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Determination of Fatty Acids, Tocopherols, Selenium and Total Carotene of Walnut Genotypes and Evaluations by Cluster, PCA and Correlation Analysis

Songul Acar[1] · **Ahmet Kazankaya**[2] · **Adnan Doğan**[3]

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Abstract

Plant genetic diversity creates unique and unrivaled resources for future climate change scenarios. Knowledge of these genetic resources and their sustainable management are essential for the development of conservation strategies. Selection studies are also the basis of these investigation. In this study, 27 selected walnut genotypes grown in the Batman region of Southeastern Anatolia were assessed for fatty acid composition, total carotene content, selenium, as well as their tocopherol content. The total fat varied between 53.25 and 68.70%. The fatty acid content was found to be 38.86–64.37% for linoleic acid, 16.27–38.20% for oleic acid, 9.58–19.38% for linolenic acid, 4.09–6.55% for palmitic acid and 1.78–4.05% stearic acid. The amount of α-tocopherol, γ-tocopherol and δ-tocopherol were found to be 2.25–6.32 mg kg^{-1}, 38.20–136.27 mg kg^{-1} and 1.65–6.21 mg kg^{-1}, respectively, and total tocopherol content was 89.85 mg kg^{-1}. Selenium contents of genotypes were between 12.28 and 65.32 mg g^{-1}. The genotypes were found to have 0.19–0.56 mg kg^{-1} total carotene. Cluster and PCA analyses were performed and two major clusters were identified. Compared to cluster A, 72-K-11, 72-K-129, 72-S-48 and 72-K-92 genotypes in cluster B generally had the highest kernel ratio (52.609%), oleic acid (30.26%), linoleic acid (55.02%), linolenic acid (18.14%), γ-tocopherol (122.31 mg kg^{-1}), δ-tocopherol (4.45 mg kg^{-1}) and selenium (50.25 mg g^{-1}). The present results emphasize that the walnut genotypes selected from the Southeastern Anatolia Region can make an important contribution to human nutrition with their rich fatty acids, tocopherols and selenium contents.

Keywords *Juglans regia* L. · Selection · Nutritional content · Genetic resources · Variation

Introduction

Walnut, which is the main fruit of Türkiye, is grown economically in many parts of the world and has a highly respected place among the hard-shelled fruits (Sen et al. 2011; Kazankaya et al. 2022). The most important walnut producing countries in the world are China, USA, Iran, Türkiye and Ukraine (Faostat 2022). Due to the favorable climate, walnut cultivation is carried out in almost every region of Türkiye. Genotypes with superior nutritional values can be selected from this wide variety of populations. These genotypes can compete with other commercial varieties of high economic value. Therefore, identifying promising genotypes has been an important step to start breeding programs.

Walnuts have been one of the most important parts of human nutrition for thousands of years due to their high mineral, antioxidant and vitamin content. Observation studies have concluded that daily regular consumption of walnuts increases good cholesterol (HDL cholesterol) in patients (Tufail et al. 2015).

Walnut is a valuable food source that can replace animal protein source and has high unsaturated fatty acid content. It is reported that the proximate composition of walnut has 13.6–22.3% protein, 56.4–70.6% fat and 2% ash (Sahin and Akbaş 2001; Binici et al. 2021). Walnuts are appreciated for their high content of polyunsaturated fatty acids as well as high tocopherol and potassium (Segelke et al. 2020).

✉ Songul Acar
songulacar1976@gmail.com

1 Department of Breeding and Genetics, Olive Research Institute, 35100 Izmir, Turkey

2 Faculty of Agriculture, Department of Horticulture, Ahi Evran University, 40100 Kırsehir, Turkey

3 Faculty of Agriculture, Department of Horticulture, Van Yuzuncu Yıl University, 65080 Van, Turkey

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They are very rich in thiamin, vitamin B6, many vitamins including folacin and also iron, zinc, copper, magnesium, phosphorus and potassium elements. Walnuts do not contain cholesterol, they are also very rich in terms of linoleic acid and linolenic acid, which are necessary for a healthy life (Haskınacı 2003). There are 2.5 g of alpha-linolenic acid in 1 ounce (28.3 g) of walnuts. This rate is negligible in other hard-shelled fruits (TCWB 2022). Studies have concluded that daily regular consumption of walnuts increases good cholesterol (HDL cholesterol) in patients (Tufail et al. 2015).

Walnut, which is widely available in Batman due to favorable climate and soil conditions, is a common fruit species with many naturally grown genotypes with different biological and economic characteristics of the fruit. This situation reveals the genetic richness of this region. With these rich genetic variation enables success in breeding studies in a short time. Selection breeding studies form the basis of fruit breeding studies. Although important progress has been achieved as a result of walnut selection studies carried out in different regions in our country, there are still walnut populations waiting to be examined in detail. Therefore, the aim of this study is to determine the fatty acids, tocopherols, total carotene, and selenium contents of different walnut genotypes selected from Batman region of Southeastern Anatolia. These data can contribute to the selection of potential varieties with good ecological compatibility, superior nutritional properties and not lagging behind walnut varieties from other countries.

Materials and Methods

This research was carried out between 2014 and 2016 in the Sason and Kozluk Districts of Batman Province, which have rich walnut gene resources in the Southeastern Anatolia region (Fig. 1). Walnut trees in the region are usually produced from seedlings. Therefore, walnut trees are not uniform and do not qualify as any standard walnut variety.

A total of 27 promising walnut genotypes was preselected from 150 genotypes studied in the Batman region for 3 years (2014–2016) using the "Weigh Ranked Method" by their fruit quality, lateral bud flowering and late leafing characteristics. Mature walnut fruits of walnut genotypes were harvested from Batman province of Türkiye in November 2016. The samples were stored in shell in paper bags at ambient temperature in room conditions for 1–2 months until the chemical analysis. Fruit samples of 27 walnut genotypes were randomly prepared with three replicates for chemical analysis.

Fruit Characteristics

Pomological properties such as nut weight, kernel weight, kernel ratio and shell thickness were determined in 20 fruits for each genotype (Şen 1980; Yarılgaç 1997; Muradoğlu 2005; Yılmaz 2007).

Chemical Analysis

Fatty Acid (FAMEs) Analysis

Oil fatty acids were determined according to the standard AOAC (1990) procedure. For gas-chromatographic (GC) analysis, fatty acid methyl esters (FAMEs) were prepared by dissolving 0.4 g oil in 4 mL of isooctane and methylated in 0.2 mL 2 M methanolic KOH. Analysis of FAME was performed on an Agilent 6890 series gas-chromatography equipped with flame-ionization detector and a 60-m capillary column (ID ¼ 0.25 mm) coated with 0.25 µm of 50% cyanopropyl-methylpolysiloxane (JandW Scientific, Folsom, CA, USA). Helium was used as a carrier gas at a flow



Fig. 1 Location map of the study area

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rate of 1.5 mL/min and a split ratio of 1:10. Injector temperature was 250 °C, detector temperature was 260 °C and the oven temperature was programmed at 120 °C for a hold of 5 min and increased to 240 °C at a rate of 15 °C/min and hold at the final temperature for 20 min (Kirazci and Javidipour 2008). FAMEs were identified by comparison of their retention time and equivalent chain length with respect to standard FAMEs (Supelco, 47885-U). FAMEs in samples were quantified according to their percentage area (AOAC 1990). All the samples were analyzed in triplicate.

Tocopherols

The tocopherol profiles of the samples were determined using a high performance lipid chromatography (HPLC) technique as previously described by Surai et al. (1996) and Surai (2000). A total of 0.5 g of sample was saponified with ethanolic KOH in the presence of pyrogallol and the tocopherols, tocotrienols and carotenoids were extracted from the mixture with hexane (5 mL). The extraction was repeated twice more with 5 mL hexane. Hexane extracts were combined, evaporated and re-dissolved in a mixture of methanol/dichloromethane (1:1, v/v). The extract was dried under nitrogen, redissolved in methanol and injected onto a Spherisorb S30DS2, 3 µm C_{18} reverse phase HPLC column, 15 cm x 4.6 mm (Phase Separations, Clwyd, UK).

Chromatography was performed using a mobile phase of methanol/water (97:3, v/v) at a flow rate of 1.1 mL/min. Fluorescence detection utilized excitation and emission wavelengths of 295 and 330 nm, respectively. Calibration was performed using standard solutions of tocopherols (Sigma, Poole, UK).

Total Carotenes

Total carotene analysis was carried out as described by Karadaş et al. (2005) using the same HPLC system from the same extract but fitted with a Spherisorb type S5NH2, 5 µm C_{18} reverse phase HPLC column, 25 cm x 4.6 mm (Phase Separations, Clwyd, UK). Chromatography was performed using a mobile phase of methanol/water (97:3 v/v) at a flow rate of 1.5 mL/min. Total carotene was identified as a single peak at 445 nm and concentrations were calculated in relation to a lutein standard (Sigma-Aldrich, Poole, UK) (Karadaş et al. 2005).

Selenium

Selenium concentration was determined using hydride formation atomic fluorescence spectroscopy of samples digested with nitric acid/perchloric acid/water/sulfuric acid (50/25/17.4/7.6 by volume) (Hershey et al. 1988). Selenium in the subsequent residue was dissolved in 5 mL HCL (6 mol/L) and heated at 120 °C for 30 min. Hydride generation utilized solutions of NaBH4 (0.32 mol/L)/NaOH (1 mol/L) and HCl (3 mol/L) at flow rates of 3.8 and 8 mL/min, respectively. The HCl solution, the sample, and the NaBH4 solution were pumped through a pump head and mixed together in a solenoid valve. The boosted discharge hollow cathode lamp (Superlamp Se, Photon PTY Ltd., Australia) was set at 25 mA and 196 nm wavelength as the excitation source. Atomic fluorescence was measured by a detector (Model 10.033, PS Analytical Ltd., Kent, UK) and the output recorded using Avalon™ Software (PS Analytical Ltd., Kent, UK). Concentrations in the samples were calculated from the linear relationship ($r^2 = 0.999$) obtained using Spectrosol Selenium Standard Solution (BDH, Poole, UK).

Data Analysis

Data from three replications for chemical analysis were analyzed by two-way analysis of variance using SPSS 20.0 for Windows program. Significance level was established at $P < 0.05$. Hierarchical clustering was performed by the Ward method using in JMP® Pro 13.2.1 program (SAS Institute, Cary, NC) (Murtagh and Legendre 2014). Correlation, cluster and principal component analysis (PCA) were performed to determine relationships of features. The R Studio statistical software was used in the analysis and data visualization. The package 'corrplot' (Wei and Simco 2017) was used to perform correlation analysis, and the package 'ggplot2' (Wickham 2016) was used for PCA.

Results and Discussion

Fatty Acid Methyl Esters

Fatty acid composition of selected walnut genotypes was shown Table 1. The total oil content of the kernels was in the range of 53.25–68.70%, with an average fat content of 62.92%. These results were similar to those reported previously Gulsoy et al. (2019) and Jaćimović et al. (2020). It was stated by Martinez et al. (2010) that walnut is a nutritious food due to its oil content (740 g/kg in some commercial varieties), which can be easily extracted and consumed unrefined. Pandele (1968) reported that the oil rate should be at least 65% and the protein rate should be at least 16% in varieties to be considered as breeding material.

The contents of eight fatty acids in this study were determined and presented Table 1. As the FAMEs percentage, the walnut genotypes had 38.86–64.37% linoleic acid, 16.27–38.20% oleic acid, 9.58–19.38% linolenic acid, 4.09–6.55% palmitic acid and 1.17–4.05% stearic acid. As

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Table 1 Fatty acid composition of selected walnut genotypes

Genotype No.	Total fat (%)	Palmitic acid C16:0	Palmitoleic acid C16:1	Stearic acid C:18:0	Oleic acid C18:1	Linoleic acid C18:2	Linolenic acid C18:3	Arachidic acid C20:0	Behenic acid C22:0
72-S-03	66.34 ^{ab} *	5.26 $^{a-e}$	0.07 ^{ns}	3.05 $^{a-c}$	33.45 ^{bc}	40.47 ^{lm}	13.44 $^{c-e}$	0.08 ^{ns}	0.23 ^{ns}
72-S-04	65.83 ^{ab}	5.12 $^{a-e}$	0.12	2.48 $^{b-d}$	38.20 ^{a}	46.55 $^{h-k}$	12.30 ^{de}	0.16	0.21
72-S-05	68.70 ^{a}	5.38 $^{a-e}$	0.08	2.35 ^{cd}	38.14 a	46.55 $^{i-k}$	16.30 $^{a-c}$	0.08	0.22
72-K-11	58.42 $^{d-f}$	5.30 $^{a-e}$	0.06	2.45 ^{cd}	30.10 $^{c-e}$	46.40 ^{jk}	19.38 ^{a}	0.14	0.19
72-K-12	63.10 ^{bc}	5.92 ^{ab}	0.12	2.69 ^{bc}	26.50 ^{gh}	46.31 ^{jk}	15.56 ^{cd}	0.11	0.18
72-K-16	63.00 ^{bc}	4.59 $^{c-e}$	0.09	4.05 ^{a}	26.34 ^{gh}	47.42 $^{h-j}$	16.30 ^{bc}	0.14	0.18
72-K-17	53.25 ^{g}	5.90 ^{ab}	0.18	3.12 ^{ab}	26.32 ^{gh}	43.24 ^{kl}	13.22 ^{de}	0.08	0.17
72-K-27	68.12 ^{a}	5.40 $^{a-d}$	0.08	2.47 ^{cd}	30.10 $^{c-e}$	47.89 $^{h-j}$	11.20 ^{e}	0.12	0.19
72-K-29	60.36 ^{cd}	4.80 $^{b-e}$	0.09	2.76 ^{bc}	16.27[1]	52.31 ^{de}	15.40 ^{cd}	0.15	0.15
72-S-48	64.12 ^{b}	6.18 ^{a}	0.09	3.56 ^{a}	32.27 ^{e}	64.37 ^{a}	18.47 ^{a}	0.21	0.18
72-S-53	66.12 ^{ab}	5.30 $^{a-e}$	0.05	2.40 ^{cd}	20.41 ^{jk}	52.28 ^{e}	14.97 ^{cd}	0.06	0.16
72-K-92	64.02 ^{b}	6.24 ^{a}	0.11	2.76 ^{bc}	21.85 ^{ij}	59.85 ^{b}	18.21 ^{ab}	0.08	0.23
72-K-95	60.10 ^{d}	6.55 ^{a}	0.10	2.27 ^{cd}	27.45 $^{f-h}$	56.34 ^{c}	9.68 ^{e}	0.09	0.26
72-K-118	65.50 ^{ab}	4.26 ^{e}	0.12	2.46 ^{cd}	28.52 ^{ef}	39.55 ^{m}	16.33 $^{a-c}$	0.09	0.10
72-K-120	64.52 ^{b}	5.20 $^{a-e}$	0.09	3.21 ^{ab}	29.41 ^{de}	60.30 ^{b}	9.58 ^{e}	0.12	0.22
72-K-128	59.42 ^{de}	4.09 ^{e}	0.07	2.85 ^{bc}	20.34 ^{k}	44.89 ^{k}	12.85 ^{de}	0.08	0.14
72-K-129	55.16 ^{g}	6.05 ^{a}	0.06	2.12 ^{cd}	36.80 ^{ab}	49.45 ^{gh}	16.49 $^{a-c}$	0.08	0.15
72-K-135	65.45 ^{ab}	5.07 $^{b-e}$	0.12	3.56 ^{a}	26.14 ^{h}	50.47 ^{fg}	16.15 ^{c}	0.13	0.17
72-K-136	60.50 ^{cd}	4.36 ^{e}	0.07	1.78 ^{d}	27.61 ^{fg}	38.86 ^{m}	13.47 ^{cd}	0.09	0.21
72-K-143	65.21 ^{b}	5.10 $^{b-e}$	0.06	3.11 ^{ab}	29.45 ^{de}	53.96 ^{d}	13.85 ^{cd}	0.09	0.20
72-K-144	55.43 ^{fg}	4.53 ^{de}	0.14	2.81 ^{bc}	29.85 $^{c-e}$	47.80 $^{h-j}$	13.27 ^{de}	0.10	0.18
72-K-145	68.25 ^{a}	5.98 ^{a}	0.07	2.85 ^{bc}	29.33 ^{e}	63.12 ^{a}	13.85 ^{cd}	0.08	0.23
72-K-146	65.00 ^{b}	5.29 $^{a-e}$	0.08	2.69 ^{bc}	20.11 ^{k}	50.20 ^{fg}	13.35 ^{de}	0.09	0.20
72-K-147	65.12 ^{b}	5.44 $^{a-c}$	0.09	2.47 ^{cd}	23.14 ^{i}	50.84 ^{ef}	11.82 ^{e}	0.11	0.19
72-K-148	58.20 ^{ef}	4.84 $^{b-e}$	0.09	2.14 ^{cd}	30.84 ^{e}	60.19 ^{b}	12.10 ^{e}	0.09	0.18
72-K-149	63.22 ^{b}	5.41 $^{a-c}$	0.07	1.96 ^{d}	19.42 ^{k}	48.72 ^{hi}	13.59 ^{cd}	0.06	0.18
72-K-150	66.34 ^{ab}	5.60 ^{ab}	0.08	2.87 ^{bc}	30.41 ^{cd}	40.33 ^{m}	12.45 ^{de}	0.10	0.20
Min	53.25	4.09	0.05	1.78	16.27	38.86	9.58	0.06	0.10
Max	68.70	6.55	0.18	4.05	38.20	64.37	19.38	0.21	0.26
Mean	62.92	5.30	0.09	2.71	27.73	49.95	14.21	0.10	0.19

s Statistical analysis was not performed
*Different letters in the same column indicate significant variance in the level of 0.05. *ns* Not significant

a result of the analysis, linoleic acid took the first place with an average of 49.95% as the main fatty acid in genotypes. Linoleic acid was followed by oleic acid with an average of 27.73%, linolenic acid with 14.21%, palmitic acid with 5.30% and stearic acid with 2.71%, respectively. In addition, three different fatty acids were detected in the genotypes at rates below 1% or in trace amounts. Average values of behenic acid of 0.19%, arachidic acid of 0.10% and palmitoleic acid of 0.09% were found in all genotypes (Table 1). There was no statistically significant difference in terms of arachidic acid and behenic acid values in the examined genotypes. Similar results have been reported in previous studies conducted in different countries in walnut studies. It was reported by Bada et al. (2010) that the fatty acid composition of 15 walnut varieties in Spain varied between 59.81–64.77% for linoleic, 11.70–18.90% for oleic, 11.11–15.65% for linolenic and 6.11–7.49% for palmitic acid. Özcan and Sütyemez (2019) determined of different 10 walnut genotypes in Türkiye that the total fat content of was 62.60% and stearic acid ranged between 2.89–2.97%, myristic acid 0.02–0.03%, palmitic acid 6.87–7.06%, linoleic acid 60.93–62.23%, a-linolenic acid 11.01–11.62%, oleic acid 14.97–16.65% and palmitoleic acid 0.07–0.08%, respectively. Fatty acid composition ranges for 50 promising walnut genotypes in Türkiye noted by Uzun et al. (2021) palmitic acid 2.04–7.83%, stearic acid 0.62–6.66%, oleic acid 13.14–47.28% and linoleic acid 32.52–65.47%, respectively. The chemical compositions were studied in six walnuts varieties (cv. 'Franquette', 'Lara', 'Marbot', 'Mayette', 'Mellanaise' and 'Parisienne') produced in Portugal by Pereira et al. (2008). Linoleic acid varied between 55.51% (Franquette)—60.30% (Lara);

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oleic acid 14.92% (Franquette)—20.22% (Lara); linolenic acid 13.2% (Parisienne)—17.61% (Mayette); palmitic (5.95–6.61%) and stearic acids (2.70–3.07%) showed considerable amounts. In this study, fatty acid contents of promising genotypes were found close to or higher than important varieties.

Tocopherols

Walnut, which is an excellent source of vitamin E, has two forms of vitamin E in its oil, alpha (α) tocopherol and gamma (γ) tocopherol. There is 21 mg of (γ)-tocopherol (Vitamin E) in 100 g of walnuts and this amount meets 140% of our daily need (Sen and Karadeniz 2015). Tocopherol contents of the examined samples in this study were determined and the relevant results are given in Table 2. The walnut genotypes contained α-tocopherol 2.25–6.32 mg kg^{-1}, γ-tocopherol 38.20–136.27 mg kg^{-1} and δ-tocopherol 1.65–6.21 mg kg^{-1}, and their mean values were found to be 4.37 mg kg^{-1}, 81.78 mg kg^{-1} and 3.75 mg kg^{-1}, respectively (Table 2). Total tocopherol content was determined as 89.85 mg kg^{-1}. Among the tocopherols, gamma tocopherol (γ), alpha (α) and delta (δ) tocopherols were found to be in very high amounts. γ-Tocopherol was the most abundant tocopherol in this study (Table 2). It has been reported in previous studies that walnuts are rich in fibres, proteins and minerals and are also characterized by a high antioxidant capacity due to the phenolic compound found in the seed coat and tocopherols in the lipid fraction (Salcedo et al. 2010; Dordoni et al. 2021). The tocopherol

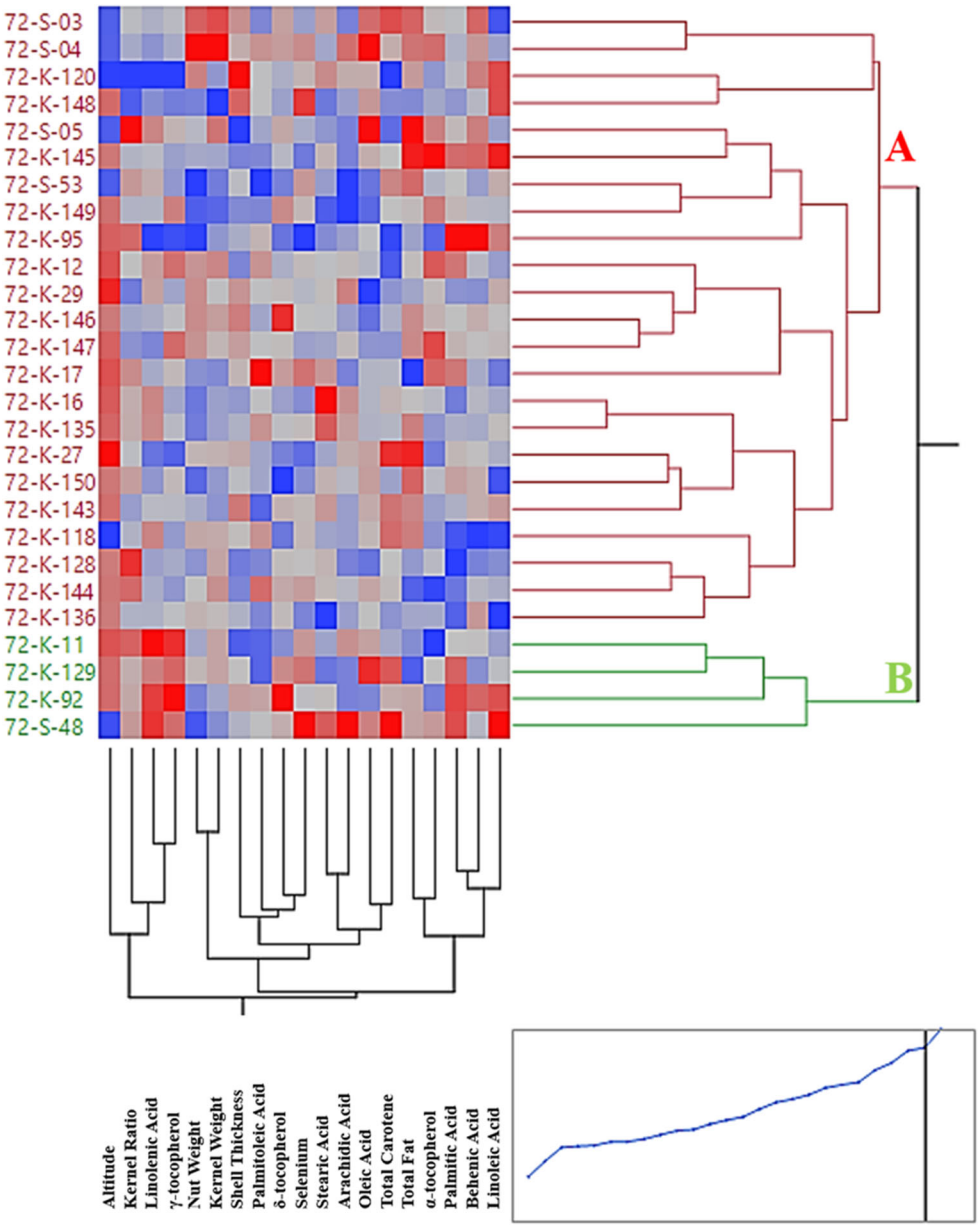


Fig. 2 Two-way cluster analysis of 27 selected genotypes according to Ward's method with high heritability

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Table 2 Tocopherol, selenium, total carotene contents and some fruit characteristics of selected walnut genotypes

Genotype No.	Altitude (m)	Nut weight (g)	Kernel weight (g)	Kernel ratio (%)	Shell thickness (%)	Tocopherols (mg kg^{-1})			Selenium (mg g^{-1})	Total carotene (mg kg^{-1})
						Alfa (α)	Gamma (γ)	Delta (δ)		
72-S-03	926	15.06 ^{ab*}	7.26 ^{ab}	48.23 $^{b-d}$	2.18 ^{ab}	4.52 $^{d-f}$	78.92 ^{gh}	4.31 $^{b-f}$	30.10 ^{jk}	0.50 $^{a-c}$
72-S-04	929	17.44 ^{a}	7.98 ^{a}	45.75 ^{cd}	2.04 ^{ab}	5.82 $^{b-d}$	72.70 ^{i}	4.40 $^{b-e}$	48.10 ^{d}	0.39 ^{cd}
72-S-05	921	11.09 ^{bc}	6.73 ^{ab}	60.76 ^{a}	1.33 ^{b}	5.51 ^{cd}	87.12 ^{f}	4.21 $^{c-f}$	33.12 ^{ij}	0.24 $^{e-g}$
72-K-11	1271	10.67 ^{bc}	6.01 ^{ab}	56.01 ^{b}	1.45 ^{b}	2.25 ^{i}	128.14 ^{b}	2.80 $^{f-h}$	46.27 ^{d}	0.32 $^{d-f}$
72-K-12	1273	13.25 ^{b}	6.48 ^{ab}	48.88 ^{bc}	1.61 ^{b}	6.10 ^{bc}	110.82 ^{d}	3.37 $^{e-g}$	39.74 $^{e-g}$	0.22 ^{fg}
72-K-16	1265	9.59 ^{bc}	4.93 ^{bc}	50.75 ^{bc}	1.70 ^{b}	4.20 ^{ef}	70.24 ^{ij}	3.11 $^{e-h}$	26.32 ^{k}	0.38 ^{cd}
72-K-17	1273	10.12 ^{bc}	4.61 ^{bc}	53.25 ^{b}	1.99 ^{ab}	5.94 ^{bc}	85.32 ^{f}	4.41 $^{b-d}$	52.16 ^{c}	0.34 $^{c-e}$
72-K-27	1322	11.95 ^{b}	5.78 ^{b}	48.40 ^{bc}	2.05 ^{ab}	3.12 ^{gh}	48.35 ^{m}	2.58 $^{f-h}$	20.12 ^{n}	0.53 ^{ab}
72-K-29	1306	13.54 ^{b}	5.45 ^{b}	40.22 ^{e}	2.16 ^{ab}	4.52 ^{ef}	70.15 ^{ij}	3.86 $^{d-f}$	35.50 ^{i}	0.29 $^{d-f}$
72-S-48	892	8.92 ^{c}	4.65 ^{bc}	52.42 ^{b}	1.83 ^{b}	5.12 ^{d}	111.30 ^{d}	4.10 $^{c-f}$	65.32 ^{a}	0.56 ^{a}
72-S-53	920	8.51 ^{c}	4.42 ^{bc}	52.41 ^{b}	1.88 ^{b}	4.09 ^{f}	65.36 ^{k}	2.56 ^{gh}	46.14 ^{d}	0.44 ^{c}
72-K-92	1252	9.81 ^{bc}	5.03 ^{bc}	51.22 ^{b}	2.11 ^{ab}	3.18 ^{gh}	136.27 ^{a}	6.21 ^{a}	37.55 ^{h}	0.39 ^{cd}
72-K-95	1261	8.60 ^{c}	4.79 ^{bc}	55.62 ^{b}	1.80 ^{b}	2.78 ^{hi}	42.60 ^{n}	2.39 ^{h}	12.28 ^{o}	0.20 ^{g}
72-K-118	860	12.52 ^{b}	5.89 ^{b}	46.95 ^{cd}	1.94 ^{b}	3.48 ^{fg}	66.32 ^{k}	2.44 ^{h}	40.65 ^{ef}	0.47 ^{bc}
72-K-120	866	13.96 ^{b}	4.80 ^{bc}	34.24 ^{g}	2.70 ^{a}	3.55 ^{fg}	38.20 ^{o}	3.38 ^{ef}	41.05 ^{e}	0.19 ^{g}
72-K-128	1238	10.19 ^{bc}	6.00 ^{b}	58.88 ^{a}	1.57 ^{b}	4.20 ^{f}	72.46 ^{i}	3.85 $^{d-f}$	38.75 ^{gh}	0.36 ^{cd}
72-K-129	1250	11.84 ^{b}	6.02 ^{ab}	50.74 ^{bc}	1.93 ^{b}	4.39 ^{ef}	113.54 ^{c}	4.67 ^{bc}	51.87 ^{c}	0.49 ^{bc}
72-K-135	1256	9.68 ^{bc}	4.94 ^{bc}	50.88 ^{b}	1.82 ^{b}	3.56 ^{fg}	78.36 ^{h}	3.69 $^{d-f}$	39.48 ^{fg}	0.34 $^{c-e}$
72-K-136	1237	12.00 ^{b}	5.60 ^{b}	46.75 ^{cd}	1.82 ^{b}	3.85 ^{f}	80.72 ^{g}	3.89 $^{d-f}$	24.39 ^{l}	0.29 $^{d-f}$
72-K-143	1249	10.78 ^{bc}	4.75 ^{bc}	44.10 ^{d}	2.29 ^{ab}	3.72 ^{f}	78.12 ^{h}	4.05 $^{d-f}$	40.10 ^{ef}	0.46 ^{c}
72-K-144	1242	11.37 ^{b}	6.32 ^{ab}	55.61 ^{b}	1.61 ^{b}	2.37 ^{i}	65.12 ^{k}	4.20 $^{c-f}$	45.36 ^{d}	0.37 ^{cd}
72-K-145	1241	10.70 ^{bc}	5.03 ^{bc}	47.03 ^{cd}	1.60 ^{b}	7.05 ^{a}	68.35 ^{j}	3.55 ^{ef}	22.39 ^{m}	0.36 ^{cd}
72-K-146	1239	13.57 ^{b}	6.12 ^{ab}	45.42 ^{d}	2.27 ^{ab}	4.69 ^{de}	80.45 ^{g}	5.85 ^{ab}	38.45 ^{gh}	0.33 ^{de}
72-K-147	1259	13.29 ^{b}	5.70 ^{b}	42.82 ^{d}	2.11 ^{ab}	6.32 ^{b}	112.08 ^{d}	3.85 $^{d-f}$	30.40 ^{j}	0.29 $^{d-f}$
72-K-148	1253	9.79 ^{bc}	3.69 ^{d}	37.18 ^{f}	2.38 ^{ab}	3.85 ^{f}	56.41^{1}	3.12 $^{e-g}$	59.87 ^{b}	0.28 $^{d-f}$
72-K-149	1247	9.10 ^{c}	4.15 ^{c}	46.99 ^{cd}	1.63 ^{b}	5.60 ^{cd}	105.37 ^{e}	4.67 ^{bc}	40.56 ^{ef}	0.36 ^{cd}
72-K-150	1241	9.29 ^{c}	4.83 ^{bc}	51.96 ^{b}	1.89 ^{b}	4.22 ^{ef}	85.36 ^{f}	1.65 ^{i}	26.45 ^{k}	0.31 $^{d-f}$
Min	860	8.51	3.69	34.24	1.33	2.25	38.20	1.65	12.28	0.19
Max	1322	17.44	7.98	60.76	2.70	6.32	136.27	6.21	65.32	0.56
Mean	1166	11.36	5.48	49.02	1.91	4.37	81.78	3.75	38.24	0.36

*Different letters in the same column indicate significant variance in the level of 0.05

content of seven red walnut genotypes and the 'Chandler' variety reported by Ozcan et al. (2020) ranged as follows: α-tocopherol from 1.18 mg·kg^{-1} to 15.25 mg·kg^{-1}, β- +γ-tocopherol from 154.76 mg·kg^{-1} to 331.27 mg·kg^{-1} and δ-tocopherol from 3.24 mg·kg^{-1} to 45.86 mg·kg^{-1}, respectively. In the selection study conducted in Türkiye, (α)-tocopherol, (γ) and (β) + (δ) tocopherol contents were determined 23.47 and 38.04 µg/g, 161.09 and 292.56 µg/g and 16.93 and 32.34 µg/g, respectively (Beyhan et al. 2017). 156 F1 genotypes belonging to the 'Chandler' × 'Kaplan'-86 walnut population and their parents were investigated in Türkiye and the content of α-tocopherol, β-tocopherol and γ-tocopherol from the walnut population were determined as 1.61–23.65 µg/g, 1.90–12.57 µg/g and 90.22–394.96 µg/g, respectively (Arcan et al. 2021). Kodad et al. (2015) noted that tocopherol contents of 10 candidate walnut selections from the high Atlas Mountains in Morocco was determined γ-tocopherol (188.1–230.7 mg·kg^{-1}), δ-tocopherol (23.3–43.4 mg·kg^{-1}) and α-tocopherol (8.9–16.57 mg·kg^{-1}). As in previous studies, (γ)-tocopherol was found to be higher than (α)- and (δ)-tocopherol in this study. The differences observed when this study is compared with other studies can be explained by the effect of different genotypes and ecological conditions.

Total Carotenes

The total carotene content of the 27 genotypes examined was found to be between 0.19 mg kg^{-1} (72-K-120) and 0.56 mg kg^{-1} (72-S-48). The average total carotene content of the genotypes was determined 0.36 mg kg^{-1} (Table 2). It has been emphasized that β-carotene as a carotenoid is a pigment of plants and one of the main safe dietary sources of vitamin A (Khatib and Vaya 2010; Gulsoy et al. 2019). In

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east of Türkiye, 15 promising walnut selections were examined and total carotenoid of these promising genotypes were found to be between 0.08 and 0.49 mg kg^{-1} (Ozrenk et al. 2012). Abdallah et al. (2015) reported that β-carotene levels of six walnut varieties ranged from 0.22 to 0.62 mg/kg. Overall, it can be said that the results of this study are in good agreement with the literature data for walnuts.

Selenium

Selenium contents of the selected genotypes were found between 12.28–65.32 mg g^{-1}, with an average of 38.24 mg g^{-1} (Table 2). It was reported in the previous study that the content of selenium was determined in nine walnut varieties in Romania varied between 0.001 and 0.005 mg/100 g. It was also cited in same study that among the many roles for which selenium (Se) was accredited, its antioxidant effect ranks first (Cosmulescu et al. 2009). The selenium content of the genotypes examined in studies conducted in Türkiye was reported to be between 11.95 and 64.52 ng g^{-1} in the Van Lake basin (Ozrenk et al. 2011), between 15.89 and 68.19 ng/g in Arras Valley (Gulsoy et al. 2019) and an average of 30.77 ng/g in the Erzincan region (Ozrenk et al. 2012). In general, the results of this study are similar to the literature data mentioned above.

Cluster, Principal Component (PCA) and Correlation Analysis

Cluster analysis was performed with the data obtained from this study in order to statistically evaluate the genotypes in terms of some pomological features and examined criteria. Using the Ward method cluster analysis was detected two major clusters and five subgroups (Fig. 2). The first cluster (A) consisted of 23 genotypes, which were mainly separated from group B cluster in terms of highest values such as nut weight (11.54 g), total fat (63.55%) and α-tocopherol (4.48 mg kg^{-1}). The second cluster (B) consisted of four genotypes (72-K-11, 72-K-129, 72-S-48 and 72-K-92). The genotypes in this group usually had the highest kernel ratio (52.609%), oleic acid (30.26%), linoleic acid (55.02%), linolenic acid (18.14%), γ-tocopherol (122.31 mg kg^{-1}), δ-tocopherol (4.45 mg kg^{-1}) and selenium (50.25 mg g^{-1}) compared to the cluster A. The second cluster (B) was seen as the group with superior values in terms of 11 criteria (Fig. 3).

PCA was performed to determine patterns of variation between genotypes and to differentiate variables from a multivariate data table (Iezzoni and Pritts 1991). The main purpose of this analysis is to classify the examined genotypes and to determine the effect levels of the examination criteria.

Using PCA analysis based on the correlation matrix, the eigenvalues (eigen), percentages of variation and load coefficients of the first eight components were calculated for the criteria examined. Components with eigenvalues greater than 1.00 were preserved to preserve the reliability of the final output in principal component analysis. It was found that the first eight main components constituted 15.66%, 13.87%, 12.56%, 11.37%, 9.70%, 7.78%, 6.37% and 5.29% of the variations, respectively, and the cumulative rate of the variation corresponded to 82.61% of the total variance (Fig. 4).

In PCA analysis, PC1 explained 15.66% of the variance and PC2 explained 13.87% of the variance. Linolenic acid has the highest effect on PC1 (0.48). Nut weight and Kernel weight PC2 (0.48) had the highest effect with their equivalents. The variables with the biggest characteristic vectors in the major component are the following: PC3—linoleic acid (0.50); PC4—behenic acid (0.53) and palmitic acid (0.50);

Fig. 3 Mean discrimination values of the groups according to the examined criteria

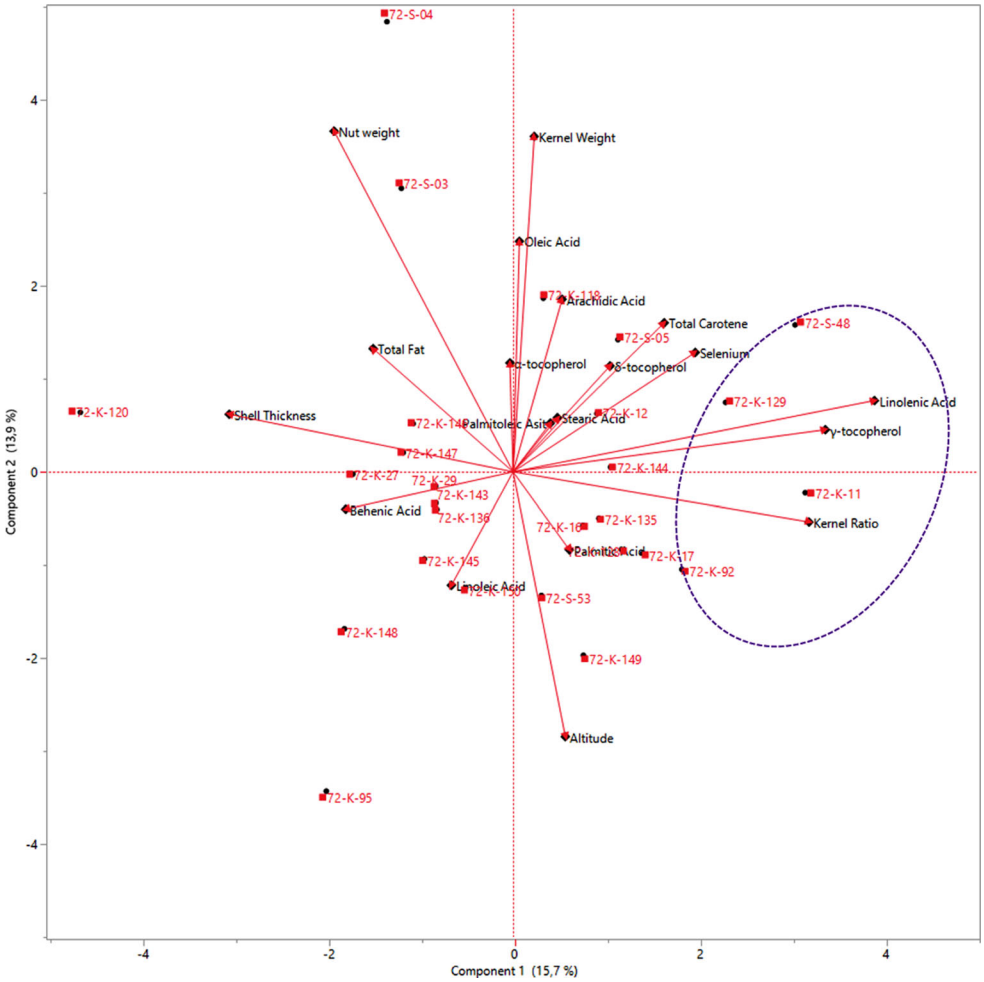
Cluster	A	B
Count	23	4
Altitude (m)	1166.26±161.52	1166.25±183.08
Nut weight (g)	11.54±2.27	10.31±1.25
Kernel Weight (g)	5.49±1.03	5.43±0.70
Kernel Ratio (%)	48.39±6.39	52.60±2.38
Shell Thickness (%)	1.93±0.32	1.83±0.28
Total Fat (%)	63.35±4.05	60.43±4.41
Palmitic Acid (%)	5.19±0.59	5.94±0.44
Palmitoleic Acid (%)	0.09±0.03	0.08±0.02
Stearic Acid (%)	2.71±0.51	2.72±0.62
Oleic Acid (%)	27.29±5.55	30.26±6.26
Linoleic Acid (%)	49.07±6.65	55.02±8.49
Linolenic Acid (%)	13.52±1.96	18.14±1.21
Arachidic Acid (%)	0.10±0.03	0.13±0.06
Behenic Acid (%)	0.19±0.03	0.19±0.03
α-tocopherol (mg kg^{-1})	4.48±1.22	3.74±1.27
γ-tocopherol (mg kg^{-1})	74.73±18.91	122.31±11.93
δ-tocopherol (mg kg^{-1})	3.63±0.92	4.45±1.41
Selenium (mg g^{-1})	36.15±11.05	50.25±11.65
Total Carotene (mg kg^{-1})	0.35±0.09	0.44±0.11

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Fig. 4 Estimates of variance, accumulated variances and of the eight principal components for 19 characters evaluated on 27 walnut genotypes. *Asterisk* Values in *bold* correspond for each variable to the factor for which the squared cosine is the largest

	PC							
Number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Eigenvalue	2.97	2.63	2.39	2.16	1.84	1.47	1.21	1.01
Percent (%)	15.66	13.87	12.56	11.37	9.70	7.78	6.37	5.29
Cum Percent (%)	15.66	29.53	42.09	53.46	63.16	70.95	77.32	82.61
ChiSquare	317.38	287.51	259.04	230.77	202.13	175.02	151.52	130.55
DF	170.24	156.77	143.23	129.90	116.85	103.10	91.37	79.19
Prob>ChiSq	>.0001	>.0001	>.0001	>.0001	>.0001	>.0001	>.0001	0.0002
	Eigenvalue							
Altitude (m)	0.07	-0.38	-0.07	-0.01	0.29	0.10	0.22	0.34
Nut weight (g)	-0.24	0.48	-0.07	-0.01	0.27	0.00	0.09	0.13
Kernel Weight (g)	0.03	0.48	-0.31	0.11	0.14	0.08	0.04	0.19
Kernel Ratio (%)	0.40	-0.07	-0.31	0.16	-0.16	0.19	-0.06	-0.01
Shell Thickness (%)	-0.38	0.08	0.34	-0.16	0.12	-0.11	-0.10	0.19
Total Fat (%)	-0.19	0.18	-0.01	0.29	-0.35	-0.28	0.35	-0.11
Palmitic Acid (%)	0.08	-0.11	0.24	0.50	0.04	0.14	-0.18	-0.08
Palmitoleic Acid (%)	0.05	0.07	0.07	-0.15	0.24	0.61	0.06	-0.22
Stearic Acid (%)	0.06	0.08	0.27	-0.07	-0.29	0.34	0.45	0.10
Oleic Acid (%)	0.01	0.33	-0.07	0.18	-0.21	0.22	-0.51	-0.02
Linoleic Acid (%)	-0.08	-0.16	0.50	0.20	-0.11	-0.01	-0.10	0.03
Linolenic Acid (%)	0.48	0.10	0.11	0.01	-0.10	-0.14	0.12	0.13
Arachidic Acid (%)	0.07	0.25	0.27	-0.07	-0.23	0.33	0.20	0.22
Behenic Acid (%)	-0.22	-0.05	0.01	0.53	-0.01	0.19	-0.11	0.28
α-tocopherol (mg kg^{-1})	0.00	0.15	0.09	0.27	0.22	-0.06	0.31	-0.66
γ-tocopherol (mg kg^{-1})	0.42	0.06	0.10	0.22	0.24	-0.17	0.08	0.12
δ-tocopherol (mg kg^{-1})	0.13	0.15	0.21	0.16	0.46	-0.13	0.06	0.23
Selenium (mg g^{-1})	0.24	0.17	0.36	-0.25	0.11	-0.03	-0.34	-0.22
Total Carotene (mg kg^{-1})	0.20	0.21	0.12	-0.10	-0.26	-0.30	-0.07	0.14

Fig. 5 Biplot obtained from the fruit characteristics and genotypes in the first two principal components



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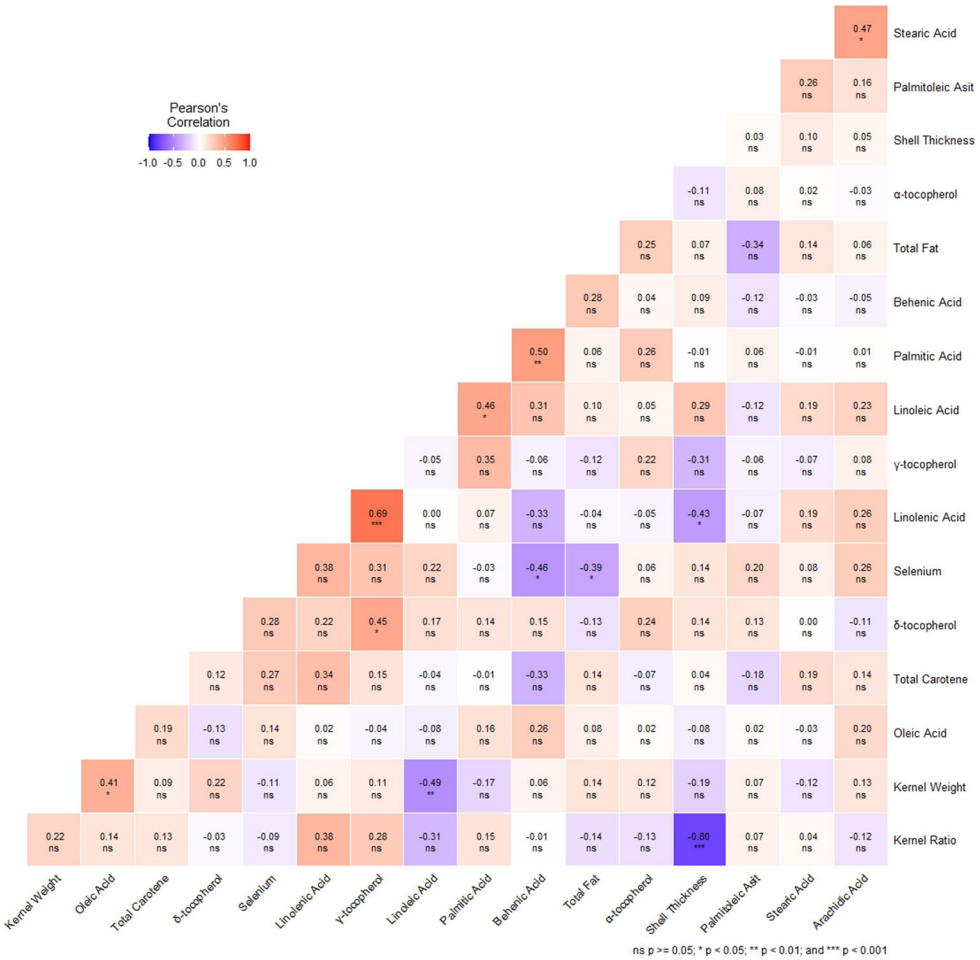


Fig. 6 Pearson correlations of walnut fruit characteristics

PC5—δ-tocopherol (–0.42); PC6—palmitoleic acid (0.61); PC7—oleic acid (–0.51) and PC8—α-tocopherol (–0.66) (Fig. 4). As can be clearly seen in Fig. 5, certain genotypes (72-K-11, 72-K-129, 72-K-92 and 72-K-48) have been distinguished in special groups supporting cluster analysis. The difference between the various PCA amounts is thought to be due to the data set differentiation (Fig. 4).

Correlation analysis was performed to determine the relationships between some fruit characteristics and the refined fatty acids and tocopherol contents. As a result of the correlation analysis, positive correlations were found between γ-tocopherol/linolenic acid' and 'kernel weight/nut weight', and significant negative correlations between 'kernel ratio/shell thickness' at $P<0.001$ level. Figure 6 reveals a strong positive correlation between 'behenic acid/palmitic acid' at $P<0.01$ level. In addition, it was determined that there was positive correlation at $P<0.05$ level between 'arachidic acid/stearic acid', 'δ-tocopherol/γ-tocopherol', 'shell thickness/linoleic acid' and 'linoleic acid/palmitic acid'; a negative correlation between 'selenium/total fat' and 'selenium/behenic acid'. None of the characteristics were statistically associated with α-tocopherol, total carotene, palmitic acid and oleic acid.

While a statistically significant relationship was observed between nut weight and the investigated pomological features, no relationship was found between other chemical features. Strong positive correlation was also observed between shell thickness and linoleic acid. The obtained results have a fairly logical explanation, as the thickness of the shell increases, the amount of linoleic acid also increases. All correlations with statistical significance levels are shown in Fig. 6.

Conclusion

Türkiye has a great genetic diversity of walnut and there are still areas pending evaluation within this natural rich walnut population. Walnuts are an excellent source of important nutritional values such as essential fatty acids, carotenoids and tocopherols. The findings presented in this study revealed important information on the nutritional benefit of the selected promising walnut genotypes from the Batman region in southeastern Türkiye. This study showed that promising walnut genotypes selected and examined for their superior properties have comparable characteristics with na-

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tional and international selected varieties. Considering that awareness of the importance of walnuts in human nutrition has increased in recent years, these walnut genotypes may be valuable for future nutritional breeding studies.

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Conflict of interest S. Acar, A. Kazankaya and A. Doğan declare that they have no competing interests. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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