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RESEARCH ARTICLE

Characterization of ornamental pumpkin (*Cucurbita pepo* L. var. *ovifera* (L.) Alef.) genotypes: molecular, morphological and nutritional properties

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Abstract In this study, 36 different ornamental pumpkin (*Cucurbita pepo* L. var. *ovifera* (L.) Alef.) genotypes were analyzed in terms of detailed morphological parameters, molecular properties, and some nutritional features. In this regard, high morphological diversity among the genotypes was observed in terms of plant, leaf and fruit characteristics. Molecular results showed that the genotype which is farthest from the other genotypes of 55% of the difference was determined and fourteen ISSR primers produced, on average, 121 bands in the accessions examined, of which 88 (73%) were polymorphic and Jaccard's similarity coefficient ranged from 0.45 to 0.96. Nutritional analysis showed that *C. pepo* var. *ovifera* seeds are rich in potassium (K) and phosphorus (P) with the concentrations of 8490–21,798 mg/kg and 13,902–28,686 mg/kg, respectively. It was also determined that the pumpkin seed oils had alpha and gamma tocopherols and no β-tocopherols. All samples had β-carotene with the range of 19.63–150.88 mg/kg oil.

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Introduction

The *Cucurbitaceae* family, which has more than 800 species, members have long been cultivated to be used as food, medicinal and also ornamental purposes. *Cucurbitaceae* family used as food is classified in five genera: *Cucurbita, Cucumis, Lagenaria* and *Sechium* (François et al. 2006). *Cucurbita pepo* was shown as a highly polymorphic species in terms of fruit characteristics such as fruit size, shape and color, and the cultivars could be grouped into eight morphotypes in two subspecies, ssp. *pepo* and ssp. *ovifera.* Pumpkin, Vegetable Marrow, Cocozelle and Zucchini correspond to ssp. *Pepo* while Scallop, Acorn, Crookneck, and Straightneck correspond to ssp. *ovifera* (Ferriol et al. 2003).

Cucurbita pepo L. (pumpkin, squash, gourd), an economically and nutritionally important member of the *Cucurbitaceae* family, consists of various vegetable crops cultivated worldwide (Paris 2004; Blanca et al. 2012). Besides the evaluation of Cucurbita species as vegetables, they are also evaluated in different fields such as alternative medicine because of their medicinal properties and nutritional values. Pumpkin seeds are also an important source of protein

(albumin and globulins) and fats (Quanhong and Caili 2005; Dalda Sekerci et al. 2017; Kirnak et al. 2019). In addition, pumpkin seeds contain minerals (especially potassium, phosphorus, and magnesium), phytosterols, carotenoids and vitamin E (tocopherols) in significant amounts and prevent chronic diseases (Seymen et al. 2016). *C. pepo* is the most economically important species within the genus and has the highest degree variation in the *Cucurbitaceae* family. While most Cucurbita members are cultivated for culinary purposes, some are grown for also decorative purposes in nearly all warm and temperate parts of the world (Paris et al. 2003). *C. pepo* is thought to contain two subspecies, each containing several cultivar-groups, ssp. *pepo* (Pumpkin, Vegetable, Marrow, Cocozelle, and Zucchini) and ssp. *ovifera* (Acorn, Scallop, Crookneck, and Straightneck) (Blanca et al. 2012). *C. pepo* is perhaps the most polymorphic species for fruit properties, fruit size, shape and color, and varieties, two subspecies, *pepo* and *ovifera* can be grouped in eight morphotypes (Ferriol et al. 2003; Barzergar et al. 2013).

Cucurbita pepo ssp. *ovifera* var. *ovifera* is known as ornamental pumpkins of Eastern USA origin (Decker-Walters et al. 2001). These pumpkins generally grown for ornamental uses and their morphological characteristics are reported as its fruits are smooth and small pear shape, bottom half is in green, and top half is in yellow color, leaves and stems of same species are prickly (Tuncer 2013). The ornamental pumpkin species have a large scale colored and various shapes such as the apple, bell, egg, bicolor, orange and pear; the surface of species may be smooth or warty, colored or plain, striped or ridged (Anonymus 2008). Based on these differences, genetic diversity between ornamental gourds is thought to be high.

Genetic diversity and similarity in the populations can be measured using molecular and morphological markers. DNA-based molecular markers are useful to provide a relatively independent estimate of genetic diversity. DNA-based molecular markers are useful for predicting the dimensions of genetic diversity. Among these, PCR-based random molecular markers, such as Inter Simple Sequence Repeats (ISSRs), are commonly used in species with a lack of DNA sequence information (Bharathi et al. 2012). Many studies have been conducted using various molecular markers such as SRAP, SSR, RAPD, ISSR and AFLP to study genetic diversity or relationship between several species of Cucurbita (Katzir et al. 2000; Ferriol et al. 2003, 2004; Inan et al. 2012; Yildiz et al. 2015).

Turkey, though not the primary center of genetic diversity for the Cucurbita species, Cucurbita species have had an important genetic diversity over the years due to geographical location (migration routes and the intersection of three genetic diversity centers) and ecological compatibility (Sari et al. 2008). Genetic diversity of major cucurbit species such as winter squash (Balkaya et al. 2010), watermelon (Yagcioglu et al. 2016), melon (Sensoy et al. 2007), bottle gourd (Yetisir et al. 2008; Tas et al. 2019) and bitter melon (Karaman et al. 2018) has been studied and reported. A study on the morphological diversity of *C. pepo* var. *ovifera* and the fatty acid compositions of the seeds was carried out by our group. However, no comprehensive characterization studies consisting of morphological, molecular and seed nutritional parameters have been carried out yet. Therefore, in the current study, it was aimed to investigate fruit morphology and some nutritional composition of *C. pepo* var. *ovifera* from different geographical zones in Turkey. Additionally, the genetic relationships and assessment of genetic diversity by ISSR markers among various genotypes were established and the genotypes were classified according to their nutritional composition using linear discriminate analysis.

Materials and methods

Plant materials

In this study, the seeds of 36 ornamental pumpkin genotypes were collected from different geographical regions of Turkey (Table 1). Seedlings of 36 different genotypes were produced in the unheated greenhouse of the Erciyes University Faculty of Agriculture Department of Horticulture. Five seedlings from each genotype were transplanted to the open field at the 2–3 true leaf stage and seeds were produced with selfing from each genotype. The plants were irrigated regularly based on plant and soil observations by the drip irrigation system. The pH of the soil was measured as 6.8 and it was sandy soil. Various morphological observations were recorded in the field conditions. The seeds were extracted from mature fruit and dried at room temperature in laboratory. The necessary amounts of seeds were ground and used in further

Table 1 Collection sites of ornamental pumpkin genotypes

Genotypes	Region in Turkey	Genotypes	Region in Turkey
G1	Eskisehir	G19	Manisa
G2	Rize	G20	Yozgat
G3	Mugla/Fethiye	G21	Rize
G4	Eskisehir	G22	Batman/Gercus
G5	Manisa	G23	Yozgat/Sefaatli
G6	Manisa	G24	Manisa
G7	Bursa/İznik	G25	Manisa
G8	Tekirdag	G26	Manisa
G9	İzmir/Sirince	G27	Yozgat
G10	Yozgat/Sefaatli	G28	Manisa
G11	Yalova	G29	Kayseri/Develi
G12	Batman	G30	Trabzon
G13	Kutahya	G31	Tekirdag
G14	Kayseri	G32	Trabzon
G15	Sanlıurfa/Bagdere	G33	İzmir/Sirince
G16	Manisa	G34	Cankırı
G17	Kayseri	G35	Burdur
G18	Igdır	G36	Adapazarı/Erenler

nutritional analyzes. Also, the samples required for DNA extraction were taken from young leaf tissue at the flowering stage.

Determination of morphological characteristics

Genotypes were evaluated for different phenotypic characteristics such as plant (growth habit, branching, degree of branching), leaf blade (intensity of green color, blistering, incisions, silvery patches) and fruit (general shape, size, color of skin, secondary color, fruit fresh color).

ISSR analysis

Genomic DNA was isolated from young leaves of the samples according to the modified CTAB method (Doyle and Doyle 1990) and purified. The quality and quantity of DNA were characterized using agarose gel electrophoresis. The genomic DNA was subjected to PCR amplification using 14 ISSR primers (Table 2). For amplification, each 15 µL of PCR components consisted of 1.5 µL of 10X PCR buffer, 1.3 µL of $MgCl_2$ (2.5 mM), 1.33 µL of dNTP (200 µM of each), 0.2 µL of Taq polymerase (500 Unit), 1 µL (5 pM) each of ISSR primers and 2 µL (20 ng) of template DNA. PCR reactions were run on a Bio-Rad C1000 thermocycler and PCR cycling parameters were the same as reported by Gulsen et al. (2007). PCR products were separated on 1.5% agarose gel at 110 V for 3.5 to 4 h, using 0.5 TBE (Tris-Boric acid-EDTA) buffer and visualized under UV light using gel documentation system (Kodak EL Logic 200). Then, DNA fragments were scored as 1–0 binary data matrix for the presence and absence of a band, respectively. Cluster analysis among the 36 genotypes of ornamental pumpkin species was based on Jaccard similarity coefficient (Jaccard 1908) using the Unweighted Pair-Group with Arithmetic Average method (UPGMA) and SAHN clustering algorithm. The analyses were performed using NTSYS-pc (Numerical Taxonomy Multivariate Analysis System, NTSYS-pc version 2.11, Exeter Software, Setauket, NY, USA) program. Thus, the variations and the level of similarity between genotypes were tried to be determined (Fig. 1).

Determination of mineral composition

Approximately 0.5 g of milled ornamental pumpkin seeds was weighed and 10 mL of buffer solution (nitric-perchloric acid) was added to detect mineral profile of the pumpkin seed samples. Then the samples were subjected to wet ashing until 1 mL of the sample remained. After the ashing procedure was completed,

Table 2 Details of amplification and polymorphic potential of ISSR markers in ornamental pumpkins

ISSR Primer	Sequence (5′ ≫ 3′)	TNF	NPF	MP (%)
1	DBDACACACACACACACA	4	3	75
2	CTCTCTCTCTCTCTCTTG	11	10	91
3	GTGTGTGTGTGTGTGTYA	9	8	89
4	CACACACACACACACAR	8	5	62
5	VHVGTGTGTGTGTGTGTG	14	11	78
6	TCCTCCTCCTCCTCCRY	10	7	70
7	HVHCACACACACACACAT	8	5	62
8	AGAGAGAGAGAGAGYC	10	7	70
9	HVHTCCTCCTCCTCCTCCTCCTCC	10	6	60
10	CACCACCACGC	11	8	73
11	GTGTGTGTGTGTGG	6	4	67
12	AGCAGCAGCAGCAGCAGCG	8	7	87
13	BDBCACACACACACACAC	9	6	67
14	GACAGACAGACAGACA	3	1	33
Total		121	88	73

TNF total number of fragments, *NPF* number of polymorphic fragments, *MP* mean polymorphism (%)

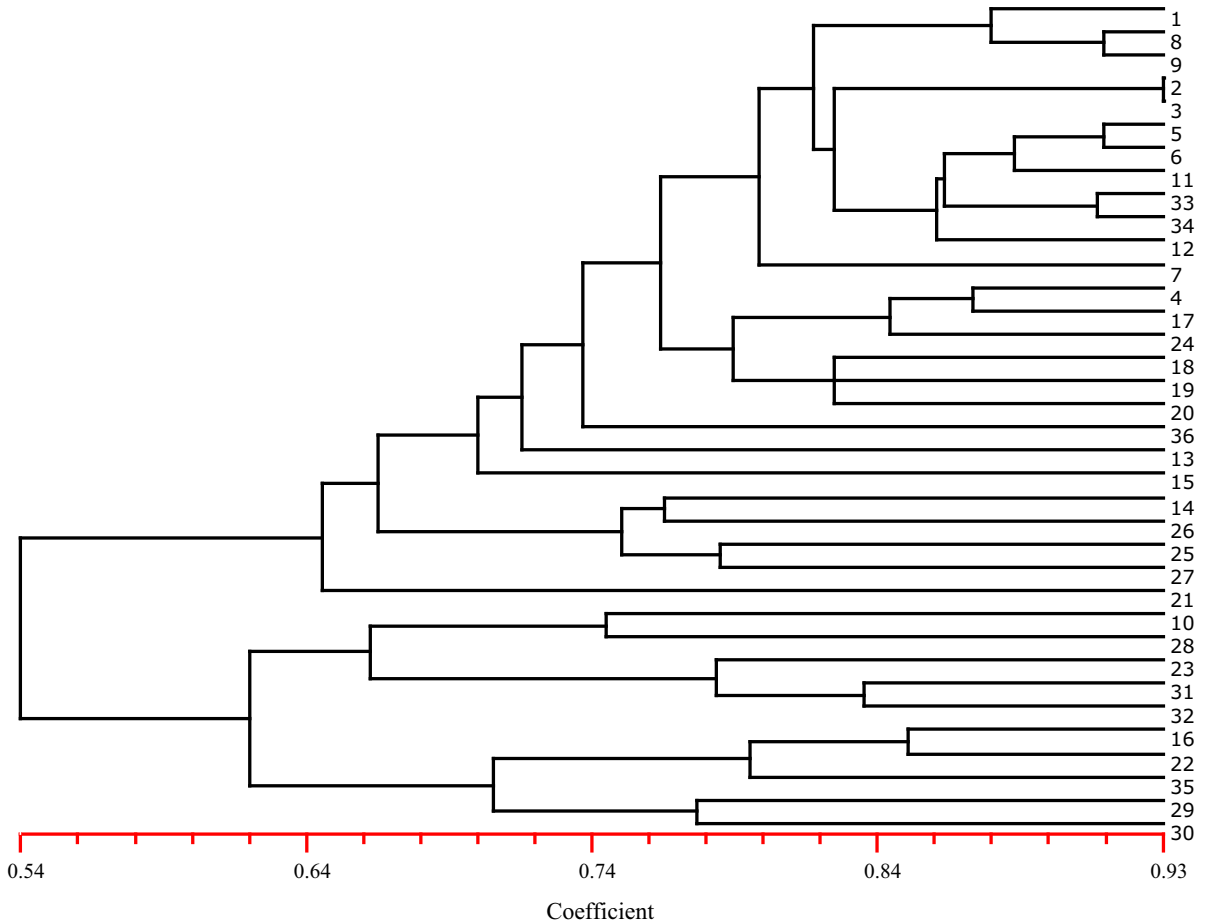

Fig. 1 The UPGMA dendrogram based on Jaccard similarity matrix

the samples were diluted with distilled water and analyzed by ICP-OES spectrometer (Perkin-Elmer, Optima 4300 DV, ICP/OES, Shelton, CT 06484-4794, USA). The contents of Mg, Na, K, P, S, Fe, Mn, Zn, Cu and B were determined (Mertens 2005a, b). The results were calculated using the calibration curves for each minerals prepared by the standard elements and expressed as mg/kg. All measurements were performed with three replications.

Determination of some major vitamin compounds

Firstly, the crude oil was extracted using n-hexane. 10 g of ground sample was mixed with 30 mL of n-hexane and mixed for 10 min on a magnetic stirrier. Finally, the sample was filtrated and the solvent was evaporated using a vacuum evaporator. The obtained crude oil was used for the vitamin analysis. The vitamin compounds of the oil samples were investigated according to the procedure of Beltrán et al. (2010). According to the method, 1.5 g of oil was dissolved in the mobile phase (10 mL) and the chromatographic separation was performed using HPLC (Agilent, 1260) equipped with a UV–Vis detector. The injection volume was 20 µL and a flow rate was 1.0 mL/min. The mobile phase was composed of 0.5:99.5% isopropanol:n-hexane and the absorbance was recorded at 295 nm. Tocopherols and carotene were quantified by an external standards method; α-, δ-, γ-tocopherols and β-carotene were purchased from Sigma Aldrich Co (St. Louis, MO, USA). The results were calculated using the calibration curves for each vitamins prepared by the standards and expressed as mg/kg oil. All measurements were performed with three replications. Limits of detection for α and γ-tocopherols were calculated as 0.66 and 0.61 while the limits of quantification were 2.22 and 2.21, respectively. Coefficient of determination (R^2) were also determined as 0.998, 0.989 and 0.991 for α and γ-tocopherols and β-carotene, respectively.

Data evaluation and modelling

To evaluate whether genotypes from different regions could be mathematically distinguished on the basis of minerals and vitamins. Linear discriminant analysis was performed with using PAST software. The statistical significance of each discriminant function was evaluated on the basis of the Wilks' Lambda factor.

Results and discussion

Morphological properties of pumpkin genotypes

In the current study, 36 ornamental pumpkin genotypes were collected from different regions of Turkey (Table 1). This population was characterized in terms of morphological, molecular and some seed nutritive properties. Morphological features are phenotypically used to determine diversity, but it is important to support these morphologically observed variations by molecular studies. Tables 3 and 4 show the morphological characteristics of the genotypes. It could be said that high morphological diversities among the ornamental pumpkin genotypes such as plant phenotype (growth habit, branching, degree of branching), leaf characteristics (intensity of green color, blistering, incisions, silvery patches) and fruit characteristics (general shape, size, color of skin, secondary color, color of flesh) were monitored. According to the results, plant growth habit was found to be semi-trailing in 23 genotypes, trailing in 7 genotypes, bushy in 6 genotypes. The degree of branching was determined as to be weak in 20 genotypes, medium in 11 genotypes and strong in 5 genotypes.

Protuberance on the leaves was absent in 11 genotypes, present in 25 genotypes (Table 4). Also, ornamental gourds showed high variation in fruit shapes, rind colors and flesh colors. Among the ornamental pumpkin of 36 different genotypes, 22 genotypes had pear-shaped, 3 genotypes had disk-shaped, 3 genotypes had globular shaped, 3 of them had cylindrical and 5 of them had elliptical-shaped fruits. Fruit rind colors were observed as cream, yellow, green and orange. The number of two-colored ornamental pumpkin was 22, and the number of three-colored ornamental pumpkin was 7 (Table 3). A higher level of intra and interspecies variation is present in *Cucurbitaceae* family because they have many cross-pollinated species. The intraspecies variations in the shape, size and rind texture of the fruit is easily observed. In many studies, it is stated that diversity is high in terms of morphological features in *Cucurbitaceae* family (Sakar 2004; Yetisir et al. 2008, Yildiz et al. 2015). Ferriol et al. (2003) performed

Table 3 Fruit morphological parameters of ornamental pumpkin genotypes

Genotypes	Color of flesh	General shape	Main color of skin	Intensity of main color	Number of color	Secondary color
G1	Yellow	Pyriform	Cream	Light	One	Absent
G2	Orange	Disc shaped	Cream	Medium	Two	Present
G3	Yellow	Pyriform	Yellow	Medium	Two	Present
G4	Yellow	Pyriform	Yellow	Medium	Two	Present
G5	Yellow	Globular	Green	Dark	Two	Present
G6	Yellow	Pyriform	Green	Medium	Three	Present
G7	Yellow	Pyriform	Green	Dark	Three	Present
G8	Yellow	Pyriform	Green	Dark	Three	Present
G9	Yellow	Pyriform	Cream	Medium	Three	Present
G10	Yellow	Cylindrical	Yellow	Medium	Three	Present
G11	Yellow	Pyriform	Yellow	Medium	Two	Present
G12	Yellow	Pyriform	Yellow	Medium	Two	Present
G13	Yellow	Pyriform	Yellow	Medium	Three	Present
G14	Yellow	Elliptical	Cream	Light	One	Absent
G15	Yellow	Elliptical	Orange	Medium	One	Absent
G16	Yellow	Pyriform	Green	Medium	Two	Present
G17	Yellow	Cylindrical	Green	Dark	Three	Present
G18	Yellow	Elliptical	Cream	Light	Two	Present
G19	Yellow	Cylindrical	Green	Dark	Two	Present
G20	Yellow	Elliptical	Green	Dark	Two	Present
G21	Orange	Globular	Orange	Medium	Two	Present
G22	Yellow	Pyriform	Orange	Medium	Two	Present
G23	Yellow	Pyriform	Orange	Medium	Two	Present
G24	Yellow	Pyriform	Yellow	Medium	Two	Present
G25	Whitish	Pyriform	Cream	Light	One	Absent
G26	Whitish	Pyriform	Green	Dark	One	Absent
G27	Yellow	Pyriform	Yellow	Medium	Two	Present
G28	Yellow	Elliptical	Orange	Medium	Two	Present
G29	Yellow	Pyriform	Green	Dark	Two	Present
G30	Yellow	Disc shaped	Cream	Light	One	Absent
G31	Yellow	Pyriform	Orange	Medium	Two	Present
G32	Whitish	Disc shaped	Cream	Light	One	Absent
G33	Yellow	Pyriform	Green	Dark	Two	Present
G34	Yellow	Pyriform	Yellow	Medium	Two	Present
G35	Whitish	Globular	Green	Medium	Two	Present
G36	Whitish	Pyriform	Orange	Medium	Two	Present

principal component analysis and differentiation in terms of two main features in morphological characterization. The pumpkin accessions were grouped fundamentally according to the fruit weight and size. Two subspecies were clearly separated, with *Cucurbita pepo* ssp. *pepo* fruits being bigger and heavier than those of *C. pepo* ssp. *ovifera*. Also, they were grouped according to the fruit shape (length/width) ratio. The morphotypes with long fruits (Crookneck, Cocozelle, Straightneck, Vegetable Marrow, and Zucchini) were separated from those with round or flattened ones (Pumpkin, Scallop, and Acorn). Ferriol

Table 4 Plant morphological parameters of ornamental pumpkin genotypes

Genotypes	Plant			Leaf blade				Stem
	Growth habit	Branching	Degree of branching	Intensity of green color	Protuberance	Silvery patches	Incisions	Tendrils
G1	Bushy	Absent	Weak	Medium	Absent	Present	Deep	Absent
G2	Trailing	Present	Strong	Medium	Present	Absent	Absent or very shallow	Present
G3	Trailing	Present	Strong	Dark	Present	Absent	Medium	Present
G4	Trailing	Present	Strong	Medium	Absent	Absent	Shallow	Present
G5	Semi-trailing	Present	Medium	Medium	Present	Present	Shallow	Absent
G6	Semi-trailing	Present	Medium	Medium	Absent	Absent	Medium	Present
G7	Trailing	Present	Medium	Medium	Present	Absent	Shallow	Absent
G8	Semi-trailing	Present	Weak	Medium	Present	Absent	Medium	Absent
G9	Semi-trailing	Present	Weak	Medium	Present	Absent	Deep	Absent
G10	Semi-trailing	Present	Medium	Medium	Absent	Absent	Shallow	Absent
G11	Semi-trailing	Present	Weak	Medium	Present	Absent	Medium	Absent
G12	Semi-trailing	Present	Medium	Medium	Present	Absent	Medium	Present
G13	Semi-trailing	Present	Medium	Medium	Absent	Present	Medium	Present
G14	Semi-trailing	Present	Weak	Light	Present	Absent	Shallow	Absent
G15	Semi-trailing	Present	Weak	Light	Absent	Present	Absent or very shallow	Absent
G16	Semi-trailing	Present	Weak	Medium	Present	Absent	Shallow	Present
G17	Semi-trailing	Present	Weak	Light	Absent	Absent	Absent or very shallow	Absent
G18	Semi-trailing	Present	Weak	Light	Absent	Absent	Medium	Absent
G19	Semi-trailing	Present	Medium	Dark	Present	Absent	Shallow	Absent
G20	Trailing	Present	Strong	Medium	Absent	Absent	Shallow	Present
G21	Trailing	Present	Strong	Medium	Present	Absent	Deep	Absent
G22	Semi-trailing	Present	Medium	Medium	Present	Absent	Shallow	Absent
G23	Semi-trailing	Present	Medium	Medium	Present	Absent	Shallow	Absent
G24	Bushy	Absent	Weak	Medium	Present	Absent	Medium	Absent
G25	Semi-trailing	Present	Weak	Medium	Present	Absent	Shallow	Absent
G26	Semi-trailing	Present	Medium	Medium	Present	Absent	Medium	Absent
G27	Semi-trailing	Present	Weak	Dark	Present	Absent	Medium	Absent

Table 4 continued

Genotypes	Plant			Leaf blade				Stem
	Growth habit	Branching	Degree of branching	Intensity of green color	Protuberance	Silvery patches	Incisions	Tendrils
G28	Semi-trailing	Present	Weak	Medium	Present	Absent	Shallow	Absent
G29	Bushy	Absent	Weak	Dark	Absent	Absent	Shallow	Absent
G30	Bushy	Absent	Weak	Dark	Present	Absent	Absent or very shallow	Absent
G31	Semi-trailing	Present	Weak	Medium	Present	Absent	Medium	Absent
G32	Bushy	Absent	Weak	Medium	Absent	Absent	Absent or very shallow	Absent
G33	Trailing	Present	Medium	Dark	Present	Absent	Shallow	Absent
G34	Semi-trailing	Present	Weak	Medium	Present	Absent	Shallow	Absent
G35	Bushy	Absent	Weak	Medium	Present	Absent	Medium	Absent
G36	Semi-trailing	Present	Weak	Medium	Present	Absent	Medium	Absent

et al. (2003) determined that the individuals in the population have different growth habits, different fruit rind colors, and fruit flesh colors.

ISSR analysis

Morphological characterization results were supported by the molecular data. ISSR markers are useful in detecting genetic diversity and simpler to use than other technique like SSR and AFLP (Reddy et al. 2002). Fourteen ISSR primers produced, on average, 121 bands in the genotypes examined, of which 88 (73%) were polymorphic (Table 2). The number of amplification per accession varied from 3 to 14, where band sizes ranged between 150 *bp* and 1100 *bp*. Jaccard's similarity coefficient ranged from 0.54 to 0.93. According to the dendrogram, genotype 1 was in a separate branch alone and located in a position away from the other genotypes. Genotype 2 and genotype 3, which were collected from different regions and showed differences in morphological characteristics, were genetically similar to each other over 90%. Similarly, genotypes 8 and 9; genotypes 33 and 34, which were obtained from different ecological regions and had some differences in morphological properties, were genetically more than 85% similar. As expected, genotype 5 and 6 from the same region had more than 85% genetic similarity. Several molecular studies have been performed for different cucurbit species or in a population in *Cucurbitaceae* family. In some of these studies conducted in *Cucurbitacaea* species; Katzir et al. (2002) used the ISSR and SSR markers; Decker-Walters et al. (2001) used RAPD primers; Paris et al. (2003) used AFLP, ISSR, and SSR molecular marker methods; Ferriol et al. (2003, 2004) used SRAP and AFLP molecular markers. Similarly, the rate of polymorphism was found to be over 60% in these studies conducted in the *Cucurbitaceae* family. Also, ISSR and SRAP markers were used by Inan (2012); SSR marker method was used by Li et al. (2013) and Barzergar et al. (2013). Li et al. (2013) stated that the genetic similarity coefficients between individuals (*C. maxima* and *C. pepo*) ranged from 0.31 to 0.77. In the present study, the high genetic similarity coefficient (0.45–0.96) between ornamental gourds showed an indicator of high variation.

In a different study, Katzir et al. (2000) characterized the presence of ISSR and SSR primers in a population containing *C. pepo* ssp. *ovifera*, *C. pepo*

ssp. *fraterna* and *C. pepo* ssp. *pepo*, and *C. pepo* ssp. *ovifera* and they were genetically located far away from others. In a study conducted by Paris et al. (2003), 48 different accessions were studied with 3 different primers (AFLP, ISSR, and SSR) and found polymorphism rates as 63% AFLP and 74% ISSR. Similarly, genetic diversity of bottle gourd and 31 other cucurbits (*Cucurbita maxima:*11, *C. moschata:*3, *C. pepo* subsp. *ovifera:* 5, *C. pepo:*10, and *Luffa cylindrica*: 2) were compared with 16 SRAP primer combinations by Yildiz et al. (2015). They reported that principal coordinate analysis separated accessions into three genera: (1) *Lagenaria*, (2) *Luffa*, and (3) *Cucurbita* and *Cucurbita* had a high genetic diversity because it contained four species (*Cucurbita maxima*, *C. moschata*, *C. pepo* subsp. *Ovifera* and *C. pepo*). They also stated that close genetic relationship between *C. maxima* and *C. pepo* subsp. *ovifera* could be associated with the domestication process of the two species.

The present study was conducted among individuals of a single sub-species (*C. pepo* var. o*vifera*), and in-species variation and genetic diversity were found to be significant. It was concluded that ornamental gourds collected from Turkey contain different types in terms of both morphological characteristics and molecular properties. This diversity is explained by the fact that ornamental gourds introduced the country from different sources by different routes and ornamental pumpkins is 100% cross-pollinated species.

Nutritional properties of pumpkin seed samples

Mineral composition of pumpkin seed samples

All pumpkin genotypes were subjected to mineral analysis and some macro and micro mineral content of the samples were determined and tabulated in Table 5. As could be seen clearly from the table that highly significant variation was observed in all investigated elements. At least two-fold difference between the highest and lowest values was determined. This difference was determined to be 2–2.5 folds for those other than Na and Zn, and 3 times for Zn and 13 times for Na. The most abundant macro mineral was found as P and it was in the range of 13,902–28,686 mg/kg. The highest P was in G5 while the lowest content was recorded in G7. Phosphorus was followed by K, Mg, and S with average of 15,160, 5898 and 5654 mg/kg, respectively. The content of macroelements were: Phosphorous (28,696–13,902 mg/kg), potassium (21,798–8490 mg/kg), magnessium (7692–3626 mg/kg) and sulfur (8010–3457 mg/kg. As is seen in Table 5, the lowest K and P levels were determined in the same genotype coded as G7 and the highest one was determined in genotype G5. The average concentration of microelements were ordered as Fe, Zn, Mn, Na, Cu, and B with 116.10, 64.21, 42.12, 35.74, 25.10, and 9.61 mg/kg, respectively. The maximum and minimum concentration of microelements were: Iron (157.40–65.34 mg/kg), zinc (96.12–31.86 mg/kg), manganese (53.28–23.04 mg/kg), sodium (85.38–6.54 mg/kg), copper (42.30–17.04 mg/kg) and boron (13.26–6.48 mg/kg). According to results there was high variation among the genotypes and it was pointed out high amount of some elements (Mg, S, K, P, B and Mn) in G1, G5 and G8 coded genotypes while G7 was poor in terms of mineral content. Similar results were obtained from our previous study about *C. pepo* ssp. *ovifera* and we concluded that G1 encoded genotype indicated differences in terms of morphological properties (Dalda Sekerci et al. 2017). The mineral content results of the current study were in accordance with the literature findings. Kwiri et al. (2014) investigated the proximate composition of *C. pepo* seeds and reported that the major element of the seed samples was P and the minor element was Zn with the concentrations of 10.408 g/kg and 0.12 g/kg, respectively. In another study about the mineral contents of pumpkin seeds, the major elements of the seed samples were reported to be P, K, and Mg which is similar to the present study results (Seymen et al. 2016). Similar results were also reported for the mineral composition of pumpkin seed samples by Lazos (1986) and El-Adawy and Taha (2001).

Some major vitamin contents of pumpkin seed samples

Table 6 shows the tocopherol and β-carotene contents of the pumpkin seed samples. As is seen, only alpha and gamma tocopherols were detected in the samples but beta-carotene was found in all samples. There was no alpha tocopherol content for half of the pumpkin seed samples (17 genotypes having no alpha tocopherol). The highest alpha tocopherol was determined in the sample of G22 as 1990.57 mg/kg oil while the lowest alpha tocopherol was in the genotype of G36 (166.10 mg/kg oil). Gamma tocopherol level changed

Table 5 Mineral composition of ornamental pumpkin genotype seeds (mg/kg)

Genotypes	B	Cu	Fe	K	Mg	Mn	Na	P	S	Zn
G1	12.72 ± 0.18	36.66 ± 0.38	100.92 ± 0.94	18,312 ± 47.4	7692 ± 74.5	51.18 ± 0.21	29.52 ± 0.41	27,846 ± 49.8	8010 ± 26.4	66.60 ± 0.23
G2	7.14 ± 0.21	24.84 ± 0.41	65.34 ± 0.91	12,804 ± 46.1	4798 ± 46.9	23.04 ± 0.18	9.66 ± 0.18	19,338 ± 52.3	6096 ± 33.3	55.50 ± 0.45
G3	12.36 ± 0.11	23.10 ± 0.33	123.36 ± 0.97	16,530 ± 41.9	7020 ± 23.4	48.96 ± 0.17	38.40 ± 0.47	26,196 ± 74.3	5618 ± 15.6	59.22 ± 0.64
G4	11.40 ± 0.20	27.84 ± 0.39	136.20 ± 0.83	18,042 ± 44.6	7122 ± 25.1	35.22 ± 0.31	49.44 ± 0.43	26,952 ± 73.9	6696 ± 46.2	68.76 ± 0.35
G5	12.24 ± 0.14	25.98 ± 0.34	118.62 ± 0.81	21,798 ± 33.8	7554 ± 54.4	39.12 ± 0.23	72.90 ± 0.35	28,686 ± 54.6	7326 ± 42.1	75.06 ± 0.27
G6	9.90 ± 0.18	28.38 ± 0.31	98.64 ± 0.78	21,744 ± 49.4	7008 ± 35.4	45.60 ± 0.14	85.38 ± 0.37	24,366 ± 35.5	7446 ± 35.8	48.72 ± 0.64
G7	6.48 ± 0.25	17.04 ± 0.39	88.86 ± 0.94	8490 ± 31.1	3626 ± 25.5	30.54 ± 0.34	6.54 ± 0.28	13,902 ± 46.8	3572 ± 26.4	44.16 ± 0.45
G8	13.26 ± 0.21	30.36 ± 0.33	151.38 ± 0.99	14,988 ± 46.2	6576 ± 41.4	53.28 ± 0.25	68.04 ± 0.43	25,650 ± 68.4	6450 ± 42.5	76.44 ± 0.43
G9	11.16 ± 0.14	29.10 ± 0.29	115.38 ± 0.84	19,752 ± 47.1	7158 ± 39.4	50.70 ± 0.27	36.12 ± 0.38	27,006 ± 35.4	6900 ± 34.8	63.54 ± 0.19
G10	10.86 ± 0.18	22.44 ± 0.27	115.26 ± 0.87	17,862 ± 26.8	6414 ± 37.4	36.24 ± 0.39	34.62 ± 0.19	23,274 ± 29.4	6090 ± 26.5	58.56 ± 0.47
G11	8.52 ± 0.17	17.70 ± 0.41	76.50 ± 0.94	13,260 ± 38.4	3990 ± 31.6	35.40 ± 0.17	41.04 ± 0.45	14,868 ± 49.5	4961 ± 42.9	31.86 ± 0.64
G12	10.20 ± 0.17	28.26 ± 0.44	134.88 ± 0.84	18564 ± 36.4	7212 ± 48.7	50.52 ± 0.19	46.20 ± 0.43	27,366 ± 37.8	5695 ± 39.4	65.52 ± 0.34
G13	8.82 ± 0.11	22.32 ± 0.42	120.12 ± 0.83	13,494 ± 31.1	6078 ± 42.1	45.18 ± 0.13	18.60 ± 0.28	22,302 ± 49.7	5703 ± 26.4	56.40 ± 0.45
G14	8.34 ± 0.13	19.08 ± 0.41	106.92 ± 0.98	13,872 ± 49.7	6072 ± 45.5	37.62 ± 0.14	20.52 ± 0.37	21,780 ± 41.6	5508 ± 41.5	56.28 ± 0.19
G15	8.34 ± 0.16	25.38 ± 0.49	79.08 ± 0.97	15,282 ± 29.8	5362 ± 57.7	40.98 ± 0.21	16.92 ± 0.18	20,700 ± 35.4	6222 ± 49.5	47.34 ± 0.64
G16	8.64 ± 0.18	26.28 ± 0.41	115.38 ± 0.90	13,392 ± 37.9	6660 ± 49.8	46.86 ± 0.25	17.58 ± 0.38	23,508 ± 29.8	5753 ± 35.7	91.08 ± 0.35
G17	9.78 ± 0.24	18.06 ± 0.43	117.60 ± 0.79	14,586 ± 34.4	5546 ± 54.4	42.24 ± 0.29	25.32 ± 0.49	20,628 ± 43.5	4652 ± 35.1	50.16 ± 0.48
G18	8.34 ± 0.14	21.84 ± 0.46	79.08 ± 0.87	12,708 ± 31.9	6624 ± 24.4	41.70 ± 0.18	27.60 ± 0.42	23,250 ± 45.7	5213 ± 29.5	45.36 ± 0.17
G19	9.00 ± 0.18	25.38 ± 0.49	99.00 ± 0.88	12,408 ± 24.8	6930 ± 19.9	46.44 ± 0.17	9.30 ± 0.18	24,936 ± 52.4	5877 ± 42.5	71.88 ± 0.42
G20	11.70 ± 0.19	26.70 ± 0.47	104.88 ± 0.80	14,118 ± 29.4	7014 ± 21.4	44.88 ± 0.22	39.78 ± 0.27	23,550 ± 46.8	6120 ± 49.5	71.58 ± 0.49
G21	9.54 ± 0.17	23.70 ± 0.41	116.76 ± 0.78	12,594 ± 24.6	6294 ± 29.9	48.54 ± 0.28	77.34 ± 0.35	24,732 ± 43.1	4897 ± 35.1	69.90 ± 0.35
G22	12.30 ± 0.10	22.44 ± 0.46	131.22 ± 0.79	21,462 ± 34.8	6276 ± 34.7	36.42 ± 0.34	47.82 ± 0.37	22,164 ± 35.8	7440 ± 36.5	65.46 ± 0.48
G23	10.98 ± 0.15	27.78 ± 0.48	157.14 ± 0.94	17,814 ± 39.7	6312 ± 17.7	46.98 ± 0.34	25.92 ± 0.25	24,282 ± 39.4	5673 ± 37.8	67.56 ± 0.41
G24	8.76 ± 0.14	17.22 ± 0.47	122.40 ± 0.97	13,746 ± 31.4	6276 ± 19.5	40.80 ± 0.16	22.68 ± 0.42	22,734 ± 56.2	5412 ± 45.9	62.82 ± 0.35
G25	8.22 ± 0.19	21.60 ± 0.41	130.74 ± 0.91	16,164 ± 43.1	5512 ± 25.5	48.36 ± 0.17	43.38 ± 0.43	20,118 ± 51.2	5577 ± 41.5	58.62 ± 0.9
G26	8.70 ± 0.17	22.80 ± 0.29	151.86 ± 0.73	15,546 ± 43.8	5333 ± 29.7	38.58 ± 0.34	34.20 ± 0.28	19,842 ± 28.4	6246 ± 15.8	75.30 ± 0.74
G27	9.84 ± 0.17	21.36 ± 0.43	121.02 ± 0.74	16,116 ± 41.8	5877 ± 34.7	44.28 ± 0.18	22.02 ± 0.21	19,620 ± 43.6	5983 ± 35.4	74.34 ± 0.46
G28	8.28 ± 0.16	20.70 ± 0.41	107.34 ± 0.79	12,594 ± 26.5	4674 ± 39.5	37.86 ± 0.18	18.60 ± 0.34	18,870 ± 42.1	4551 ± 19.6	56.82 ± 0.38
G29	8.88 ± 0.17	17.46 ± 0.38	79.50 ± 0.78	11,922 ± 29.7	4195 ± 47.8	31.26 ± 0.13	12.30 ± 0.46	16,248 ± 35.4	3457 ± 25.4	46.86 ± 0.49
G30	7.02 ± 0.13	27.12 ± 0.34	102.84 ± 0.79	12,138 ± 41.6	4722 ± 49.5	37.92 ± 0.25	11.23 ± 0.27	19,038 ± 64.2	4051 ± 36.5	69.24 ± 0.34
G31	9.60 ± 0.18	42.30 ± 0.39	136.32 ± 0.84	14,460 ± 44.5	5576 ± 34.7	46.92 ± 0.27	72.24 ± 0.19	21,042 ± 39.5	6834 ± 34.5	81.90 ± 0.48
G32	9.24 ± 0.16	30.60 ± 0.37	154.62 ± 0.81	18,420 ± 49.8	5659 ± 39.8	41.34 ± 0.29	33.48 ± 0.24	22,296 ± 48.5	5074 ± 54.5	82.50 ± 0.34
G33	6.66 ± 0.21	34.44 ± 0.34	113.46 ± 0.83	14,286 ± 47.5	5065 ± 42.5	42.30 ± 0.34	31.08 ± 0.35	19,800 ± 43.2	5487 ± 49.5	65.82 ± 0.39

Table 5 continued

Genotypes	B	Cu	Fe	K	Mg	Mn	Na	P	S	Zn
G34	7.80 ± 0.18	24.78 ± 0.31	123.54 ± 0.79	13,104 ± 41.6	5215 ± 29.8	38.88 ± 0.49	21.12 ± 0.45	20,064 ± 35.6	5422 ± 47.8	68.70 ± 0.48
G35	11.70 ± 0.23	26.40 ± 0.39	125.94 ± 0.98	14,652 ± 29.5	4479 ± 27.4	41.82 ± 0.47	76.80 ± 0.35	18,120 ± 46.9	4132 ± 46.5	65.40 ± 0.49
G36	9.24 ± 0.17	26.16 ± 0.33	155.22 ± 0.91	16,104 ± 27.8	6138 ± 45.5	48.42 ± 0.34	43.26 ± 0.29	24,150 ± 43.1	5545 ± 35.5	96.12 ± 0.37
Min	6.48	17.04	65.34	8490	3626	23.04	6.54	13,902	3457	31.86
Max	13.26	42.3	157.14	21,798	7692	53.28	85.38	28,686	8010	96.12
Average	9.61	25.10	116.1	15,364.7	5946.5	42.12	35.74	22,200.7	5713.6	64.21

in the range of 212.26–1178.40 mg/kg oil and the highest gamma tocopherol level was determined in G7 sample while the lowest value was in the genotype of G19. Gamma tocopherol was not detected in 33.3% of pumpkin genotype seed samples. It was also seen that there was a negative correlation between alpha and gamma tocopherols. And the pumpkin seed oil having no alpha tocopherol, contained γ-tocopherol which means that if there is no alpha in the sample, it had gamma tocopherol in quite high levels. This type of negative correlation between α- and γ-tocopherols was observed in vegetable oils (Kamal-Eldin and Andersson 1997). In addition to tocopherol contents, beta carotene levels of the samples were analyzed and tabulated in Table 6. As could be seen in the Table, it ranges between 19.63 and 150.88 mg/kg oil and the highest level was determined in the sample coded as G6 while the lowest value was in the sample coded as G9 to be 150.88 mg/kg oil. Murkovic et al. (1997) reported that the tocopherol composition of different pumpkin seed genotypes and they stated that the major tocopherol was gamma tocopherol and β-tocopherol was not detected in 45 of 50 genotypes as is in the current research. In another research conducted by Stevenson et al. (2007), tocopherol contents of 12 pumpkin cultivars ranged from 27.1 to 75.1 μg/g of oil for α-tocopherol, from 74.9 to 492.8 μg/g for γ-tocopherol, and from 35.3 to 1109.7 μg/g for δ-tocopherol and they reported that the samples had no β-tocopherols. As in the aforementioned study (2007), β-tocopherol was not determined in the seeds of the genotypes used in the present study.

Linear discriminant analysis (LDA)

In terms of regions, nutritional data of ornamental pumpkin genotypes were submitted to linear discriminant analysis. Figure 2 shows that the regions of genotypes were grouped with a predictive ability of 91.6% It is clearly shown that genotypes from different regions were well distinguished from each other. Only three genotypes (G15, G18 and G35) behaved differently and separated from others. These results indicated that the pumpkins from different origins can be classified based on mineral and vitamin composition. Ma et al. (2016) stated that geographic origin of food and agricultural products is topic of interest for both consumers and producers. LDA is a supervised classification technique where the

Table 6 Some major vitamin composition of ornamental pumpkin genotype seeds

Genotypes	α-tocopherol	γ-tocopherol	β-carotene
G1	–	668.50 ± 35.7	33.99 ± 5.4
G2	538.70 ± 34.7	–	37.12 ± 6.4
G3	–	812.33 ± 47.5	25.89 ± 2.9
G4	450.97 ± 25.8	665.83 ± 43.5	24.38 ± 3.5
G5	836.87 ± 41.4	–	31.75 ± 4.9
G6	–	393.50 ± 34.4	19.63 ± 4.7
G7	–	1178.40 ± 84.5	33.75 ± 3.8
G8	–	683.45 ± 42.5	82.83 ± 5.9
G9	–	790.68 ± 34.1	150.88 ± 11.1
G10	–	919.32 ± 31.2	71.23 ± 6.6
G11	1287.87 ± 35.5	–	85.49 ± 5.4
G12	–	1143.25 ± 68.5	80.13 ± 7.6
G13	–	979.50 ± 55.2	90.29 ± 4.9
G14	–	574.13 ± 34.6	68.81 ± 3.8
G15	–	1172.65 ± 65.3	89.87 ± 5.4
G16	–	1067.38 ± 58.4	72.50 ± 6.8
G17	–	705.68 ± 22.1	83.99 ± 7.3
G18	–	241.95 ± 15.5	75.71 ± 4.9
G19	1885.00 ± 43.6	212.26 ± 19.4	66.37 ± 7.2
G20	1722.33 ± 49.5	1058.27 ± 24.2	71.47 ± 4.9
G21	–	882.48 ± 22.2	88.91 ± 8.2
G22	1990.57 ± 53.6	–	84.00 ± 8.3
G23	881.36 ± 25.5	–	80.62 ± 7.6
G24	343.18 ± 24.7	921.72 ± 32.2	71.02 ± 4.9
G25	686.18 ± 32.6	792.23 ± 25.4	72.80 ± 5.8
G26	866.69 ± 41.5	–	89.17 ± 6.8
G27	899.19 ± 36.9	–	58.99 ± 3.5
G28	1162.00 ± 25.8	–	95.06 ± 5.9
G29	1064.23 ± 54.4	–	96.61 ± 8.4
G30	–	762.52 ± 29.4	74.83 ± 4.9
G31	741.12 ± 32.6	–	71.99 ± 4.8
G32	–	552.53 ± 19.7	84.94 ± 6.8
G33	796.20 ± 25.5	–	80.35 ± 7.3
G34	869.48 ± 21.5	–	68.95 ± 4.9
G35	729.43 ± 15.5	1156.57 ± 65.5	78.64 ± 6.8
G36	166.10 ± 11.5	702.66 ± 42.8	96.69 ± 8.7

–, not detected

number of categories and the samples that belong to each category are previously defined (Liu et al. 2006). In several studies, LDA was used successfully and agricultural products were classified regionally (D'Archivio and Maggi 2017; Sun et al. 2011; Silvano et al. 2014). This is the first report about *C. pepo* var. *ovifera* landraces classification using mineral and vitamin data.

Conclusion

Among genotypes, some differences were observed in molecular and nutritional characterization data. The application of multivariate analysis is adequate to classify samples according to geographical zones. Morphological variation was proved with molecular analysis since polymorphism was 73%. The results

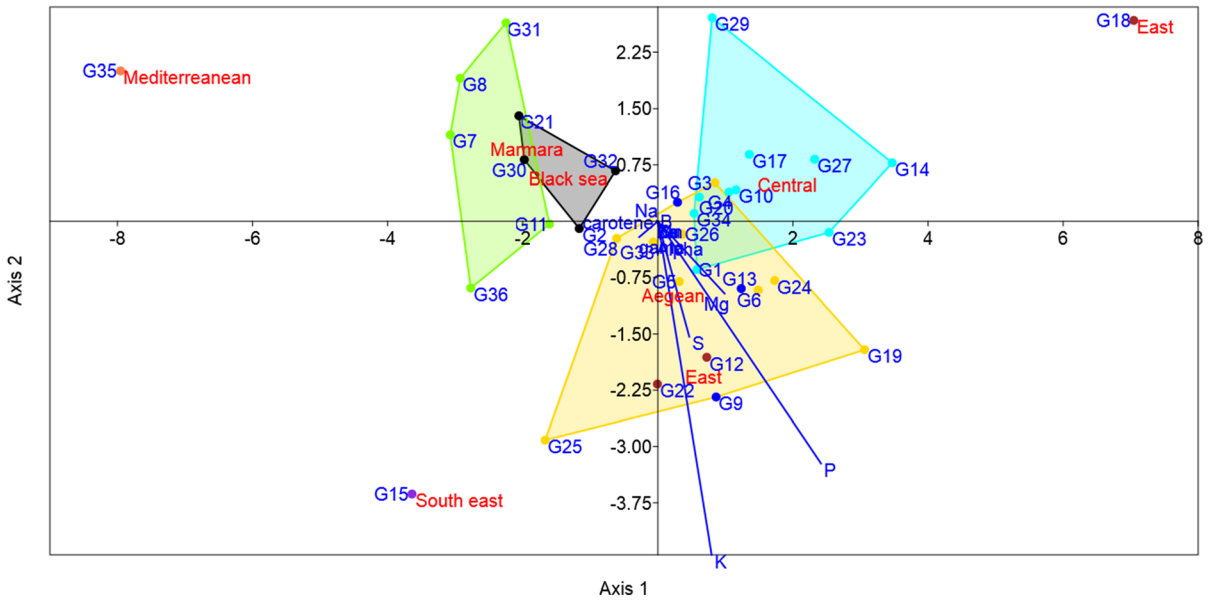

Fig. 2 Linear discriminate analysis image for the distribution of pumpkin genotypes according to mineral and vitamin contents based on regional factors

showed that *C. pepo* var. *ovifera*, which has been neglected to this day, can be considered as an alternative food source due to the vitamin and mineral contents. Studies should be planned to develop genotypes with high seed yield by using current germplasm and consequently, we could conclude that G1, G5, G7 and G8 coded genotypes could be used in future breeding studies.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

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