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# INVESTIGATION OF GENETIC PARAMETERS AND PHYTOCHEMICAL CHARACTERISTICS IN PLUM UNDER ALTITUDE CHANGE

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Suitability of ecological factors is the most important factor affecting the productivity of agricultural activities. Sensitivity of the phytochemical characteristics that are direct the product quality to the changing environmental conditions is very high. In the present study, how climate change depending on the altitude change affects phytochemical properties in plum cultivars 'Friar' and 'Fortune' fruits which were harvested at two different altitudes (200 m and 800 m a.b.s.), during two consecutive years (2017 and 2018) was investigated. In addition, under environmental and genotype modeling, genetic parameters of the properties were determined. In line with the obtained results from the study, it was determined that the phytochemical characteristics varied parallel to the altitude change within the same latitude. Generally, amount of individual phenolic compounds and organic acids were increased with altitude increase while general phytochemical characteristics such as total phenolic content and antioxidant activity were decreased. Heritabilities of general characteristics were found lower, due to high difference between genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) than organic and phenolic acids. Ranking of organic acids in both altitudes was found as malic acid> acetic acid> oxalic acid> ascorbic acid in both cultivars. In terms of phenolic acids, a stable order could not be determined while chlorogenic acid has come to the front in both cultivars. Since cultivar/location interactions found significant according to bi-plot segregation, investigating the changes at genotype level would be more accurate.

*Keywords:* Heritability, phenolic compounds, organic acids, altitude

## INTRODUCTION

Phenolic compoundss are commonly found in plum fruits (CELIK *et al.*, 2017) show high antioxidant activity by preventing the start of oxidation and peroxidation reactions (DAI and MUMPER, 2010; WEI *et al.*, 2010). It has been stated that this antioxidative effect reduces the risk of many chronic diseases including cancer and cardiovascular diseases (LE MARCHAND, 2002;

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KRIS-ETHERTON *et al.*, 2002). So, the positions of these compounds in the daily diet are very important in terms of health, adequate and balanced nutrition. In societies with a high awareness level, consumption of products that are defined as functional food due to their superior phytochemical properties has been increasing (DEMIR and AKTAS, 2018).

Phytochemical compounds with antioxidant properties have a high sensitivity to environmental conditions (GUNDUZ and OZBAY, 2018; USANMAZ *et al.*, 2018). Since superior phytochemical properties are only obtaining by cultivating the cultivars in the suitable ecology, researches on the identification and breeding of cultivars suitable for ecologies have increased recently (MDITSHWA *et al.*, 2013; MEZZETTI *et al.*, 2016; BLANDO *et al.*, 2019).

Altitude, one of the most important factors shaping ecological factors, is expressed as the vertical distance of any place to sea level. Ignoring the effects of other factors affecting the climate, it has been observed that there are substantial changes in climatic characteristics related to altitude change. In general, with the increase in altitude, day and night temperature difference, atmospheric layer thickness, the angle of the sun rays, light intensity, radiation, precipitation, and wind activity are increased whereas atmospheric temperature and atmospheric pressure are decreased (KORNER, 2007; MUNIZ *et al.*, 2018).

During the flowering and fruit set stages, cool weather allows increased gibberellic acid and cytokinin production in growth cones (KHEW *et al*., 2020). The cellular increase in the calyx and pedicel lobes leads to longer and heavier fruits. However, this leads to a decrease in biochemical properties in the unit area, by increasing the intercellular spaces. Phytochemical accumulation increases with the increase of day and night temperature difference thanks to increased intensity of photosynthesis. Reduction in air or soil moisture is triggered pytochemical accumulation due to rising abscisic acid (VILLALOBOS-GONZALEZ *et al.*, 2016). MERTOGLU *et al.* (2018) reported that day from full bloom to harvest was shorter when temperature was higher and longer when the temperature decreased. Phytochemical accumulation is reported to be higher in fruits that remain more on the tree (USENIK *et al.*, 2013). Parallel to these differences caused by altitude, serious changes are observed in phytochemical content in many fruit species including plums (GUERRA and CASQUERO, 2009; VAGIRI *et al.*, 2013; MDITSHWA *et al., 2013*; MIKULIC-PETKOVSEK *et al.*, 2015; HWANG and DO THI, 2016; LU *et al.*, 2017; PALMIERI *et al.*, 2017).

Therefore, the main objectives of this work were (1) to determine how phytochemicals are react to changing climatic conditions caused by altitude difference, in two different plum cultivars, (2) revealing the inheritance pattern of these compounds for breeding studies, to achieve the desired targets by choosing the right parent and methods and (3) examine the variation sources and interralitons among the properties in order to provide useful informations.

## MATERİALS AND METHODS

Study was carried out in 2017 and 2018 in two different experimental fields which are located at different altitudes belonging to the Faculty of Agriculture of Eskisehir Osmangazi University in Eskisehir-Turkey. ‘Friar’ and ‘Fortune’ plum cultivars were used as the material. Cultivars were planted in 2011 after grafting onto ‘Myrobolan’ seedling rootstocks. 40 days after full blooming, thinning was performed on all trees in order to balance the crop load (MERTOGLU *et al.*, 2019). The fruits were harvested at technological maturity accordingto the sense of taste

and colour (VANGDAL and FLATLAND, 2008). The fruit of each tree were harvested without mixing with the fruit from other trees. Fruits of previously harvested cultivars were homogenised and stored at –20°C. To prepare the fruit extracts for TPC, TFC and antioidant activity analysis, 150 g fruit mixture from each replicate was extracted with 100 mL of 80% acetone containing 0.2% formic acid by using a homogenizer for 2 min. Then centrifuged at 20,000 rpm for 20 min at 4°C. For organic acids and phenolic compounds analysis, 150 g mixture of each replicate was weighed and homogenized for 5 min with 50 mL of deionized water and then shaken for 30 min. The homogenates were centrifuged at 20,000 rpm for 20 min at 30°C and the obtained supernatant was filtered through a membrane filter having 0.45 μm pore size (SELCUK and ERKAN, 2016).

*Table 1. Average weather data in related months of experimental areas (2017–2018) years*

| Center - High Altitude (800 m) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 2017 | | | | | | | | |
| | March | April | May | June | July | August | September | October |
| Wind speed (m.san$^{-}$) | 3.4 | 3.0 | 3.1 | 3.0 | 3.6 | 3.7 | 2.9 | 2.6 |
| Temperature (°C) | 7.6 | 9.6 | 14.4 | 19.1 | 23.1 | 22.0 | 19.6 | 10.8 |
| Actual pressure (hPa) | 923.2 | 924.9 | 923.1 | 923.9 | 922.3 | 923.0 | 924.7 | 926.6 |
| Humidity (%) | 68.7 | 66.9 | 73.2 | 73.4 | 59.5 | 67.3 | 57.0 | 72.9 |
| Precipitation (mm·m$^{-2}$) | 16.2 | 62.0 | 50.8 | 44.8 | 13.4 | 31.4 | 2.6 | 46.4 |
| 2018 | | | | | | | | |
| Wind speed (m.san$^{-}$) | 3.5 | 2.9 | 2.9 | 2.9 | 3.4 | 3.1 | 3.1 | 2.6 |
| Temperature (°C) | 9.2 | 13.8 | 16.8 | 19.9 | 22.3 | 22.9 | 18.6 | 13.3 |
| Actual pressure (hPa) | 920.0 | 924.9 | 922.4 | 921.1 | 920.8 | 922.5 | 925.9 | 927.9 |
| Humidity (%) | 73.5 | 61.6 | 74.8 | 69.5 | 65.5 | 63.5 | 65.4 | 77.4 |
| Precipitation (mm·m$^{-2}$) | 53.6 | 12.6 | 62.2 | 46.6 | 39.2 | 18.0 | 2.8 | 29.2 |
| Sarıcakaya - Low altitude (200 m) | | | | | | | | |
| 2017 | | | | | | | | |
| Wind speed (m.san$^{-}$) | 1.9 | 1.8 | 1.9 | 2.1 | 2.4 | 2.7 | 2.1 | 1.6 |
| Temperature (°C) | 11.0 | 14.2 | 19.1 | 23.6 | 26.7 | 26.8 | 24.9 | 15.3 |
| Actual pressure (hPa) | 983.1 | 984.4 | 981.4 | 981.2 | 978.7 | 979.5 | 981.0 | 985.3 |
| Humidity (%) | 67.2 | 64.5 | 94.7 | 77.0 | 49.9 | 64.9 | 62.9 | 75.5 |
| Precipitation (mm·m$^{-2}$) | 32.1 | 58.4 | 90.4 | 48.9 | 0.8 | 49.4 | 2.0 | 39.4 |
| 2018 | | | | | | | | |
| Wind speed (m.san$^{-}$) | 1.8 | 2.1 | 2.0 | 2.0 | 2.4 | 2.4 | 2.1 | 1.7 |
| Temperature (°C) | 13.3 | 18.4 | 21.4 | 23.6 | 26.0 | 26.7 | 22.1 | 16.9 |
| Actual pressure (hPa) | 978.7 | 982.9 | 979.7 | 977.6 | 976.7 | 978.7 | 983.2 | 986.3 |
| Humidity (%) | 77.3 | 70.6 | 76.0 | 64.9 | 50.9 | 50.4 | 54.2 | 66.8 |
| Precipitation (mm·m$^{-2}$) | 73.2 | 14.9 | 113.3 | 75.8 | 37.1 | 6.2 | 13.4 | 44.0 |

One of the experimental orchards covering the study material is located in Eskişehir-Central region in where typical temperate climate is seen, while the other is located in Eskişehir-Saricakaya region which is close to the Mediterranean climate and has micro-climate characteristics. Climatic data of the study areas are given in Table 1. While temperature and pressure were higher in Sarıcakaya region, wind speed was higher in Center in all months of both years. Humidity and precipitation were higher in Sarıcakaya, where was warmed earlier in the first months of the years, while higher values were found in the Center in the following months.

*Determination of general phytochemical compoundss*

Folin–Ciocalteu method was used for the determination of total phenolic content as defined by SINGLETON and ROSSI (1965). The fruit extracts were mixed with Folin–Ciocalteu reagent and distilled water at a ratio of 1:1:18 and left to rest for 8 min, then 7% sodium carbonate was added. After 2 h of incubation in a dark place, the absorbance of the solution was measured at 750 nm. Gallic acid was used as an external standard for the calibration curve and the results were expressed as mg GAE $kg^{-1}$ fresh weight basis.

Total flavonoid content of the samples was determined by aluminum chloride colorimetric method according to CHANG *et al.* (2002). 50 µl of juice, 950 µl of methanol and 6400 µl of deionized water were transferred to 10 ml tubes, then 300 µl of sodium nitrite (5% $NaNO_2$) solution was added. Then 300 µl of aluminum chloride (10% $AlCl_3$) solution was added to the mixture and it was left for 5 minutes, then 2000 µl sodium hydroxide (4% NaOH) solution was added to the mixture. The mixture was allowed to stand in the dark for 15 minutes and its absorbance was measured at 510 nm using a UV spectrophotometer (UV 1800, Shimadzu, Kyoto, Japan). Catechin was used as standard, and the total flavonoid content was expressed as mg catechin equivalent per kg of fresh weight (mg CAE $kg^{-1}$).

Antioxidant activity was performed using DPPH (2.2-diphenyl-1-picrylhydrazy) method. Firstly, 50% inhibition concentration (IC50) was calculated by drawing percent inhibition against the sample concentrations. Then, up to IC 50 value samples were taken and ability to remove the DPPH radical was determined according to the method specified by POLAT *et al.* (2018). Results were expressed as a percentage (%).

*Determination of phenolic and organic acid compositions*

The polyphenols were seperated with an Agilent 1260 series HPLC system equipped with Ace C18 (4.6 mm × 150 mm, 5 µm) column. The flow rate of the mobile phase was kept at 0.5 mL $min^{-1}$. Mobile phase A was water containing 0.02% trifluoroacetic acid (TFA), and phase B was methanol containing 0.02% TFA. The gradient conditions were as follows: 0-5 min, 25% B; 5-10 min, 25-30% B; 10-16 min, 30-45% B; 16-18 min, 45% B; 18-25 min, 45-80% B; 25-30 min, 80% B; 30-40 min, 80-25% B. The temperature of the column was controlled at 25°C. Injection volume was 10 µL. The detection wavelengths of diode array detector (DAD) were set at four positions: 254, 275, 305, and 320 nm (WEN *et al.*, 2005).

The chromatographic separation was performed at 35°C for organic acids with an Agilent 1260 series HPLC system equipped with Ace C18 (4.6 mm × 150 mm, 5 µm) column. The flow rate of the mobile phase was kept at 0.6 mL $min^{-1}$. 0.01 M ammonium hydrogen phosphate $(NH_4)_2HPO_4$ was pepared as mobile phase and pH of the mobile phase was adjusted with $H_3PO_4$

to 2.4. Injection volume was 10 µL. Ultraviolet detection (UV) at 210 nm was used for determination of all organic acids (SELCUK and ERKAN, 2016).

*Statistical method*

Study was designed in accordance with the completely randomize experimental design with three replicates. The presence of statistical differences in the investigated properties of each plum cultivar in different altitudes were investigated using the t-test procedure in the Minitab-17 package program. Homogeneity of variance was checked for the all investigated characteristics before the analysis. Principal component analysis (PCA) was used to examine the interrelations among the observed set of variables to identify the similarities and differences of characteristics. In addition, scatter plot based on the first two principal components (PC1 and PC2) was generated. First component describes most of the variation in the data. The second principal component is orthogonal and covers much of the remaining variation (ZAR, 2013). Variance components of investigated properties were determined in the ASReml-R package program using the REML (Restricted Maximum Likelihood) method (GILMOUR *et al.*, 2006). Mathematical model and its elements used for predictions are given in formula (1).

$$y_{ijk} = \mu + L_i + G_j + (LG)_{ij} + e_{ijk} \qquad (1)$$

Here;

$y_{ijk}$ ; phenotypic value of the property (total phenol, antioxidant activity etc.),
$\mu$ ; population average,
$L_i$ ; i. effects of ith location (i = high altitude and low altitude),
$G_j$ ; j. effects of jth cultivar (j = 'Friar' and 'Fortune'),
$(LG)_{ij}$ ; effect of ith location and jth genotype together (interaction),
$e_{ijk}$ ; vector of random residual effect (error).

Calculated variance components were used for determination of broad-sense heritability ($h^2$), error (environmental effect) ($e^2$), genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) according to formulas (2, 3, 4 and 5).

$$h^2 = \frac{\sigma_G^2}{\sigma_P^2} \quad (2),$$

$$e^2 = \frac{\sigma_e^2}{\sigma_P^2} \quad (3),$$

$$GCV\% = \sqrt{\frac{\sigma_G^2}{\bar{X}}} X100 \quad (4),$$

$$PCV\% = \sqrt{\frac{\sigma_P^2}{\bar{X}}} X100 \quad (5).$$

In these formulas:

$h^2$; portion of genotypic variance in phenotypic variance,
$e^2$; portion of error in phenotypic variance,

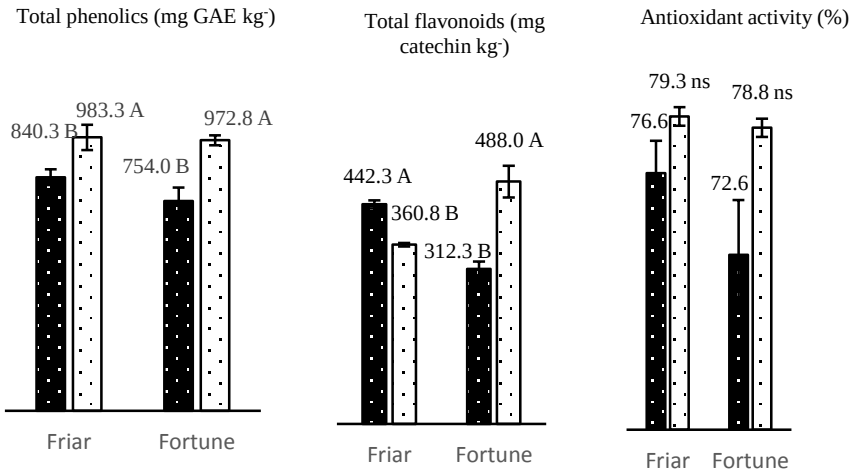
$\sigma_G^2$; genotypic variance,
$\sigma_p^2$; phenotypic variance,
$\sigma_e^2$; error variance,
$\bar{X}$; average of property.

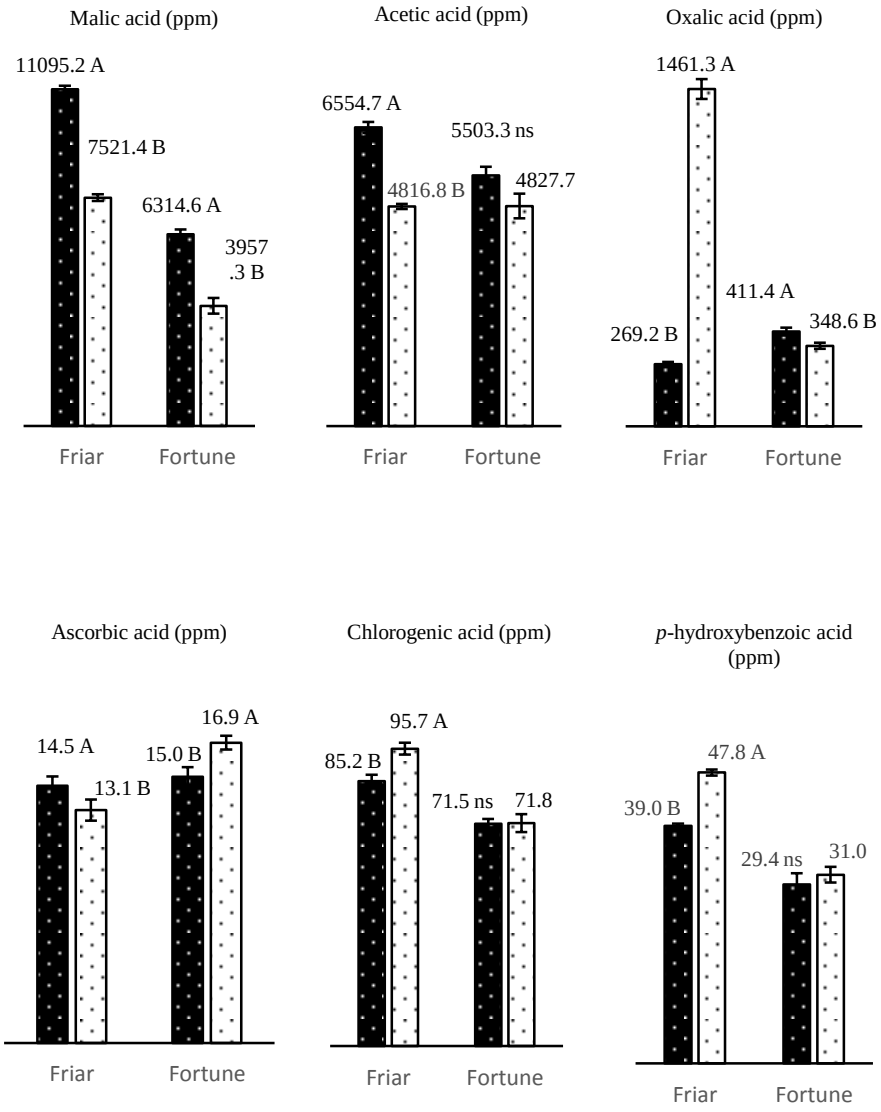
## RESULTS AND DISCUSSION

Findings of the investigated phytochemical compounds are given in Figure 1. In line with the obtained results, total phenolic content, total flavonoid content and antioxidant activity were varied from 754.0 ('Fortune') to 983.3 ('Friar') mg GAE $kg^{-1}$, from 312.3 ('Fortune') to 488.0 (Fortune) mg CAE $kg^{-1}$ and from 72.6% ('Fortune') to 79.3% ('Friar'), respectively.

In studies conducted with different plum genotypes, total phenolic content was varied between 635.7–705.8 mg GAE $L^{-1}$ (HARMAN and SEN, 2016); 380–8410 mg GAE $L^{-1}$ (SAHAMISHIRAZI *et al.,* 2017); 356.5–1300.0 mg GAE $L^{-1}$ (MUZZAFFAR and MASOODI, 2018); 642.8–1745.0 mg GAE $L^{-1}$ (TAITI *et al.,* 2019) and 854.9–1056.0 mg GAE $L^{-1}$ (MERTOGLU *et al.,* 2019). Similar results were recorded in study of COSMULESCU *et al.* (2015) (22.2–1606.7 mg quercetin $L^{-1}$) and DHINGRA *et al.* (2014) (2.06–47.96 mg quercetin $g^{-1}$) for total flavonoid content. Antioxidant activity levels determined by DPPH method were stated within the ranges of 15–65% (KİM *et al.,* 2012) and 49.10–87.94% (NAJAFABAD and JAMEİ, 2014).

Obtained results are in line with previous studies. Although the differences are thought to be mainly caused by genotype, differences in climate and soil characteristics, the geographical position/location, type and time of harvest, storage or processing of the crop, method or periodic differences of the applied cultural practices, cause significant differences on the fruit phytochemical composition (Lİ *et al.*, 2012; TİWARİ and CUMMİNS, 2013; GUNDUZ and OZBAY, 2018).




Malic acid (ppm)
11095.2 A
7521.4 B
6314.6 A
3957
.3 B
Friar
Fortune
Acetic acid (ppm)
6554.7 A
4816.8 B
5503.3 ns
4827.7
Friar
Fortune
Oxalic acid (ppm)
1461.3 A
269.2 B
411.4 A
348.6 B
Friar
Fortune
Ascorbic acid (ppm)
14.5 A
13.1 B
15.0 B
16.9 A
Friar
Fortune
Chlorogenic acid (ppm)
85.2 B
95.7 A
71.5 ns
71.8
Friar
Fortune
p-hydroxybenzoic acid (ppm)
39.0 B
47.8 A
29.4 ns
31.0
Friar
Fortune

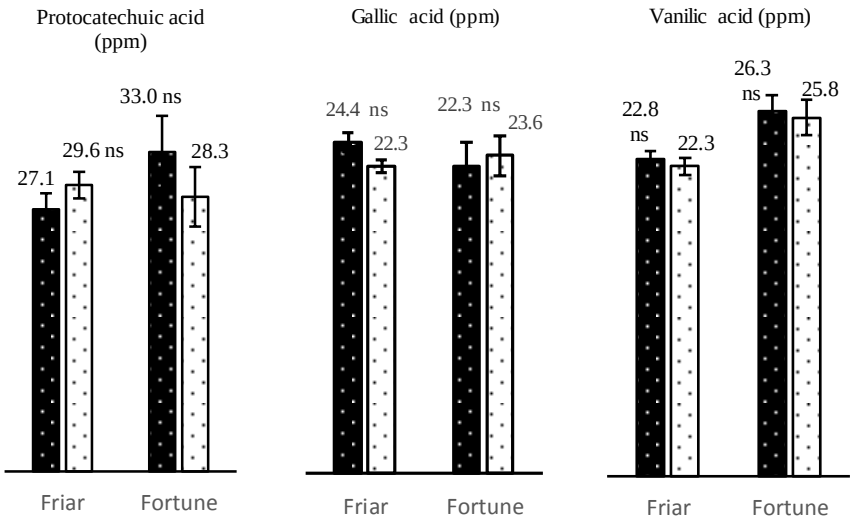



Figure 1. Phytochemical compounds of plum cultivars depending on altitudes (While the black columns represent the high altitude, white columns represent the lower altitude. (Different letters are mean significant difference between altitudes for each cultivar and ns represents no significance)

Malic acid was determined as the dominant organic acid of plum cultivars by MELGAREJO *et al.* (2012) and İONİCA *et al.* (2013) and the result of this study is similar with theirs. Ranking of organic acids at both altitudes was found as malic acid> acetic acid> oxalic acid> ascorbic acid both in 'Friar' and 'Fortune'. Quantities of malic and acetic acids were responded positively to altitude increase in both cultivars increasing by 48% and 36% in 'Friar' and 60% and 14% in 'Fortune', respectively. Oxalic acid was increased in 'Fortune' with the increase of altitude from 348.6 to 411.4 ppm, while 'Friar' showed a decrease from 1461.3 to 269.2 ppm. On the other hand, opposite situation was observed for ascorbic acid. Decline was determined with altitude increase in 'Fortune' from 16.9 to 15.0 ppm, while it was increased in 'Friar' from 13.1 to 14.5 ppm.

In terms of phenolic acids, a stable order could not be determined, while chlorogenic acid was the most abundant in both cultivars. Also, amounts of protocatechuic acid, *p*-hydroxybenzoic, gallic and vanilic acids, had higher content than *p*-coumaric, caffeic, syringic and rutin in both plum cultivars (Figure 1). Obtained results are in agreement with DONOVAN *et al.* (1998) and USENIK *et al.* (2013). Amount of *p*-hydroxybenzoic acid was detected lower at higher altitude in both cultivars, while chlorogenic, vanilic, syringic, caffeic and *p*-coumaric acids were found higher at high altitude. Protocatechuic acid and rutin were higher at higher

altitude in 'Fortune', while these compounds had higher values at low altitude in 'Friar'. On the contrary, gallic acid was found higher level in 'Friar' at higher altitude while this sontent was higher in 'Fortune' at low altitude.

Differences between diurnal and nocturnal temperatures and the light intensity are increase at higher altitudes. These conditions provide more synthesis of the primary and secondary metabolite such as organic and phenolic acids, during increased photosynthesis (MDITSHWA *et al.,* 2013; GUNDUZ and OZBAY, 2018). Amount of all organic and phenolic acids examined in the study, were found at higher level with the increase in altitude at least in one cultivar except for p-hydroxybenzoic acid (Figure 1). This may also be caused by higher UV rays at higher altitudes. As a defense mechanism against the harmful effects of high UV rays, plants produce phenolic compounds with high UV absorbing ability in epidermal tissues, in the phenylpropanoid pathway (VERDAGUER *et al.,* 2017). JOSUTTIS *et al.* (2010) reported that the quantity of phenolic acids in strawberries grown in open field were determined higher than those cultivated inside UV-blocking plastic tunnels because of exposed to higher UV. Although the amount of individual phenolic acids increased with the increase of altitude in general, there were decrease in the total phenolic content and antioxidant activity in both cultivars. This may be caused by other phenolic compounds that have not been examined. The total flavonoid content was higher at low altitude in 'Fortune' while it was higher at high altitude in 'Friar' cultivar.

Genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV) and heritability of investigated characteristics are given in Table 2. PCV refers to the range of variation for any property (LYNCH and WALSH, 1998; INGVARSSON and STREET, 2011). In this context, low variation was found in antioxidant activity (4.42%), total phenolic content (8.79%), acetic acid (10.32%), gallic acid (10.56%), vanillic acid (12.38%) and ascorbic acid (13.26%), while higher variations were observed for *p*-coumaric acid (40.31%) and oxalic acid (76.26%). Middle group consists of chlorogenic acid (17.41%), protocatechuic acid (18.48%), total flavonoid content (19.82%), syringic acid (20.07%), caffeic acid (22.34%), malic acid (24.26%) and *p*-hydroxybenzoic acid (26.51%) in terms of variation.

Less difference between GCV and PCV is interpreted as low environmental effect in the heredity (ISLAM *et al.,* 2010; RAKONJAC *et al.,* 2015). High broad-sense heritabilities of *p*-coumaric acid (95.80%), *p*-hydroxybenzoic acid (90.62%) and chlorogenic acid (87.31%) that have close GCV and PCV values confirm this information. Lowest heritabilites were determined in general chemical characteristics such as total phenolic content (7.68%), total flavonoid content (8.68%) and antioxidant activity (11.30%). This situation is thought to be caused by the fact that these features are cumulative effect of many characters for instance phenolic compounds and antioxidative enzymes etc. (DE SOUZA *et al.,* 2012). KARAAT and SERCE (2020), reported that the inheritance of general phytochemical characteristics varies greatly in apricot and heritabilities of these characteristics were found at lower level in raspberry (STEPHENS *et al*., 2009). Ranking of heritabilities for other individial compounds found as caffeic acid (28.90%) < acetic acid (35.22%) < malic acid (34.25%) < oxalic acid (45.32%) < ascorbic acid (%53.33) < syringic acid (63.29%) < vanillic acid (63.30%) < protocatechuic acid (66.72%) < gallic acid (71.52%). Broad-sense haritabilities of phytochemical properties are comparable with the literature data (ONOMO *et al.,* 2015; KARAAT and SERCE, 2020).

*Table 2. Genetic parameters of investigated chemical compounds*

| Characters | GCV (%) | PCV (%) | $h^2$ (%) | $e^2$ (%) |
|---|---|---|---|---|
| Total phenolic content | 2.44 | 8.79 | 7.68 | 92.32 |
| Antioxidant activity | 1.48 | 4.42 | 11.30 | 88.70 |
| Total flavonoid content | 4.58 | 19.82 | 8.68 | 91.32 |
| Ascorbic acid | 9.69 | 13.26 | 53.33 | 46.67 |
| Oxalic acid | 51.34 | 76.26 | 45.32 | 54.68 |
| Malic acid | 14.20 | 24.26 | 34.25 | 65.75 |
| Acetic acid | 6.12 | 10.32 | 35.22 | 64.78 |
| *p*-hydroxybenzoic acid | 25.23 | 26.51 | 90.62 | 9.38 |
| Chlorogenic acid | 16.27 | 17.41 | 87.31 | 12.69 |
| *p*-coumaric acid | 39.46 | 40.31 | 95.80 | 4.20 |
| Gallic acid | 7.90 | 10.56 | 71.52 | 28.48 |
| Protocatechuic acid | 13.22 | 18.48 | 66.72 | 33.28 |
| Vanillic acid | 9.85 | 12.38 | 63.30 | 36.70 |
| Caffeic acid | 12.01 | 22.34 | 28.90 | 71.10 |
| Syringic acid | 15.97 | 20.07 | 63.29 | 36.71 |

GCV: Genotypic coefficient of variation, PVC: Genotypic coefficient of variation, $h^2$: Broad-sense heritability, $e^2$: error (environmental effect)

Principal component analysis (PCA) was used in order to reveal the relations among chemical caharacteristics and variations caused by the characteristics investigated. Same method was prefered in lots of fruit species such as apricot (Ruiz and Egea, 2008), peach-nectarine (CANTIN *et al.*, 2010), almond (SORKHEH *et al.*, 2009), strawberry (KAFKAS *et al.*, 2007) and plum (BOZOVIC *et al.*, 2017) for the determination of genetic relations, better cultivar or correlations and interrelations among investigated properties. Results from the PCA, indicate that the first three components explain about 73.8% of the total variability which is similar to results of KHALILI *et al.* (2019) and GUNDUZ and SARACOGLU (2012) in plum (Table 3). PC1 represent mainly *p*-hydroxybenzoic acid, chlorogenic acid, *p*-coumaric acid and rutin characteristics and explained 37.4% of the total variation in the data set while PC2 explained 21.7% of the total variation with ascorbic acid, oxalic acid and malic acid content. Total phenolic content, antioxidant activity and total flavonoid content were the most important compounds which affected on third component that corresponds to 14.7% of total variation (Table 3). These results can be interpreted as the fact that variation caused by altitude difference occurs largely in phenolic compounds followed by organic acids and in general phytochemical characteristics.

Scatter plot belonging to PC1 and PC2 (59.1% of total variance) for the analysed compounds of plum fruit were showed in Figure 2. Phytochemical properties were predominantly gathered on the right side of the chart (positive side of PC1) along with 'Friar' cultivar means that 'Friar' was found richer in general than 'Fortune' in terms of phytochemical characteristics. Expecially for total phenolic content and antioxidant activity that are important parameters for selecting cultivars with more significant health promoting characteristics intended for production of functional foods and breeding new superior genotypes by using as a parent. In

addition, organic acids that ensure the stability of the products and limit the activity of microorganisms that causing decays in products were found at a higher level in ‘Friar.

Total phenolic and flavonoid contents were found in positive correlation with antioxidant activity, due to the antioxidative effects of phenolic compounds (DAI and MUMPER, 2010). This finding is in agreement with the study conducted in plum by NAJAFABAD and JAMEİ (2014). On the other hand, negative correlation was detected between ascorbic acid and antioxidant activity, as reported by OKATAN (2020). This situation probably caused by the lower amount of askorbic asit. Since all other organic acids were positively correlated with antioxidant activity. This suggesting that increasing acidity seems to be very important in terms of increasing antioxidant activity as stated by MERTOGLU and EVRENOSOGLU (2019). Also, HUANG *et al.* (2018), said that relatively high acidity is an important breeding objective by affecting the flavor, color, clarity and the overall sensory acceptance.

*Table 3. Eigenvalues, proportion of total variability and correlation between the variables at the first three principal components (PCs).*

| | PC1 | PC2 | PC3 |
|---|---|---|---|
| Total phenolic C. | 0.150 | -0.049 | -0.507 |
| Antioxidant activity | 0.204 | -0.007 | -0.336 |
| Total flavonoid C. | 0.049 | 0.393 | -0.400 |
| Ascorbic acid | -0.271 | 0.371 | -0.245 |
| Oxalic acid | 0.236 | -0.367 | -0.102 |
| Malic acid | 0.204 | 0.433 | -0.000 |
| Acetic acid | 0.074 | 0.366 | 0.449 |
| *p*-hydroxybenzoic acid | 0.369 | -0.103 | -0.047 |
| Chlorogenic acid | 0.363 | -0.131 | 0.034 |
| *p*-coumaric acid | 0.357 | 0.038 | 0.242 |
| Gallic acid | 0.046 | 0.319 | -0.027 |
| Protocatechuic acid | -0.129 | -0.292 | 0.166 |
| Vanillic acid | -0.316 | 0.099 | -0.069 |
| Caffeic acid | -0.219 | 0.125 | 0.222 |
| Syringic acid | 0.274 | 0.116 | 0.243 |
| Rutin | -0.347 | -0.231 | 0.030 |
| Expl.Var | 5.99 | 3.47 | 2.35 |
| Prp.Totl | 0,374 | 0,217 | 0,147 |
| Cumulative (%) | 37.4 | 59.1 | 73.8 |

Both phenolic and organic acids were distributed on both sides of the chart in themselves. Some of them diagonally positioned such as oxalic acid and ascorbic acid or vanilic acid and chlorogenic acid means that they are showed negative correlation with each other. Similar tendency were reported in different fruit species (SHA *et al.*, 2011; OKATAN, 2020). This case may be due to the fact that these compounds transform into each other when needed, although the co-location of major quantitative trait loci (QTLs) for these characteristics were reported on the same linkage groups (LG1 and LG6) (SALAZAR *et al.*, 2017).

Positioning of cultivars in top and bottom according to locations shows the importance of cultivar/location interaction. In line with the change of location, it has been reported that quality parameters including phytochemical composition have substantially undergone a change in plums (GUERRA and CASQUERO, 2009). Since cultivar/location interactions found significant, investigate the changes at genotype level would be more accurate.

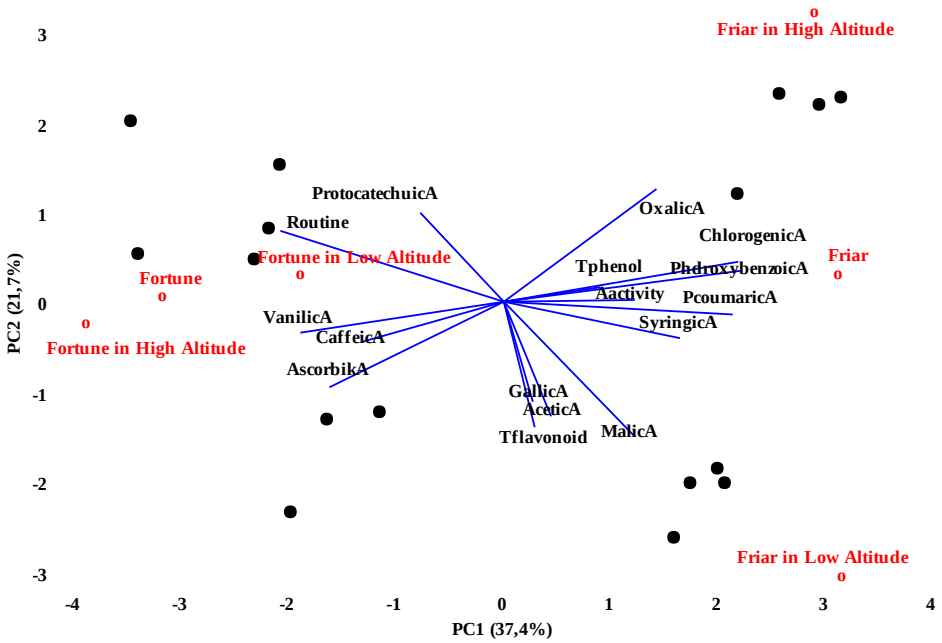



Figure 2. Bi-plot segregation of phytochemical characteristics along with cultivars

According to the obtained results, phytochemical content were changed in line with the altitude change. However, due to the significant effect of cultivar/location interaction on these parameters, it was concluded that the phytochemical difference should be examined at the genotype level. Thus, it is very important to identify cultivars specific to ecological conditions when laying out garden in order to obtain high quality fruits. Owing to the contribution of individual organic acids to antioxidant activity, high acidity must be taken into consideration for the development of cultivars with exceptional phytochemical content. Since the heritabilities of the features are not low with the exception of general properties, genetic advance could be achieved by selection after hybridizations in breeding programmes.

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# ISTRAŽIVANJE PROMENE GENETSKIH PARAMETARA I FITOHEMIJSKIH KARAKTERISTIKA ŠLJIVE SA PROMENOM NADMORSKE VISINE

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Izvod

Pogodnost ekoloških faktora je najvažnija za poljoprivrednu produktivnost. Osetljivost fitohemijskih karakteristika koje određuju kvalitet proizvoda u promenljivim uslovima sredine je veoma visoka. U ovoj studiji istraženo je kako klimatske promene (promena nadmorske visine), utiču na fitohemijske osobine kod sorti šljive 'Friar' i 'Fortune' koje su ubrane na dve različite nadmorske visine (200 m i 800 m n.v.), tokom dve uzastopne godine (2017. 2018). Pored toga, u okviru ekološkog i genotipskog modela, određeni su genetski parametri osobina. U skladu sa dobijenim rezultatima, utvrđeno je da su fitohemijske karakteristike varirale sa promenom nadmorske visine unutar iste geografske širine. Generalno, količina pojedinačnih fenolnih jedinjenja i organskih kiselina je povećana sa povećanjem nadmorske visine, dok su opšte fitohemijske karakteristike kao što su ukupan sadržaj fenola i antioksidativna aktivnost smanjene. Heritabilnost opštih karakteristika je niža, zbog velike razlike između genetičkog koeficijenta varijacije (GCV) i fenotipskog koeficijenta varijacije (PCV) u odnosu na organske i fenolne kiseline. Utvrđeni rang organskih kiselina na obe nadmorske visine u obe sorte je jabučna kiselina> sirćetna kiselina> oksalna kiselina> askorbinska kiselina. Što se tiče fenolnih kiselina, nije mogao da se utvrdi stabilan redosled, dok je hlorogena kiselina došla u prvi plan u obe sorte. Pošto je interakcija sorta/lokacija bila značajna prema bi-plot segregaciji, ispitivanje promena na nivou genotipa bi bilo tačnije.

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