Euphytica (2024) 220:96
https://doi.org/10.1007/s10681-024-03357-8

RESEARCH

Exploring nut quality traits in almond varieties: a comparative study of local and foreign cultivars and their F1 hybrid offsprings

Hatice Özcan · Müjgan Güney · Harun Karcı · Habibullah Tevfik · Salih Kafkas · İzzet Acar · Ebru Kafkas

Received: 13 March 2024 / Accepted: 22 May 2024
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Abstract Almonds are one of the most popular and widely consumed tree nuts in the world, prized for their taste, texture, and nutritional benefits. However, the quality and characteristics of almonds can vary significantly depending on the variety and growing conditions. In this context, a comparative study was conducted to explore the nut quality traits of almond local and foreign cultivars, as well as their F1 hybrid offspring resulting from crossbreeding. "Nut quality" is a dynamic concept changing based on consumer needs and perceptions that reflect sociocultural evolution. The aim of this study was to identify the most promising almond varieties and hybrids based on some nut quality traits including macro–micro elements and tocopherol contents. To accomplish this goal, samples were collected from local (Gulcan 2 and Nurlu) and foreign (Lauranne and Guara) almond cultivars, as well as F1 hybrid offspring resulting in two cross-breading ("Gulcan 2×Lauranne" and "Guara×Nurlu"), and conducted a comprehensive analysis using high-performance liquid chromatography and atomic absorption spectroscopy. Our results showed that the nut quality traits of almonds can vary significantly among different cultivars and hybrids, with some varieties and hybrids exhibiting superior characteristics compared to others. These findings provide important insights into the potential for improving almond quality through crossbreeding and the development of new varieties with improved characteristics which can help inform breeding programs and improve the quality of almonds available in the market.

Keywords Almond · Macro · Micro · Nutrient element · Tocopherol · Progeny

Introduction

Almond (*Prunus dulcis* Mill.) belongs to *Prunus* genus from the Prunoideae subfamily of the Rosaceae family (Paizila et al. 2022). It is one of the most popular drought-tolerant nuts which attracted great interest worldwide due to food, medicinal, and cosmetic applications. Almond originated from Southwest Asia and then distributed to China, India, Iran, Syria, and Mediterranean countries (Cevher et al. 2022).

Originally, almond orchards were planted with a genetic diversity primarily comprising bitter seedlings. Later, the discovery of a genetic mutation that

H. Özcan · H. Karcı · H. Tevfik · S. Kafkas · E. Kafkas (✉)
Department of Horticulture, Faculty of Agriculture, Cukurova University, Adana, Turkey
e-mail: ebruyasakafkas@gmail.com

M. Güney
Department of Horticulture, Faculty of Agriculture, Yozgat Bozok University, Yozgat, Turkey

İ. Acar
Department of Horticulture, Faculty of Agriculture, Harran University, Sanlıurfa, Turkey

Published online: 02 June 2024

determined kernel sweetness facilitated the domestication of almonds for culinary use. The cultivated variety, Prunus dulcis, likely emerged from ancient populations through interspecific hybridization selected for its superior taste. More recently, efforts to redomesticate almond germplasm have focused on enhancing its nutritional value and ensuring food safety (Massantini and Frangipane 2022). Based on pomological traits, almonds are classified into two groups which are sweet almonds, referred to as "*Prunus amygdalus dulcis*", and bitter almonds referred to as "*Prunus amygdalus amarus*". Bitter almond is mostly used as rootstock for almond seedlings (Yada et al. 2011) and is also used in cosmetics and medicine for its special oil (Geng et al. 2016). Some studies have reported a large variation in the chemical composition of almonds, depending on the cultivars and fractions analyzed (Čolić et al. 2019; Yada et al. 2011; Oliveira 2019). Chemically, almond is characterized by high contents of fat (~50%) (Amarowicz et al. 2017), proteins (~20%), carbohydrates (~20%), and low moisture (~6%), with a fatty acid profile composed mainly of monounsaturated (60%) and polyunsaturated (30%) fatty acids (Roncero et al. 2016). Moreover, almond is a rich source of dietary fiber, phytosterols, tocopherol, and many other health-promoting micronutrients, vitamin E, ash, riboflavin, and polyphenols. In nature, vitamin E consists of four tocopherols (α-, β-, γ-, and δ-tocopherol). Among these forms, α-tocopherol is considered the most biologically active form of vitamin E and utilized preferentially in the human body, compared to other forms (Zamany et al. 2017; Ergun and Bozkurt 2020; Guney 2020). Vitamin E acts as an inhibitor against lipid oxidation during processing and storage and traps free radicals in the cell membrane with its antioxidant effect (Garcia-Pascual et al. 2003). This protective effect is important for the health of all cells and prevents many diseases including cancer and cardiovascular diseases as well as lowering cholesterol (Dubois-Deruy et al. 2020). Recent studies showed that the use of gamma-tocopherol prevents colon, prostate, and gall bladder cancer (Jiang et al. 2022). Almonds are also rich in K and Mg nutrient content. Studies have shown that Mg helps the arteries and veins relax by accelerating the flow of blood and oxygen level in the blood, thus preventing free radicals from damaging the heart. K is an important electrolyte like Mg, and it has been reported that it is an important nutrient element for normal blood pressure and heart functions, especially in the contraction of muscles and in the nervous system (Kelly and Sabate 2006). Calcium, present at significant levels in almond kernels, is a vital mineral for bone formation and neurological function. In the past 50 years, studies on almonds have generally focused on total fat and fatty acids, and phytochemical content of almonds and the effect of environmental and genetic factors and varieties on these characteristics (Yada et al. 2011; Özcan et al. 2020).

In recent years, almond breeding has mostly focused on breeding characteristics such as developing new varieties, late flowering, increasing fruit quality, regular productivity, resistance to cold, self-adaptation, resistance to diseases, tree habitus, and early ripening. In addition, it draws attention that it focuses on breeding varieties with a high content of phenolic compounds and bioactive compounds, which are scientifically proven to reduce the risk of heart and cancer diseases. Studies on almond breeding in Turkiye started in the 1960s. Dokuzoğuz et al. (1968) started the first breeding study by selecting the genotypes with high agricultural value, with a wide selection study on almond trees grown from seed in Western Anatolia.

Most of the almond studies are focused on flowering time, self-compatibility, and genetic characterizations. Recent efforts in almond breeding have incorporated genomic approaches by various teams. Notably, chromosomal-scale reference genomes for the Texas and Lauranne almond cultivars were published (Sánchez-Perez et al. 2019; Alioto et al. 2020). Utilizing these genomes, numerous studies have developed markers linked to key agricultural traits, leading to more accurate results (Esgandaripirmorad et al. 2022; Paizila et al. 2022; Karcı 2023). However, there are very few studies about the chemical compositions of almond cultivars (Yada et al. 2011; Oliveira 2019). And to the best of our knowledge, there is no study conducted in almond populations. Therefore, it is aimed to evaluate traits such as mineral and tocopherol in two populations of preferred almond cultivars by crossing ("Gulcan 2 × Lauranne" and "Guara × Nurlu").

Materials and methods

Plant material

In this study, "Gulcan 2×Lauranne" and "Guara×Nurlu" almond progenies (103 and 105 F1s, respectively) and their parent's fruits were used as material (Table 1, Fig. 1). The breeding field, managed by Pistachio Research Institute in Gaziantep, locates at the at the transition point of the Mediterranean and continental climates. Summers are characterized by hot and dry conditions, while winters bring cold and rainy weather. Temperatures typically fluctuate between −1 and 35 °C, rarely dipping below −5 °C or exceeding 38 °C over the past decade. The soil predominantly consists of sandy clay. Positioned on the southern side, the plants bask in sunlight throughout the day. F1 plants are meticulously pruned using the tapestry pruning method, spaced at 3×2 intervals. Regular irrigation is conducted using the drip irrigation technique.

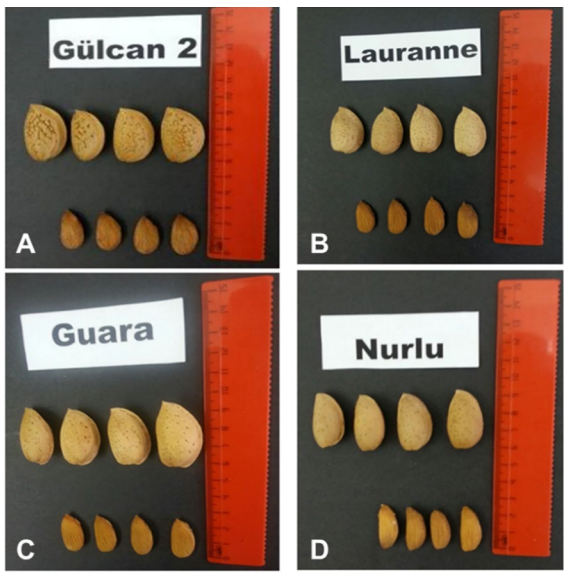


Fig. 1 The nuts of the Gulcan 2 **A**, Lauranne **B**, Guara **C** and Nurlu **D** almonds cultivars

The almond samples from both parents and progenies were harvested at the stage when their outer husks showed signs of splitting open, denoting optimal ripeness in August 2015. Subsequently, the almonds underwent a controlled drying process. Firstly, samples were dried at room temperature after removing the outer hulls for 24 h. Thereafter, they were subjected to a one-day resting period at a temperature of 65 °C before analysis. This carefully regulated drying period facilitated the removal of excess moisture from the almonds, ensuring their stability and quality for subsequent processing (Table 1).

Determination of tocopherol content

The amount of tocopherol contents was determined in three replicates. Ten kernels of 30 fruits from each genotype were used for each replicate. After removing the shells, kernels were ground and homogenized.

Table 1 Traits of parent cultivars used as materials in this study (Açar et al. 2012)

Traits	Gulcan 2	Lauranne	Guara	Nurlu
Improved by	A selective breeding program from the Aegean region	INRA Avignon Institute (France) by the crossing of "Ferragnes×Tuono"	Spain's CITA Zaragoza Institute	Selected in Datça, Turkiye
Plant strength	Medium	Medium	Medium	–
Tree Vigor	Intermediate	Intermediate	Strong	–
Compatability	Self-Incompatible	Self-Compatible	Self-Compatible	–
Productivity	High	Medium–High	-	–
Resistance	Good Resistance to Bacterial Canker	Fungal Disease	A Certain Resistance to Cold	–
Juvenile period	Short	Short	Short	–
Yield	28%	29–35%	30–34%	31%
Rate of double almonds	10%	5–20%	10–20%	0%
Flowering	Late	Late	Late	Early
Shell hardness	Hard-Shell	Semi-Hard-Shell	Hard-Shell	Hard-Shell

The extraction of tocopherols was done by HPLC grade Hexane. The extracted solvents containing α-, β-, and γ-tocopherol were analyzed by the HPLC (Agilent 1100, USA) according to the methods developed by Surai (2000). Analyses were done by the fluorescent detector and C18 reverse-phase column (15 cm × 4.6 mm, 3um; Supelco). The mobile phase was methanol–water (97:3 v/v) with a flow rate of 1.05 per minute. The results were determined quantitatively using external standards (Surai 2000).

Determination of macro and micronutrient content.

Firstly, samples were dried in the oven at 65 °C for 48 h and then two hundred milligrams of the ground and homogenized samples were burned at 550 °C for 8 h for analysis of phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), sodium (Na), iron (Fe), manganese (Mn) and zinc (Zn) contents of the samples. The resulting ash was dissolved in 3.3% (v/v) HCl acid. The solvents containing elements were analyzed in emission mode for K, Ca, Mg, and P, and in absorbance mode for determining Fe, Mn, and Zn readings using the Varian FS220 model Atomic Absorption Spectrophotometer. Phosphorus analyzes were carried out by Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer according to the Barton method using the extract prepared above.

Statistical analysis

Population distribution graphs and the average and standard deviation values of each studied compound were calculated by SPSS 22.0 version statistical package program. Correlation among the macro-micro-elements and tocopherols, hierarchical clustering of the cultivars and genotypes and principal component analysis (PCA) with biplot were then performed with R 3.6.2 statistical program. Library 'corrplot' was used for correlation analysis, 'FactoMineR' library was used for hierarchical clustering and the PCA + biplot analysis was performed with 'factoextra' library.

Results and discussion

Macronutrient content (%) of hybrid lines

Potassium was the major macronutrient in almond kernels in both populations. This result was in agreement with previous reports which showed that potassium content is generally higher than the Mg and P (Olatidoye et al. 2011; Özcan et al. 2011). The amount of K in Gulcan 2, Lauranne, Guara, and Nurlu was 1.27, 1.43, 1.29, and 1.22%, respectively (Tables 2 and 3).

In "Gulcan 2 × Lauranne" population, potassium amount ranged from 1.03 to 1.93%, and the maximum and minimum values were obtained from 109 and 150 lines, respectively. The results showed that 44 of 103 lines (approximately 43%) varied between 1.33 and 1.43%, 33 genotypes were lower than these values and 36 genotypes were higher. The calcium content ranged from 0.58 to 1.11% and the maximum

Table 2 Phytochemical content of F1 progenies and their parents ("Gulcan 2 × Lauranne'')

Nut traits	F1 average	F1 min	F1 max	Quartile 1 (%25)	Quartile 2 (%50)	Quartile 3 (%75)	Gulcan 2	Lauranne
Mn (ppm)	30.11 ± 5.12	10.67	38.34	28.34	31.67	33.34	4.34	8.00
Zn (ppm)	39.07 ± 5.48	28.00	65.00	35.84	38.34	41.84	47.34	48.34
Fe (ppm)	34.16 ± 7.94	18.34	70.34	29.51	33.00	39.17	48.00	33.00
Ca (%)	0.84 ± 0.07	0.58	1.11	0.79	0.84	0.88	0.72	0.77
K (%)	1.37 ± 0.14	1.03	1.93	1.29	1.37	1.43	1.27	1.43
Mg (%)	0.12 ± 0.03	0.05	0.19	0.10	0.12	0.13	0.20	0.18
P (%)	0.44 ± 0.08	0.29	0.79	0.39	0.43	0.47	0.38	0.42
α-tocopherol (mgg^{-1})	539.44 ± 111.38	216.75	852.13	467.82	534.97	599.02	448.02	507.35
β-tocopherol (mgg^{-1})	4.76 ± 2.06	1.51	16.87	3.45	4.50	5.84	3.03	2.32
γ-tocopherol (mgg^{-1})	5.81 ± 3.78	1.63	23.83	3.35	4.86	6.52	8.00	5.37

Table 3 Phytochemical content of F1 progenies and their parents ("Guara × Nurlu")

Nut traits	F1 average	F1 min	F1 max	Quartile 1 (%25)	Quartile 2 (%50)	Quartile 3 (%75)	Guara	Nurlu
Mn (ppm)	11.6 ± 8.90	1.00	34.00	6.67	9.00	12.60	7.34	3.67
Zn (ppm)	39.32 ± 5.53	21.00	58.00	36.67	39.67	42.67	38.00	36.67
Fe (ppm)	38.31 ± 7.77	19.34	68.34	33.67	37.34	43.00	40.34	43.67
Ca (%)	0.78 ± 0.05	0.63	0.91	0.75	0.77	0.81	0.77	0.71
K (%)	1.31 ± 0.27	0.96	2.58	1.14	1.24	1.37	1.29	1.22
Mg (%)	0.21 ± 0.08	0.07	0.35	0.14	0.25	0.28	0.17	0.18
P (%)	0.35 ± 0.053	0.17	0.53	0.32	0.35	0.38	0.37	0.40
α-tocopherol (mgg^{-1})	539 ± 110.87	144.85	1013.48	474.51	532.44	589.30	691.75	424.85
β-tocopherol (mgg^{-1})	3.89 ± 1.65	1.26	10.05	2.77	3.44	4.94	2.21	5.91
γ-tocopherol (mgg^{-1})	6.48 ± 4.38	1.33	22.47	3.04	5.62	8.33	10.72	7.18

and minimum values were obtained from 194 and 108 lines, respectively. Thirty-six percent of lines were in the range of average and only 29 lines had a higher amount of calcium. Phosphorus amounts ranged from 0.29 to 0.79% and the maximum and minimum values were obtained from 199 and 136 lines, respectively. The phosphorus content of lines showed that 52 of 103 lines were lower than average. Maximum and minimum amounts of magnesium were obtained from 18 and 115 lines, respectively. Thirty-two of 103 lines had a higher amount of magnesium than average (Table 2; Fig. 2). Macro element contents (Ca, K, Mg, P) of lines in "Gulcan 2 × Lauranne" population were analyzed by PCA in scatter biplot analysis and PC 1 (1st component) 61.36%, PC 2 (2nd component) 22.40%, total variation was calculated as 83.76%. Among the macro contents, several lines had higher values, while some lines produced higher values in only one macro content. In this population, line 109 was included in the group between calcium and potassium in PCA analysis, and the values of these elements were determined above the average values, while the magnesium and phosphorus contents were determined below the average values. In addition, potassium content of this line was calculated as the highest value. While phosphorus content was found to be the highest in lines 198 and 199, magnesium and potassium values were below the average values, although calcium contents were found to be stable (Fig. 3).

In "Guara × Nurlu" population, potassium amounts ranged from 0.96 to 2.58%, and the maximum and minimum values were obtained from 349 and 254 lines, respectively. The calcium content ranged from 0.63 to 0.91% and the maximum and minimum values were obtained from 256 and 261 lines, respectively. Phosphorus amount ranged from 0.17 to 0.53% and the maximum and minimum values were obtained from 346 and 273 lines, respectively. Maximum and minimum amounts of magnesium were obtained from 358 and 247 lines, respectively. The potassium content of 43 out of 105 progenies (approximately 36%) varied between 1.33 and 1.43%, 31 genotypes were lower than these values and 32 genotypes were higher. Approximately 36% of progenies contained 0.82–0.88% calcium, 37 lines had lower and 29 progenies had a higher content of calcium. The average value of calcium content was higher in progenies than in their parents. Although, approximately half of the progenies contained phosphorus in the range of average, remained 41 progenies had higher P content versus 19 progenies with lower content.

The results showed that in both populations, the rate of heterosis in progenies was high in terms of macronutrient elements, especially in K and Ca in "Gulcan 2 × Lauranne" and P and Mg in "Guara × Nurlu" population (Table 3; Fig. 4). In this study, biplot PCA analysis was performed using the data of macro elements (Ca, K, Mg, P) of the "Guara × Nurlu" F1 population. The classification of the macro elements according to the lines and the variation of the lines according to the macro elements were given in Fig. 5. In the scatter biplot analysis, PC 1 (1st component) was calculated as 86.47%, PC 2 (2nd component) as 27.89%, and total variation was 94.30%. Although the relationship between macro elements is positive, it is not very high. Macro elements showed significant variation among lines.

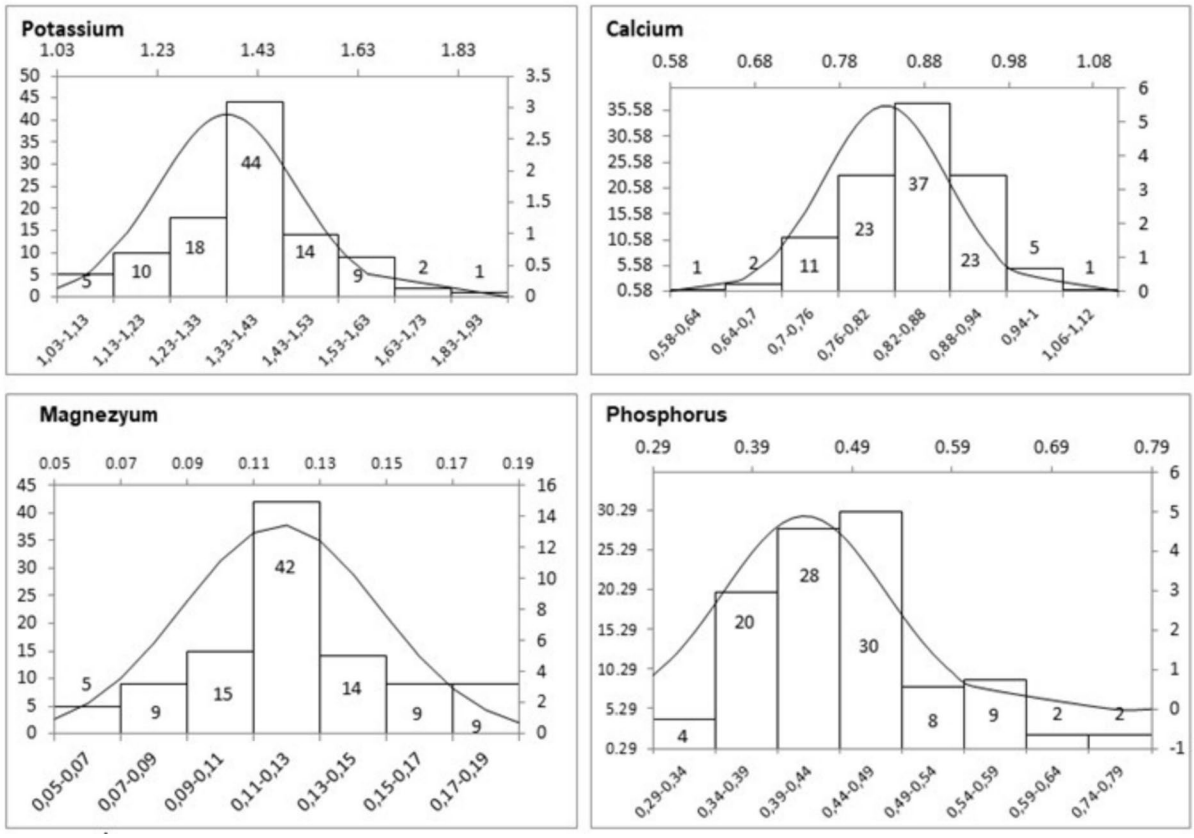


Fig. 2 Distribution of Macronutrient of "Gulcan 2×Lauranne" population

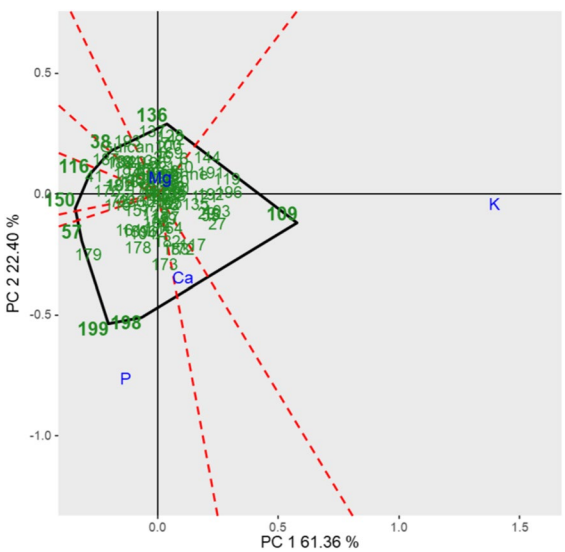


Fig. 3 PCA biplot cluster for macro elements (Ca, Mg, K, P) in 'Gulcan 2×Lauranne' F1 population

While the lines had a high value in terms of any macro element, they were stable in terms of other elements. In the "Guara×Nurlu" population, potassium was highest in line 349, while lines 338, 343, 345 and 346 outperformed other lines in terms of potassium and calcium elements. On the other hand, 203 and 214 were determined to be promising according to phosphorus and calcium elements.

Micronutrient content (ppm) of hybrid lines

In "Gulcan 2×Lauranne" population, manganese amount ranged from 10.64 to 38.34 ppm and the maximum and minimum values were obtained from 171 and 150 lines, respectively. The results showed that the amount of Mn in half of the progenies was below the average and only 21 progenies contained a higher amount of Mn. The zinc content ranged from 28 to 65 ppm and the maximum and minimum values were obtained from 116 and 136 lines, respectively.

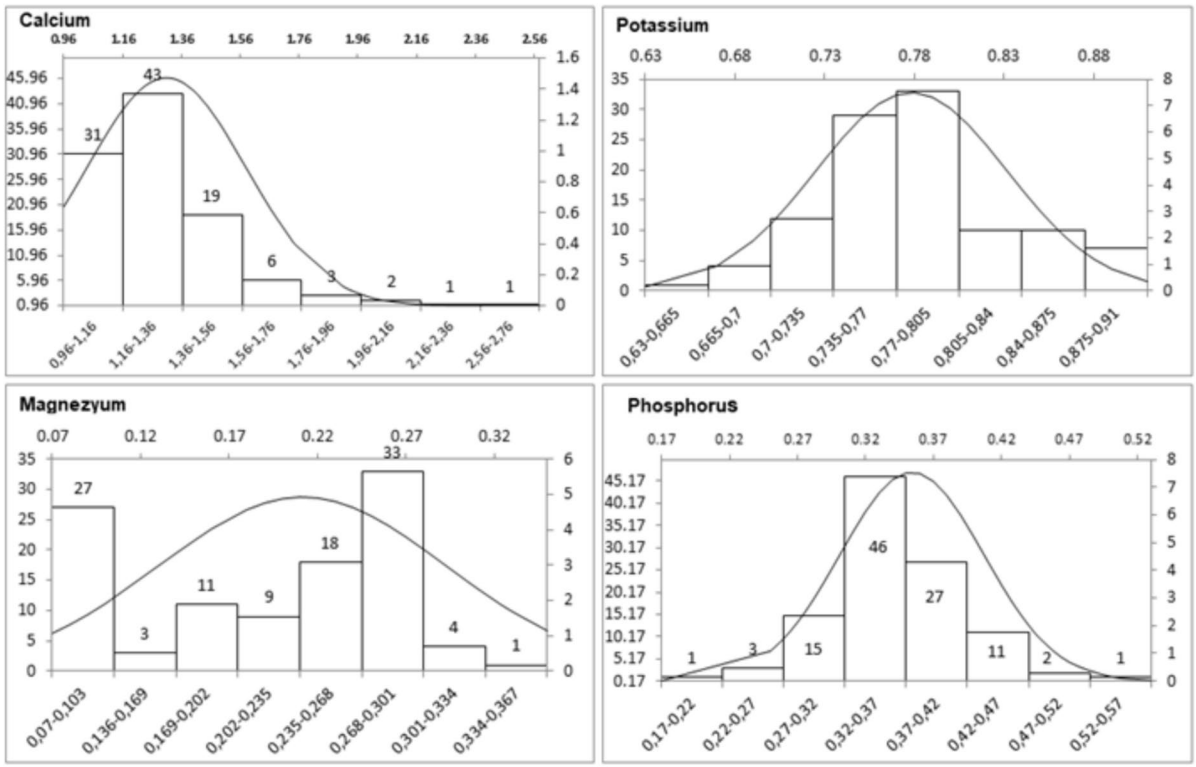


Fig. 4 Distribution of macronutrient of "Guara×Nurlu" population

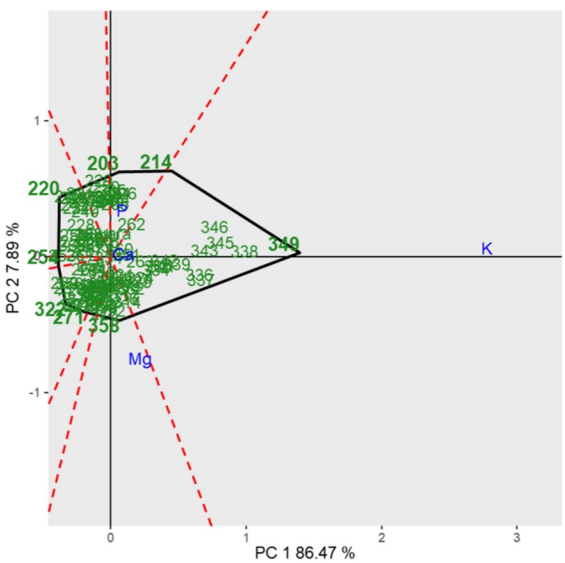


Fig. 5 PCA biplot cluster for macro elements (Ca, Mg, K, P) in "Guara×Nurlu" F1 population

Contrary to Mn, most of the progenies had a higher amount of Zn than the average value (approximately 36%). The iron amount ranged from 18.34 ppm to 70.34 ppm and the maximum and minimum values were obtained from 135 and 66 lines, respectively. Similar to Zn, half of the progenies had higher Fe content than the average range (28% in the range of average). The concentration of Mn was much higher in progenies than in their parents. Contrary to Mn, parents' Zn amount was higher than the average of progenies. However, iron amount in Gulcan 2 was higher than the average of progenies, but Lauranne contained Fe in the range of average values obtained from progenies (Table 2; Fig. 6). In PCA analysis of microelements, PC 1 (1st component) was 54.46%, PC 2 (2nd component) was 27.09%, and total variation was 81.55%. Lines showed variation according to different microelements and lines followed a wide distribution. While the Zn content values of the Gulcan 2 cultivar and 116 were determined as promising, the manganese content was determined very low in both parents. In addition, line 135 had higher values

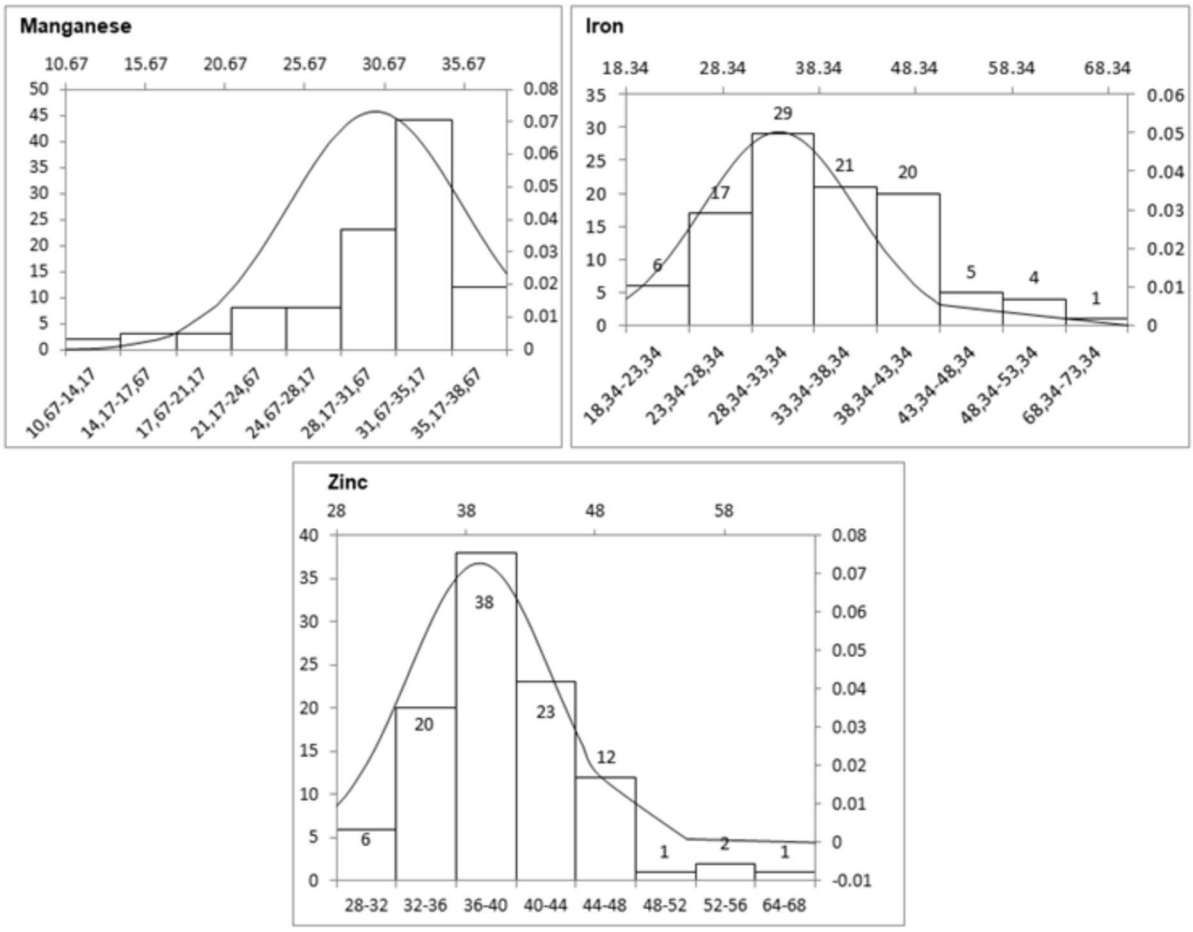


Fig. 6 Distribution of micronutrient of "Gulcan 2 × Lauranne" population

in terms of iron content, while other component values remained stable (Fig. 7).

Compared to "Gulcan 2 × Lauranne", in "Guara × Nurlu" population, the average of Mn concentration (11.6 ppm) is less and ranged from 1.00 to 34 ppm and the maximum and minimum values were obtained from 204 and 353 lines, respectively. The population distribution graph analysis showed that the amount of Mn in 39 out of 105 progenies (approximately 38%) varied between 5.5 and 10.0 ppm, 44 genotypes were lower than these values and 22 genotypes were higher. The content of Zn ranged from 21 to 58 ppm and the maximum and minimum values were obtained from 271 and 239 lines, respectively. In approximately 45% of progenies, the concentration of Zn was in the range of the average amount. The iron amount ranged from 19.34 to 68.34 ppm and the maximum and minimum values were obtained from 250 and 239 lines, respectively. In terms of Iron concentration, a large number of progenies had a higher amount of Fe than the average (approximately 43%). Contrary to Mn and Zn, the amount of Iron in parents was higher than the average concentration of progenies (Table 2; Fig. 8). In the scatter biplot analysis of microelements, PC 1 (1st component) was calculated as 62.06%, PC 2 (2nd component) as 23.85%, and total variation was 85.91%. Microelements (Mn, Zn, Fe) were determined that iron and zinc contents were detected in correlation in the "Guara × Nurlu" population. The 256 line had higher for iron and zinc contents than other lines. However, 313, 353 and 356 were identified at quite a low level in terms of all microelement contents

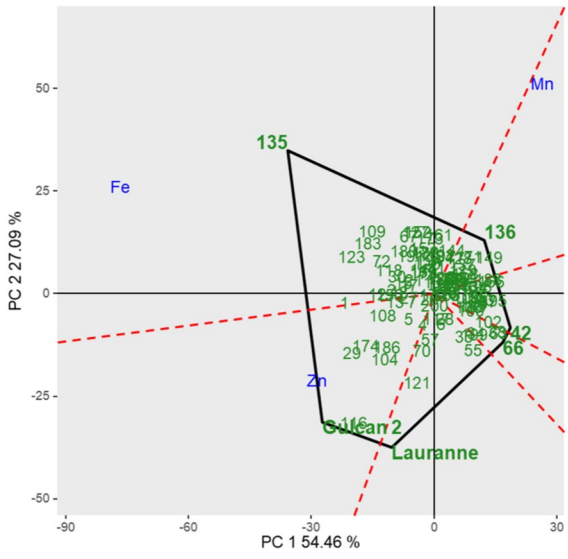


Fig. 7 PCA biplot cluster for micronutrients (Ca, Mg, K, P) in "Gulcan 2 × Lauranne" F1 population

and they can be eliminated during the selection stage in the breeding program (Fig. 9).

However, the average concentration of Mn, K, and P were higher in "Gulcan 2 × Lauranne", while "Guara × Nurlu" had a higher amount of Fe and Mg. Moreover, results proved that the crossing of Gulcan 2 and Lauranne produced a superior population, especially in terms of Mn and Mg in which all of the progenies had much higher concentrations of these minerals than parents (Tables 2 and 3).

Nutrient elements may be affected by genetic factors, genotypes, ecological conditions, irrigation, and fertigation practices (González-Fontes et al. 2017). Kodad et al. (2006) reported that K content of almonds kernel varied from genotype to genotype, but constituted 0.6 to 1.2% of the dry weight. Yada et al. (2011) studied the amount of macronutrient elements from different genotypes from different regions (California, Spain, Tunisia) and reported that genotypes from different regions showed different Ca, Mg, and P concentrations in almond kernels which varied between 0.28–0.30%, 0.23–0.61% and 0.36–1.05%, respectively. In another study, K, P, Ca, and Mg contents of 72 almond varieties of genetic resources from France, Greece, and Italy were investigated and the results proved that mineral concentration varied among varieties. Researchers stated that the concentration of nutrient content was strongly affected by the variety and growing conditions (Drogoudi et al. 2013). Chen et al. (2006) reported that Mn concentration in the almond kernel was 0.25% of the dry weight and the concentrations of Ca, Mg, and P were also identified as 248, 275, and 474 mg per 100 of the dried kernel, respectively. Despite some differences, the results obtained in this study were in accordance with previous studies. However, Ca concentrations in this study were higher than reported previously. Mn elements were partially higher than the studies of Agunbiade et al. (2006) and the Fe and Zn values were partially lower than the findings of Agunbiade et al. (2006). Olatidoye et al. (2011) reported that the concentration of K, Ca, Mg, Mn, Zn, Fe, and P in tropical almond nuts from southwest Nigeria was 42.17, 27.17, 35.92, 1.60, 0.80, 6.38, and 24.26 mg/100 g, respectively. Olatidoye et al. (2011) also determined the minerals ($mg100g^{-1}$) content in almond kernels as Na (27.72 ± 0.2), K (42.17 ± 0.1), Ca (27.17 ± 0.3), Mg (35.92 ± 0.2), Mn (1.6 ± 0.2), Cu (0.4 ± 0.1), Zn (0.8 ± 0.1), Fe (6.38 ± 0.1) and P (24.26 ± 0.2). Roncero et al. (2020) compared the mineral content of almond kernels in different studies and reported that the amount of K, Ca, P, Mg, Fe, Zn, and Mn varied between 435–2051, 98–539.2, 119–800, 2.58–14.6, 2.02–9.53 and 1.1–4.76 $mg100g^{-1}$. According to the results obtained in this study, there is a wide variation in the concentrations of minerals among genotypes along with previous studies. As mentioned above these differences may be affected by genotypes, and ecological and agricultural factors.

In "Gulcan 2 × Lauranne" population, PCA analysis was performed with all macro–micro elements together, PC 1 (1st component) was calculated as 54.45%, PC 2 (2nd component) as 27.08% and total variation as 81.53%. In biplot analysis, microelements created more variation than macro elements. In particular, line 135 was located at the end of the separate group due to its high iron content. While the content of Gulcan 2 zinc microelements was high, lines 42 and 66 produced below the average values of microelements. On the other hand, the macro and microelement contents were evaluated together in "Guara × Nurlu" population, it was determined that the contribution of microelements to the variation was higher. Although line 250 generated above the average in Mn and Zn microelements contents, the highest iron content was carried out in this line.

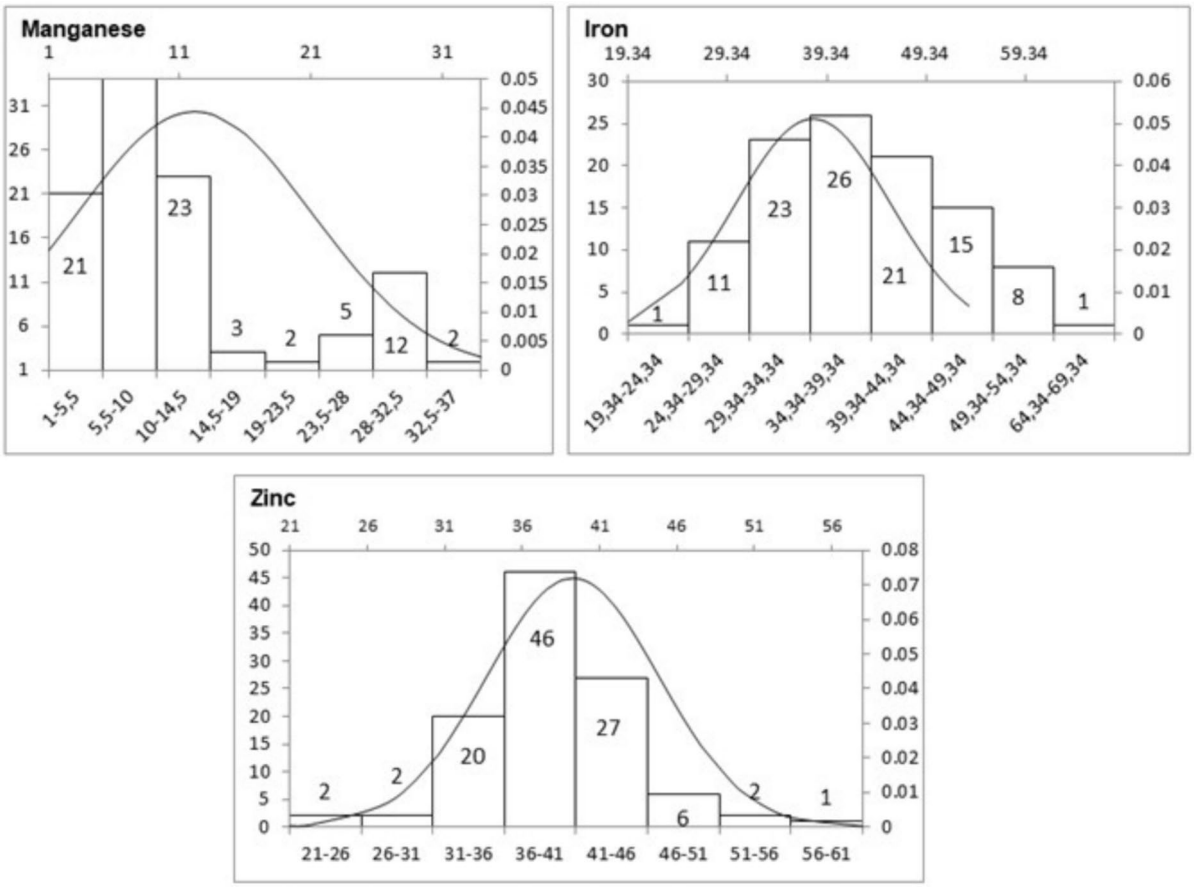


Fig. 8 Distribution of micronutrients of "Guara × Nurlu" population

On the other hand, while the lines 313 and 353 gave more stable results in terms of macro elements, they remained below the average microelements values. However, 239 "Guara × Nurlu" line had low zinc and iron content, while manganese microelement values were detected higher than the other compounds (Fig. 10).

Correlation of macro-microelements in almond populations

Correlation analysis in "Gulcan 2 × Lauranne" almond population with macro-microelements was performed and positive and negative relationships were detected. The highest positive correlation was calculated between magnesium macro nutrient and iron micro nutrient. Another positive relationship was identified among zinc and iron micro nutrients while the highest negative correlation was carried out among magnesium and manganese nutrients (Fig. 11). The macro-microelements values of "Guara × Nurlu" population was demonstrated that the highest positive relationship was calculated between phosphorus and manganese nutrients and the highest negative correlation was determined between magnesium and manganese compounds (Fig. 11).

Tocopherol content (mgg^{-1}) of hybrid lines

In agreement with the results of previous studies, α-tocopherol is determined as the major isomer of tocopherols found in almonds in this study, whereas, β-, γ- tocopherols were present in minor quantities (Zamany et al. 2017; Zhu et al. 2017; Fernandes et al. 2017; Estopañán et al. 2018; Kodad et al. 2018; Wang et al. 2019; Özcan et al. 2020).

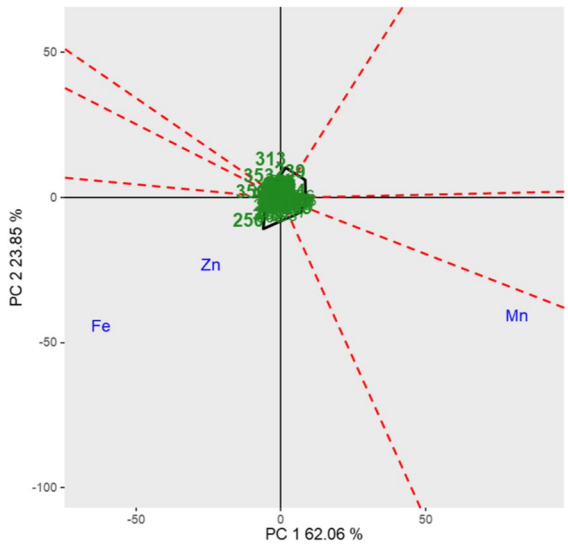


Fig. 9 PCA biplot cluster for micronutrients (Ca, Mg, K, P) in "Guara×Nurlu" F1 population

In "Gulcan 2×Lauranne" population, α-tocopherol, β-tocopherol, and γ-tocopherol ranged between 216.75–852.13, 1.51–16.87, and 1.63–23.83 mgg^{-1} among progenies, respectively. The maximum values of α-, β- and γ-tocopherol were obtained from 26, 100 and 183 progenies, respectively. The minimum values of α-, β- and γ-tocopherols were obtained from 152, 13 and 152 progenies, respectively. The results of distribution analyzes showed that approximately 48, 57, and 59% of progenies contained more concentration of α-, β- and γ-tocopherol than the average value. However, the amount of all tocopherol isomers of parents was lower than the average value (Table 2; Figs. 12 and 13).

In "Guara×Nurlu" population, α-tocopherol, β-tocopherol, and γ-tocopherol ranged between 144.85–1013.48, 1.26–10.05 and 1.33–22.47 mgg^{-1} among progenies, respectively. The maximum values of α-, β- and γ-tocopherol were obtained from lines 251,221 and 300, respectively. The minimum values of α, β- and γ-tocopherol were obtained from lines 335, 335, and 336, respectively. Approximately 44, 45, and 38% of progenies contained more concentration of α-, β- and γ-tocopherol than the average value. At least one of the parents contained more concentration of tocopherol isomers than the average value. Although the concentration of α-tocopherol in Guara (691.75 mgg^{-1}) and Nurlu (424.85 mgg^{-1}) is higher than the average value, some superior progenies were obtained with much higher values than parents (1013.48 mgg^{-1} from 251 progeny) (Table 3; Figs. 14 and 15).

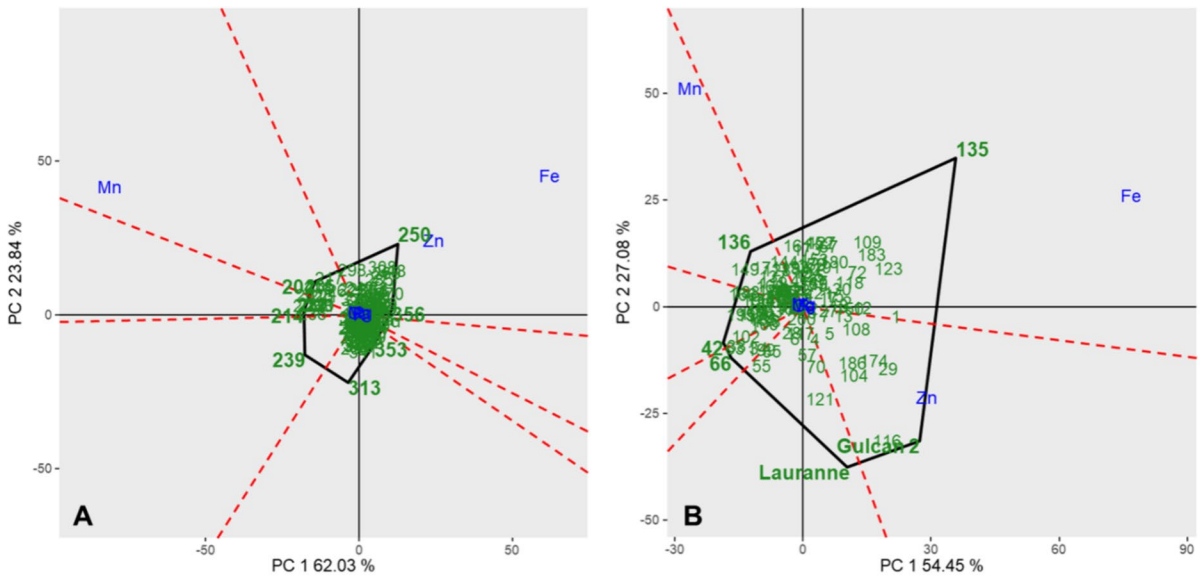


Fig. 10 **A** PCA biplot cluster for macro and micronutrients together (Ca, Mg, K, P) in "Guara×Nurlu" and **B** "Gulcan 2×Lauranne" F1 population

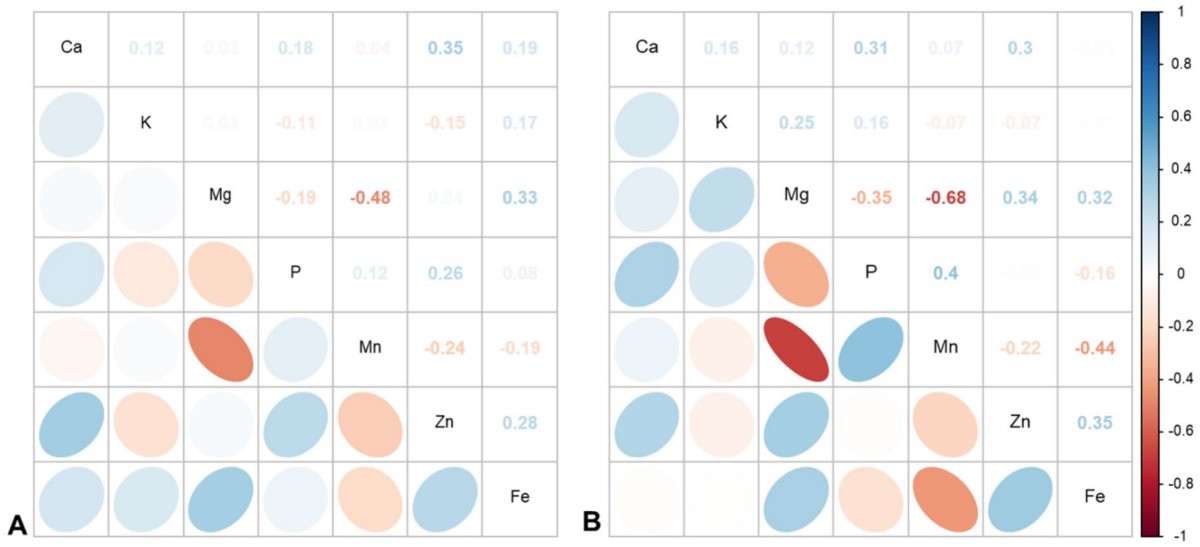


Fig. 11 The correlation coefficients of macro and micronutrients in "Gulcan 2×Lauranne" **A** and "Guara×Nurlu" **B** populations

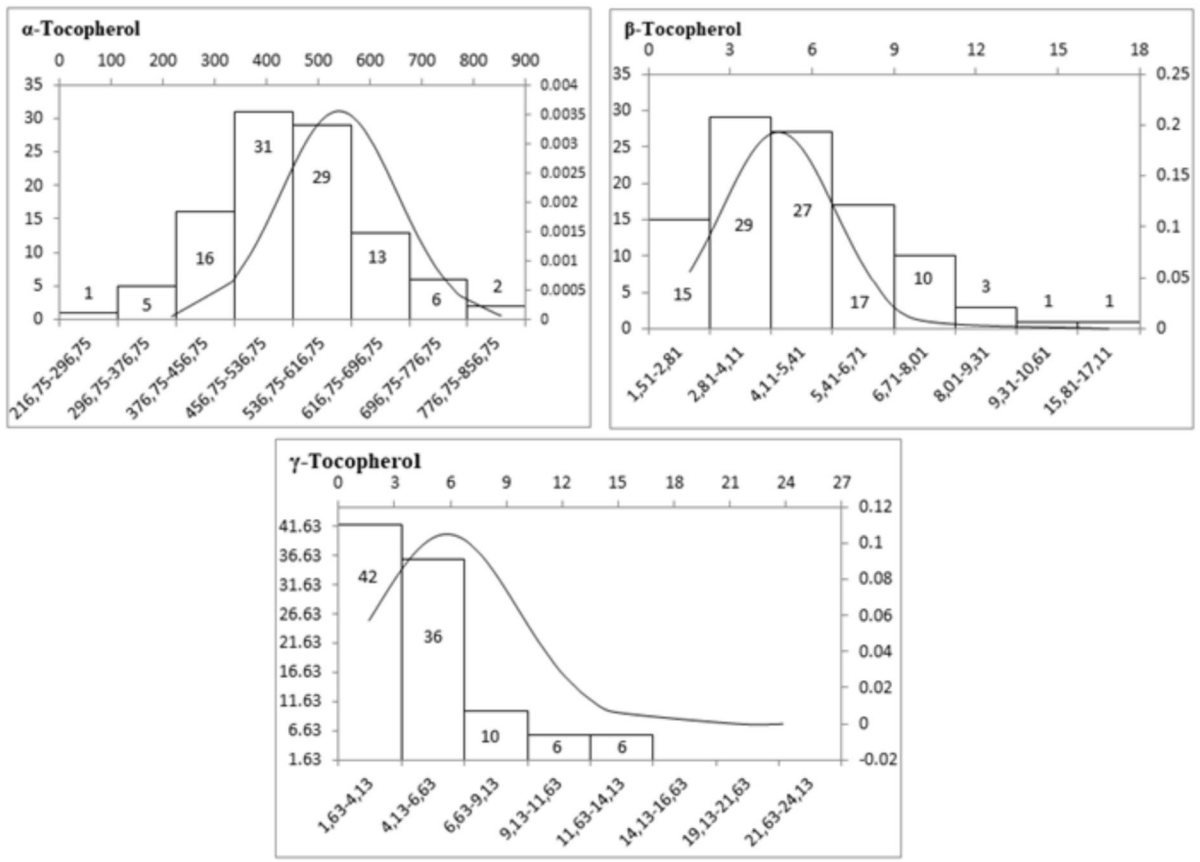


Fig. 12 Distribution of tocopherol concentration in "Gulcan 2×Lauranne" progenies

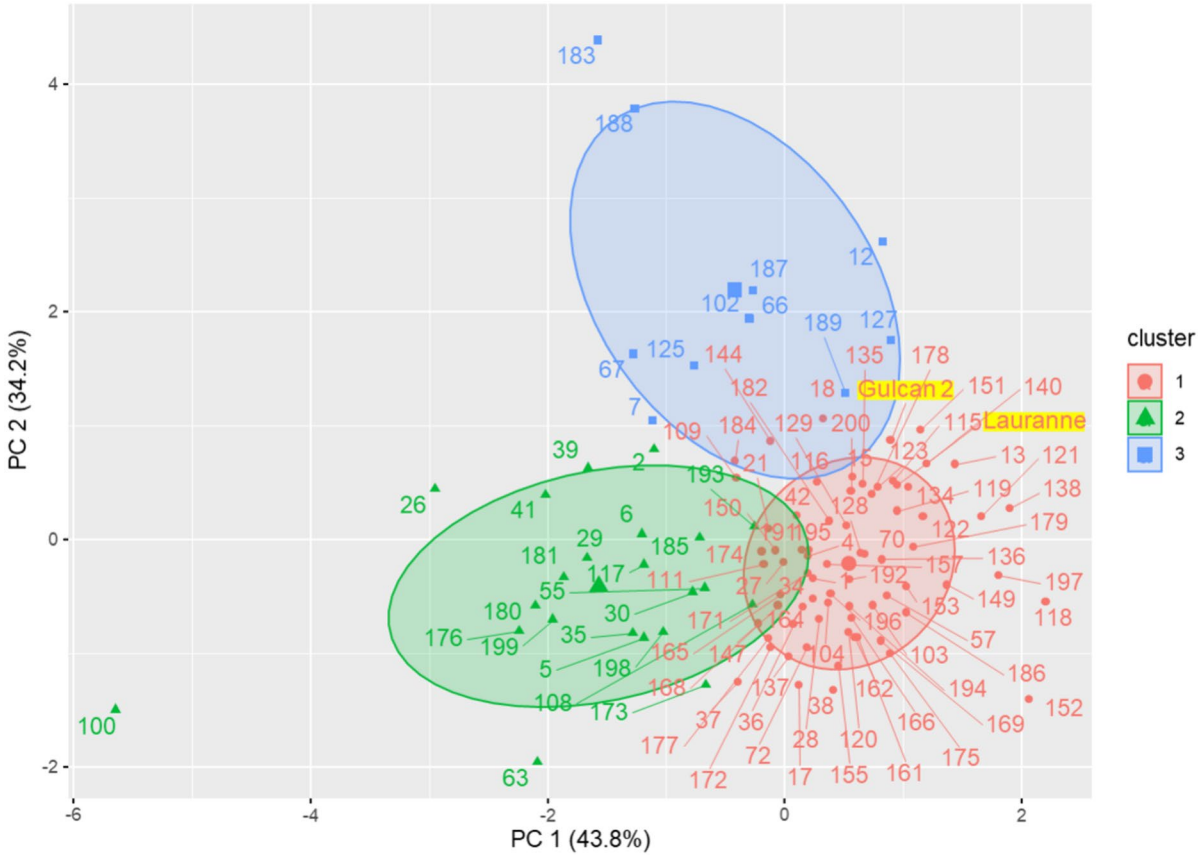


Fig. 13 PCA analysis results of the α, β and γ tocopherols in "Gulcan 2 × Lauranne" population

Zhu et al. (2017) investigated the amount of tocopherols in 24 different almond varieties from Australia and reported that the concentration of alpha and beta tocopherol ranged from 8.00 to 20.90 mg /100 g and from 0.02 to 0.12 mg/100 g among varieties. Kodad et al. (2011) studied the concentration of different tocopherol isomers of 17 different almond varieties grown in Spain and Morocco in two successive years and reported the concentration variation of α-β and γ tocopherols among varieties as 210.9 to 553.4, 4.64 to 14.92 and 0.2–1.02 $mgkg^{-1}$, respectively. The researchers stated that differences may be due to the ecological conditions, especially temperature differences in both countries, as well as cultural factors such as precipitation, irrigation, and fertilization. Several studies have been done to investigate the effect of genotypes from different regions on the concentration of tocopherol in almond kernels. For example, the α-tocopherol concentration of 20 Almond Cultivars from Afghanistan (Kalogiouri et al. 2021), 23 cultivars from Majorca (Estopañán et al. 2018), different Portuguese cultivars (Oliveira et al. 2019), 71 genetic resources of almonds selected from Eastern and Western of Türkiye (Çelik et al. 2019), was determined as 139.1–355.0 μgg^{-1}, of 258.5–500.2 $mgkg^{-1}$, 5.96–19.42 mg/100 g, and non-detected-891.71 $mgkg^{-1}$ oil, respectively. Çelik et al. (2019) also reported a wide range of variations (non-detected to 130.03 $mgkg^{-1}$ oil) in gamma-tocopherol among 71 genetic resources. Wang et al. (2019) studied the concentration of five wild almonds and reported that the concentration of α-, γ- and δ-tocopherol varied between 5–492.4, 160–600, and 15–31.9 $mgkg^{-1}$, respectively. Yada et al. (2011) studied the geographical location of almond trees influence on tocopherol content and determined α-tocopherol contents in California (1.60–19.00 mg/100 g), Italy (25.00–84.00 mg/100 g), and Spain

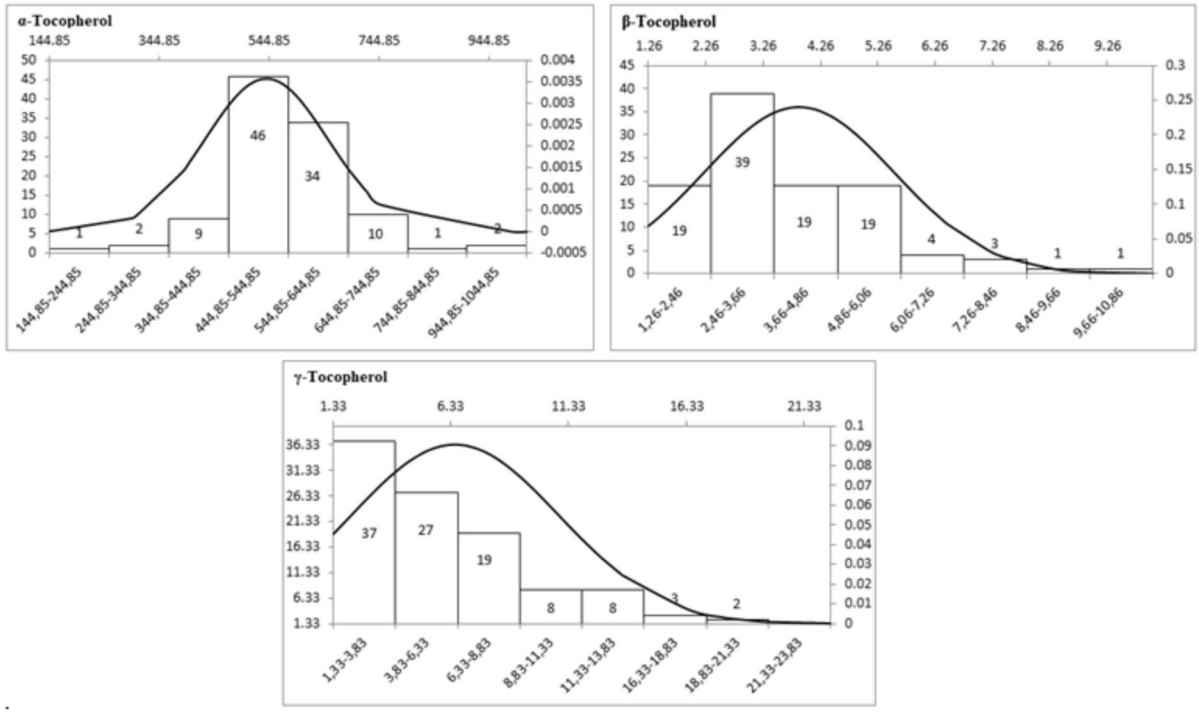


Fig. 14 Distribution of tocopherol concentration in "Guara × Nurlu" F1 progenies

(8.50–19.00 mg/100 g). Besides the cultivar and location factors such as year of harvest (Lopez-Ortiz et al. 2008) and irrigation management (Zhu et al. 2017) also affect the content of α-tocopherol in almonds. In other studies, the effect of agricultural operations and different extraction methods on the tocopherol concentration of almond kernels have been evaluated.

Matthäus et al. (2018) reported that harvest time influence on tocopherol content of almond kernels. The authors determined the concentration of α-β-δ-tocopherol as 10.44, 0.08, 0.681 mg/100 g in the first stage of harvesting which decreased partially in other stages with 5-day intervals, especially after stage 4. El Bernoussi et al. (2020) stated that nut storage negatively affects the content of tocopherol in sweet and bitter almonds. They reported the amount of alpha-tocopherol in sweet almonds as about 371.13 $mgkg^{-1}$. Aires et al. (2018) studied the effect of fertilization with N and reported that the levels of α-tocopherol (0.155 mgg^{-1}) content were higher with lower N doses (25 and 50 kg N ha^{-1}). Lipán et al. (2020) assessed the impact of different irrigation strategies in terms of almond tocopherol content on three commercial almond cultivars (Guara, Marta, and Lauranne) under Mediterranean semiarid conditions and concluded that the irrigation did not drastically affect the fruit almond quality, although it is possible to improve several key parameters when a moderate RDI strategy is applied. Kodad et al. (2018) reviewed the genotypic and environmental effects on tocopherol content in almonds and reported that different alpha-, gamma- and delta-tocopherol homologs varied between 335–657, 2–50 and, 0.1–22 $mgkg^{-1}$ respectively. The authors also mentioned that drought and heat have been the most important stresses affecting tocopherol content in almonds, with increased levels at higher temperatures and in water deficit conditions. Rabadán et al. (2018) evaluated the concentration of alpha-tocopherol in 10 genotypes including Guara in terms of crop year effects. They reported that significant variability has been reported in almond and pistachio oils as a result of the crop year and the interaction between crop year and genotype. The authors emphasized that the genotype itself remains the main factor determining oil chemometrics and α-tocopherol concentration varied between 160 and 490 $mgkg^{-1}$ and Guara had the highest amount. Al Juhaimi et al. (2018) investigated the effect of oil

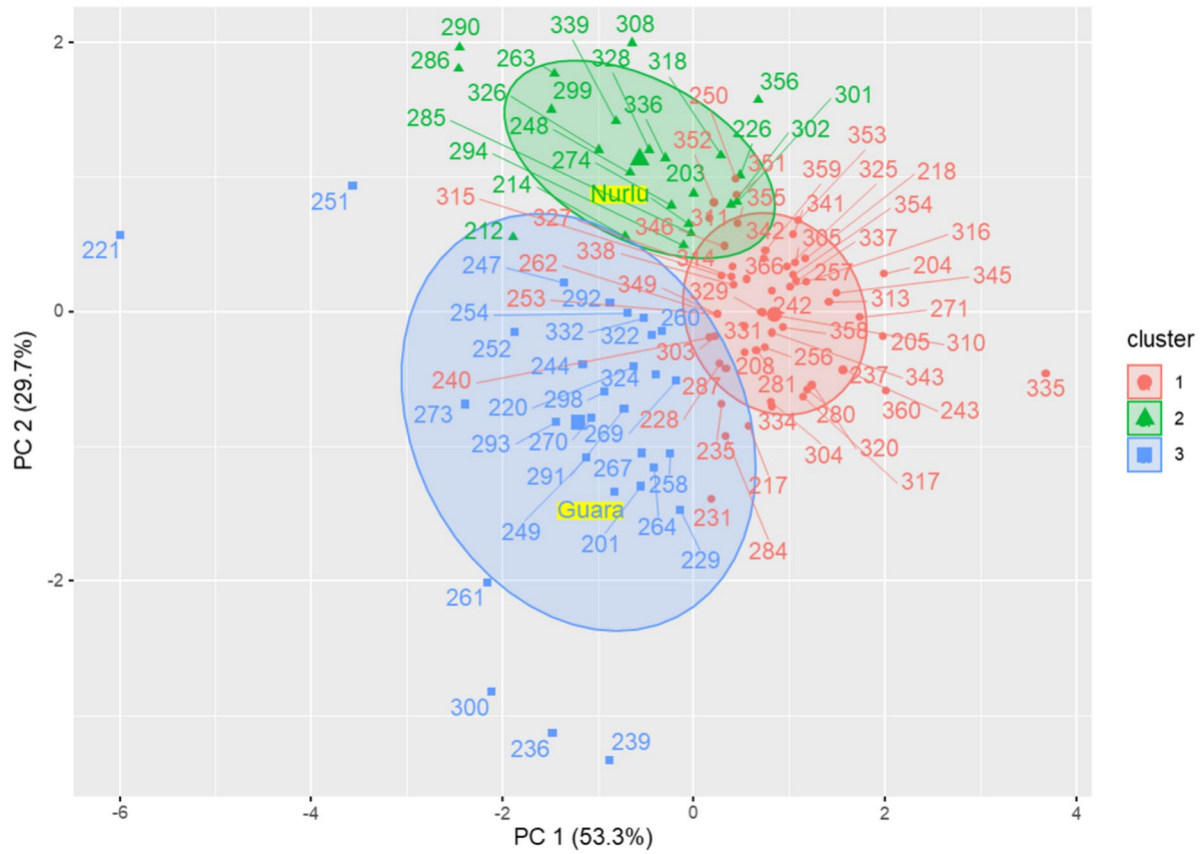


Fig. 15 PCA analysis results of the α, β, and γ tocopherols in "Guara × Nurlu" population

extraction method (Cold press and Soxhlet extraction) on tocopherol concentration of almond kernels and suggested that cold press extraction method is better to prefer. The authors determined the concentration of α, β, γ, and δ-tocopherols as 198.37, 6.83, 11.57, and 0.31 $mgkg^{-1}$ in cold press and 158.85, 5.62, 9.56, and 0.19 $mgkg^{-1}$ in Soxhlet extraction, respectively.

Conclusion

The development of new genotypes with improved phytochemical traits is an aspect of modern plant breeding to be noted. In this study, two populations from crossing four preferred almond cultivars were investigated in terms of mineral and tocopherol content. A wide range of variability was obtained in the concentration of minerals and tocopherols. The results of the present study showed that almond kernels constitute a viable source of certain health-beneficial phytochemical compounds. The kernels of almonds contain high levels of macro and micro minerals, making them excellent sources of essential nutrients in the human diet. Consequently, most of the progenies showed heterosis in studied traits. The results of this study can be used for future breeding programs by forming a new population with selected F1 progenies with superior characteristics and improving the chemical quality of the almond kernel. The results also demonstrate the importance of maintaining and characterizing the genetic diversity of almonds in a gene bank collection for further utilization, mainly for identifying interesting parents to be included in a breeding program.

Acknowledgements This work was supported by Çukurova University Scientific Research Projects (Turkiye) [grant numbers FYL-2016-5604] and the General Directorate of

Agricultural Research and Policies [grant numbers TAGEM/14/AR-GE/01].

Author contribution EK and SK contributed to the study conception, design and supervision. Methodology and formal analysis was done by HO and MG. Data retrieval and synthesis were performed by HO, MG, HK, HT and IA. The first draft of the manuscript was written by MG and all authors commented on subsequent versions of the manuscript. All authors read and approved the final manuscript.

Funding Open access funding provided by the Scientific and Technological Research Council of Türkiye (TÜBİTAK). This research received no external funding.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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