatment doses and the values respectively reached 160.07, 134.81 and 213.17 mg/g 8 mg/g Se treatment. In Lival and Tekirdağ Seedless cultivars, selenium treatments decreased skin anthocyanins. In Alphonse Lavallée, Bilecik İrikarası, Royal and Tekirdağ Seedless cultivars, selenium biofortification reduced pulp anthocyanin contents.

Table 1. Effects of selenium treatments of total phenolics (mg/g) of the cultivars

Selenium Treatments	Cultivar								
	Alphonse Lavallé	Bilecik İrikarası	Cardinal	Lival	Royal	Tekirdağ Seedless	Victoria	Italia	Sultani Seedless
CONTROL	156.99	98.81 ^{b}	211.88	202.24	170.61 ^{a}	80.29 ^{b}	111.86 ^{a}	75.34 ^{b}	105.75
4 PPM	144.64	157.31 ^{a}	199.41	214.67	157.05 ^{a}	82.03 ^{b}	68.01 ^{b}	93.34 ^{a}	101.64
8 PPM	160.07	134.81 ^{ab}	213.17	205.20	91.99 ^{b}	108.90 ^{a}	101.06 ^{a}	70.01 ^{c}	129.60
LSD (0.05)	N.S.	35.93	N.S.	N.S.	39.64	4.74	12.77	2.85	N.S.

NS= non-significant.

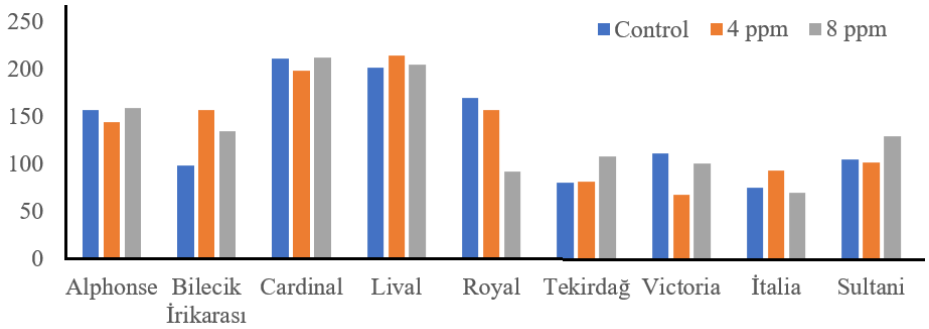

Figure 1. Total phenolics of the cultivars at different selenium doses.

Table 2. Effects of selenium treatments on skin-pulp anthocyanin contents of the cultivars

	Selenium treatments	Cultivars								
		Alphonse Lavallée	Bilecik İrikarası	Cardinal	Lival	Royal	Tekirdağ Seedless	Victoria	Italia	Sultani Seedless
Skin (mg/g)	Control	6,71 ^{b}	6,22 ^{b}	3,18 ^{c}	12,20 ^{a}	8,06	5,98 ^{a}	-	-	-
	4 ppm	6,71 ^{b}	6,76 ^{b}	4,42 ^{b}	11,38 ^{b}	8,38	2,05 ^{b}	-	-	-
	8 ppm	11,22 ^{a}	8,17 ^{a}	7,22 ^{a}	7,71 ^{c}	6,15	2,14 ^{b}	-	-	-
	LSD (0.05)	3,66	1,41	1,14	0,72	N.S.	2,38	-	-	-
Pulp (mg/g)	Control	2,50 ^{a}	1,39	1,19	1,14	1,11 ^{a}	2,83 ^{a}	-	-	-
	4 ppm	1,50 ^{b}	1,17	3,30	2,23	0,77 ^{b}	0,55 ^{b}	-	-	-
	8 ppm	2,43 ^{a}	0,93	2,92	1,21	0,70 ^{b}	0,90 ^{b}	-	-	-
	LSD (0.05)	0,72	N.S.	N.S.	N.S.	0,17	1,52	-	-	-

The greatest skin flavonoid content (Table 3) was obtained from 4 ppm treatment of Royal cultivar (177.41 mg/g) and the lowest from the control treatment of Sultani Seedless cultivar (87.60 mg/g). Compared to the control treatments, the greatest skin flavonoid contents of the cultivars were observed in 4 ppm Se treatment of Alphonse Lavallee cultivar (139.00 mg/g), 4 ppm Se treatment of Bilecik İrikarası cultivar (141.31 ppm), 8 ppm Se treatment of Cardinal cultivar (140.59 mg/g), 8 ppm Se treatment of Lival cultivar (131.50 mg/g), 4 ppm treatment of Royal cultivar (177.41 mg/g) and 4 ppm Se treatment of Sultani Seedless cultivar (96.25 mg/g). In Alphonse Lavallée cultivar, 8 ppm Se treatments reduced skin flavonoid content (120.50 mg/g). The greatest pulp flavonoid content was observed in the control treatment of Sultani Seedless cultivar (39.15 mg/g) and the lowest in 4 ppm Se treatment of Alphonse Lavallée cultivar (7.94 mg/g). In Bilecik İrikarası cultivar, pulp flavonoid contents increased with increasing treatment doses and the pulp flavonoid content of 17.54 mg/g in control treatment increased to 19.37 mg /g at 4 ppm Se treatment and 21.36 mg/g at 8 ppm Se treatment. In Sultani Seedless cultivar, pulp flavonoid contents decreased with increasing treatment doses and the pulp flavonoid content of 39.25 mg/g in control treatment decreased to 31.95 mg/g at 4 ppm Se treatment and 21.44 mg/g at 8 ppm treatment.

Table 3. Effects of selenium treatments on skin-pulp flavonoid contents of the cultivars

	Selenium treatments	Cultivars								
		Alphonse Lavallée	Bilecik İrikarası	Cardinal	Lival	Royal	Tekirdağ Seedless	Victoria	Italia	Sultani Seedless
Skin (mg/g)	Control	133,00 [a]	112,90 [b]	117,50 [b]	118,00 c	124,00 [c]	119,15	98,85	106,53	87,60 [b]
	4 ppm	139,00 [a]	141,31 [a]	108,06 [c]	150,31 a	177,41 [a]	107,63	97,70	103,66	96,25 [a]
	8 ppm	120,50 [b]	128,54 [ab]	140,59 [a]	131,50 b	141,35 [b]	118,36	99,70	103,81	95,75 [a]
	LSD (0.05)	10,80	24,54	7,17	8,06	12,80	N.S.	N.S.	N.S.	3,24
Pulp (mg/g)	Control	12,41 [a]	17,54	17,58	21,32	21,78	18,26	13,92	12,77 [b]	39,25 [a]
	4 ppm	7,94 [b]	19,37	23,71	19,93	16,72	18,03	16,99	16,66 [a]	31,95 [b]
	8 ppm	9,68 [ab]	21,36	14,58	23,65	17,87	17,73	12,60	11,23 [b]	21,44 [c]
	LSD (0.05)	2,94	0,00	N.S.	N.S.	N.S.	N.S.	N.S.	3,35	3,99

Present findings about the effects of selenium biofortification on total phenolics of different table grape cultivars comply with the findings of previous studies conducted with other plants (onion, broccoli) (Pöldma et al., 2013; Bachiega et al., 2016). Effects of selenium biofortification in selenate and selenite forms on total phenolics of apples were not found to be significant (Groth et al., 2020). Present findings obtained from Alphonse Lavellee, Cardinal, Lival and Sultani Seedless cultivars comply with those earlier findings.

Health benefits of foodstuffs have recently become prominent issues in Türkiye and the world. Plants offer such benefits through several secondary metabolites (total phenols, antioxidants, anthocyanins, etc.) they contain. Selenium is an important source of antioxidants. It was reported that selenium reduced risk of cancer, cardiovascular diseases, scavenged free radicals, improved resistance against lipid peroxidation, boosted immune system and slowed down the aging process (Bors and Saran, 1987; Ip et al., 1992; Rice – Evens, 2001; Whanger, 2004; Flores-Mateo et al., 2006; Keskinen et al., 2009; Perez-Corona et al., 2011; Kiskova et al., 2014). Such health benefits of selenium come from being a component of peroxidase and iodothyronine deiodinase enzymes (WHO, 2003; Gül, 2000). Selenium is at the forefront of defense mechanism and such a case is practiced through fighting against hydrogen peroxides with oxidative damage on cells and protecting cell membrane (Djanaguiraman et al., 2005; Gong et al., 2005; Germ et al., 2007; Yao et al., 2009; Cartes et al., 2010).

In the present study, total phenolics, total anthocyanins and total flavonoids of 9 different grape cultivars were investigated. While total phenolics of the whole berry was presented, skin and pulp total anthocyanins and total flavonoids were presented separately. With selenium treatments, total phenolics increased in Italia, Tekirdağ Seedless, Lival and Bilecik İrikarası cultivars. Skin total anthocyanins were high in Alphonse Lavalle, Bilecik İrikarası and Cardinal cultivars. This was an expected case since anthocyanins are metabolites mostly encountered in colored species and present cultivars were not included in cultivars with colored flesh. Selenium treatments increased skin flavonoids in Bilecik İrikarası, Lival, Royal and Sultani Seedless cultivars. Such an increase is quite significant since vitamin E is activated only with the existence of trace quantity of selenium with a great contribution to antioxidant mechanism (Cakmak and Marschner, 1988; Uluozlu, 2005; Kabirov et al., 2008). Zhu et al. (2017) reported that selenium treatments did not change resveratrol compound of the grapes, but increased procyanidin contents. Flavonoids are among the key stones of procyanidin, thus present findings comply with the results of that study. Assuncao et al. (2018) reported increasing antioxidant enzyme activity of wine yeasts with selenium treatments. In another study with selenium treatments to wine yeasts, increases were reported in SOD, CAT and GPX enzyme activities and as a negative case, dose-dependent increases were also observed in lipid peroxidation (Talbi et al., 2018). Although total phenolics, total anthocyanins and total flavonoids were not compared one-on-one, these compound represent one another since all of them are oxidation-preventing compounds.

4. Conclusion

In present study, effects of selenium biofortification on total phenolics, total anthocyanins and total flavonoids of 9 different grape cultivars were investigated. Selenium treatment doses differently influenced total phenolics, skin-pulp total anthocyanins and flavonoids of the cultivars. Differences in findings may have resulted from treatments doses or selenium treatments forms or cultivar-specific characteristics.

In total phenolics analyses, Victoria, Royal (control treatment), Bilecik İrikarası, Italia (4 ppm selenium treatment), Tekirdağ Seedless (8 ppm selenium treatments) cultivars were found to be prominent. In total anthocyanin analyses, it was observed that selenium treatments increased total skin anthocyanins of Tekirdağ Seedless and Lival (control), Alphonse Lavallé, Bilecik İrikarası and Cardinal (8 ppm selenium treatment) cultivars.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	S.S.	N.T.A.	A.Y.	D.K.	S.Ş.	R.C.
C	30	10	30	10	10	10
D	80		20			
S	100					
DCP	40	20	20	5	15	5
DAI	50	10	10	10	10	10
L	40	30	15	5	5	5
W	35	35	10	5	10	5
CR	30	10	30	10	10	10
SR	70	10	5	5	5	5
PM	60	10	10	10	5	5

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because there was no study on animals or humans.

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