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Oil contents and fatty acid composition of walnut genotypes selected from Central Anatolia region and assessments through GT biplot analysis

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Walnuts with quite rich nutrient contents and several benefits on human health are among the significant nut fruits. Turkey is among the gene centers of walnut and thus has diverse walnut populations throughout the country. Therefore, it is highly significant to assess this rich source and to identify superior genotypes. Walnuts are rich in oil content and have several positive impacts on human health. In this study, 50 walnut genotypes having superior characteristics were selected from Kayseri province in Central Anatolia region of Turkey. Total oil (crude oil) and fatty acid composition of these genotypes were determined. The recorded data were subjected to variance analysis and assessed through a biplot method. Crude oil contents of walnut genotypes varied in the range of 61.22-74.00% and linoleic acid (32.52-65.47%) was identified as the major fatty acid. Unsaturated fatty acid contents of walnut genotypes varied between 88.20 and 96.16%. Genotype Trait (GT) biplot graph showed that the sum of the first two principal components explained about 59.5% of total variation. While the genotypes KW24, KW10, KW41, KW2, KW6, KW17, KW31 and KW25 were found to be prominent with their crude oil contents, the genotypes KW32 and KW34 were found to be prominent with unsaturated fatty acids. Present findings revealed that the GT biplot method could efficiently be used in selection of walnut genotypes prominent with their crude oil contents and fatty acid composition. It was also concluded that walnuts rich in unsaturated fatty acids could be used as functional food and the present superior genotypes could be used as parent materials in further walnut breeding studies.

Keywords: *Juglans regia*, nutritional characteristics, fatty acids

INTRODUCTION

The genus *Juglans* includes 20 species and *Juglans regia* L. has long been cultured worldwide [1]. This species with large, tasty and thin-shelled fruits is the most significant one cultured for fruits in different countries of the world including Turkey [2]. Gene center of *Juglans* species is believed to be Central Asia and surroundings [3], and Anatolia is also among these gene centers [4]. China, USA, Iran, Turkey and Ukraine are the leading walnut producers of the world. World annual production is around 3.75 million tons. Annual production of Turkey is 195 000 tons [5]. Walnuts have quite high oil contents (50-80%) and are rich in proteins (12-15%), minerals, vitamins. They have lower sugar contents (2.5-4%), thus they are consumed as an important dietary supplement [6]. Walnut oil is largely composed of oleic and linoleic acids [7]. Polyunsaturated fatty acids, like linoleic acid, reduce the risk of coronary hearth diseases. High natural antioxidant contents of walnuts also have preventive effects against some cancer types [8]. Walnuts green husks have also some antimicrobial activity [9].

The material used in walnut breeding should be true-to-name and its characteristics should be well-defined. Morphological and physiological descriptions either take long time because of youth sterility or are influenced by environmental conditions [10]. Identification of fatty acids of walnut populations in Turkey will put forth significant data for the identification of walnut genotypes with high oil contents. There are several seed-propagated walnut populations in Turkey with quite different genetic characteristics. So, there is huge genetic diversity in walnut populations. Yield and fruit characteristics of these genetic sources have been studied [11-19], and genetic diversity researches have been conducted [20-22]. However, there is a limited information available about fatty acid composition and variations in this attribute of the walnut genotypes.

Biplot analysis allows visual inspection of data and thus is commonly used in various sciences including economy, sociology, medicine, engineering, genetic and agriculture. Biplot is a two-way table design and presents graphically row and column factors. In this method of analysis, row and column factors can be assessed one by one

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through the relationships between each pair of them and mutual interactions can also be presented visually [23]. Graphical presentation of more than one characteristic of the genotypes allows researchers to visually compare several genotypes and several attributes of these genotypes [24]. This study was conducted to determine oil contents and fatty acid compositions of walnut genotypes collected from Central Anatolia region of Turkey. The research results were assessed through biplot analysis. The prominent genotypes will be able to be used as a functional food and be also used in further breeding studies.

EXPERIMENTAL

Plant material

About 800 walnut genotypes, already known as seed-propagated from Kayseri province and surrounding town in Central Anatolia, were assessed in this study. With these assessments, 50 genotypes without any disease symptoms and prominent with yield and fruit quality attributes were selected and used as the plant material of the present experiments. Fruits were collected from the specified trees at harvest period. The shells of collected walnuts were removed and the walnuts were dried at shade.

Oil extraction and fatty acid composition

Deshelled walnut samples were cleaned from the foreign materials and ground in a hand-mill. Oil extraction was performed with hexane/isopropanol (2:1 v/v) [25]. Ten grams of ground walnut samples were weighed in a cellulosic cartridge and placed in a Soxhlet extractor for 5 h to provide oil extraction under continuously flowing solvent. At the end of the duration, the extracted oils were centrifuged at 10.000 *g* for 5 min to separate some possible strange material and the solvent was evaporated at 40 °C with a rotary evaporator. Finally, the crude oil content of the samples was calculated using the mass balance. The measurements were repeated two times with three replicates.

For quantification of fatty acids, methylation was initially performed. This process was carried out in accordance with AOAC (1990) (Official methods of analysis, method 969-33). About 100 mg of oil sample was weighed into centrifuge tubes and 3 ml of hexane was added to completely dissolve the oil. Then 100 µl of potassium hydroxide (2 N KOH in methanol) was added and the tubes were vortexed for one min. Finally, the samples were centrifuged at 5000 rpm for 5 min and the upper phase (1 ml) was taken in vials and analyzed by a gas chromatograph (Agilent GC, model 6890 N) equipped with a GC column (HP 88 capillary column - 100 m × 0.25 mm ID, 0.2 µm) and a flame ionization detector. Injection volume was 1 µl and injection temperature was set at 250 ºC. A temperature gradient was arranged for GC oven temperature (held at 130 ºC for 1 min, increased to 170 ºC with 6.5 ºC/min, increased to 215 ºC with 2.75 ºC/min, held at 215 ºC for 12 min, increased to 230 ºC with 4.0 ºC/min and held at 230 ºC for 5 min). Total analysis duration was recorded as 40.89 min. Detector temperature was 280 ºC, detector H_2 flow rate was 40 ml/min, dry air flow rate was 450 ml/min, column H_2 flow rate was 1.3 ml/min and split rate was 50/1 [26, 27]. Fatty acid components were identified according to the retention times of the compounds presented by peaks of the standards (FAME mix 37 components; Matreya, Sigma-Aldrich, Milan, Italy) containing C4-24 fatty acids. The analysis was repeated two times with two replicates.

Statistical analyses

Oil and fatty acid composition data of the genotypes were subjected to variance analysis (ANOVA) with SAS [28] software in accordance with randomized blocks experimental design and means were compared using the LSD multiple comparison test. GT biplot analysis was performed to group fatty acid substances and for visual assessment of the relationships between fatty acids. A polygon was formed through connecting the furthest genotypes from the origin of the graph. A graph can also be formed through the lines drawn from the origin perpendicular to polygon edges. Resultant data were analyzed visually with GT biplot analysis [29, 30]. Biplot was first proposed by Gabriel [31] as a graphical analysis method to present the results of principal component analysis (PCA). This chemometric method is a scatter plot graphically displaying a rank-2 matrix by both rows (entries) and columns (testers) [32]. Using the matrix, PCA-based genotype and feature-oriented Genotype-trait biplot analysis was performed to visually evaluate the data matrix, interpret the relationships between traits and group genotypes. The second module of the GGE-biplot program was used in the biplot analysis [33]. In this module, the figures obtained using the paired data based on the SVD values of the standardized data obtained using the standard deviations and standard errors of the numerical values of the genotype and traits were carried on the same plot (biplot). Information on the model used is given in the upper right corner of the biplot graphics. Visual evaluation was made

according to the location of the features and genotypes on the biplot [34].

RESULTS

Mean values of three replicates for oil contents and fatty acids of walnut genotypes are provided in Table 1. The differences in all investigated parameters of walnut genotypes were found to be highly significant ($P<0.01$). Crude oil levels of local walnut genotypes varied between 61.22 and 74.00% and the contents of palmitic acid between 2.04-7.83%, stearic acid between 0.62-6.66%, oleic acid between 13.14-47.28%, linoleic acid between 32.52-65.47%, α-linolenic acid between 7.08-17.28% and docosanoic acid between 0.00-0.34%. Docosanoic acid was not encountered in several genotypes. Unsaturated fatty acid contents of the walnut genotypes varied between 88.20 and 96.16%. The highest total unsaturated fatty acid content was obtained for KW32 genotype and the lowest total unsaturated fatty acid content was obtained for KW24 genotype.

Oil contents and fatty acids of the investigated walnut genotypes were used in GT biplot analysis to get chemical composition vectors and to determine the separation power of the relevant attributes. The attribute with a longer vector has a quite high capacity for the separation of the genotypes. While crude oil content and docosanoic acid had low separation capacity, other fatty acids had high separation capacity for the present genotypes (Fig 1). Image of biplot vectors provides information about the relationships between the investigated attributes. While there were positive correlations between linoleic acid and α-linolenic acid and between crude oil and oleic acid, the linoleic acid and α-linolenic acid negatively correlated with crude oil and oleic acid (Fig. 1).

In biplot analysis, there may be deviations in some attributes of the genotypes. In this sense, according to Table 2 in addition to Figure 1, there were significant positive correlations between palmitic acid and linoleic acid, between stearic acid and oleic acid and between linoleic acid and α-linolenic acid; while there were significant negative correlations between palmitic acid and oleic acid, between oleic acid and linoleic acid and between oleic acid and α-linolenic acid.

GT-biplot polygon also reveals which genotype is prominent with which attributes (GTI: genotype trait interaction) (Fig. 2). It was observed that the genotypes KW49, KW39 and KW4 were prominent with their oleic acid contents, KW22 and KW 24 were prominent with their total saturated fatty acid contents, KW47 with palmitic acid content, KW13, KW9, KW11 and KW36 with linoleic acid and α-linolenic acid content, KW32 and KW34 with total unsaturated fatty acid content.

In another study carried out in Moldova, oil contents of walnut genotypes were reported to be between 55 and 72% [37]. In Morocco, total oil contents of walnut genotypes were reported between 54.04 and 67.48% [38]. Previous studies carried out in different regions indicated variations in oil contents of walnut genotypes. Such differences were mostly resulted from genetic differences and ecological conditions.

Significant differences were observed in fatty acid components of the walnut genotypes. Linoleic acid had the highest quantity (32.52-65.47 %) followed by oleic acid (13.14-47.28 %), linolenic acid (7.08-17.28 %), palmitic acid (2.04-7.83 %) and stearic acid (0.62-6.66 %). Similar findings were also reported by previous studies carried out in different countries. In a study carried out in Turkey, fatty acids composition of walnuts was respectively reported as linoleic (55.3 %), oleic (13.4 %), linolenic (8.7 %) and palmitic acid (6.4 %) [39]. Linoleic acid (58%) was also reported as the major fatty acid in walnuts of Moldova [37]. In another study carried out in Serbia, linoleic acid (57.2–65.1%) was again identified as the major fatty acid followed by oleic acid (15.9–23.7%) [40]. Uzunova *et al.* [41] in a study carried out in Bulgaria reported the major fatty acids of walnut genotypes as linoleic acid (63.52-64.69%) and oleic acid (15.61-17.12%).

Biplot analysis allows researchers to test one or more characteristics under different environmental conditions. Through the use of two-way data like genotype-trait, the genotypes can be scanned for desired attributes [23, 29, 42]. In this study, local walnut genotypes selected from Central Anatolia were scanned and grouped for oil contents and fatty acids. GT-biplot analysis allows the researchers to identify the genotypes prominent with investigated traits (Fig. 2) [29]. It is also possible to identify ideal genotypes for entire traits (Fig. 3) [43]. Narrow angles between the vectors of any two traits indicate positive correlations between the traits (Fig. 1) and such a correlation can be visualized with biplot analysis [29]. Such characteristics of biplot analysis were also taken into consideration while finding out the correlations between the present traits (Fig. 1).

Table 1. Fatty acid composition and crude oil content of walnut genotypes (%).

Genotypes	*CO*	*PA*	*SA*	*OA*	*LA*	*a-LA*	*DA*	*TUSFA*	*TSFA*
KW1	64.11± 1.30r-t	5.40± 0.03o-q	3.82± 0.05d	24.35± 0.03m-o	54.97± 0.11mn	11.46± 0.15op	nd	90.78± 0.02su	9.22± 0.02c-e
KW2	71.5± 1.08a-e	5.18± 0.04qr	2.10± 0.05jk	31.43± 0.09e	51.10± 0.06u-w	10.19± 0.06q-s	nd	92.72± 0.09i-l	7.28l± 0.09-o
KW3	65.49± 1.91l-s	4.89± 0.06rs	2.29± 0.05ij	21.48± 0.26u	60.12± 0.19de	11.22± 0.07op	nd	92.82± 0.01h-l	7.18± 0.01l-p
KW4	65.05± 1.43o-s	4.63± 0.08st	4.81± 0.12c	41.53± 0.21c	40.76± 0.17b*	8.27± 0.42u	nd	90.56± 0.04tv	9.44± 0.04b-d
KW5	67.32± 0.94h-r	6.03±0.07 i-m	2.58± 0.05g-i	30.66± 0.29e-g	47.94± 0.11yz	12.79± 0.38k l	nd	91.39± 0.03np	8.61± 0.03h-j
KW6	72.02± 1.59a-d	5.66± 0.12m-o	0.97± 0.03o-t	20.06± 0.06wx	61.4± 0.08c	11.91± 0.01m-o	nd	93.37± 0.15dg	6.63± 0.15q-t
KW7	67.48± 0.42g-p	6.68± 0.23b-e	0.99± 0.07o-t	20.96± 0.16u-w	56.96± 0.62j-l	14.41± 0.62d-g	nd	92.33± 0.16k-m	7.67± 0.16k-m
KW8	67.60± 0.12g-p	6.65± 0.29b-e	1.03± 0.12o-s	30.37± 0.23fg	51.22± 0.96u-w	10.72± 0.79pq	nd	92.32± 0.41k-m	7.68± 0.41k-m
KW9	69.59± 1.23b-i	6.13± 0.18h-k	1.26± 0.14m-o	14.93± 0.20za*	61.76± 0.20c	15.670± 0.01b	0.24± 0.07b	92.61± 0.32j-l	7.39± 0.32l-n
KW10	70.66± 0.89b-f	6.06± 0.23i-l	1.08± 0.18o-r	29.27± 0.23hi	53.40± 0.61p-r	10.18± 0.43q-s	nd	92.86± 0.41g-k	7.14± 0.41n-q
KW11	68.11± 0.18g-n	6.76± 0.33b-d	0.87± 0.10p-t	17.55± 0.40y	60.66± 0.33cd	14.17± 0.30d-h	nd	92.37± 0.43k-m	7.63± 0.43k-m
KW12	67.54± 2.03g-p	6.17± 0.13g-k	0.67± 0.10t	32.68± 0.15d	51.3± 0.16u-w	9.18± 0.22t	nd	93.16± 0.23f-j	6.84± 0.23n-r
KW13	69.34± 0.35b-j	6.53± 0.19d-g	0.72± 0.10rt	15.30± 0.28z	65.47± 0.59a	11.8± 0.00no	0.19± 0.06c	92.75± 0.30h-l	7.25± 0.30l-p
KW14	69.57± 0.04b-i	5.98± 0.09i-m	2.36± 0.12h-j	21.24± 0.09uv	57.77± 0.32g-j	12.64± 0.38lm	nd	91.66± 0.03no	8.34± 0.03i-j
KW15	69.13± 0.14d-j	5.67± 0.17m-o	2.40± 0.13h-j	20.00± 0.18wx	58.56± 0.12f-h	13.36± 0.11h-l	nd	91.93± 0.05mn	8.07± 0.05jk
KW16	68.69± 1.11e-l	6.16± 0.24g-k	2.31± 0.25ij	25.79± 0.30kl	52.54± 0.54q-t	13.19± 0.35i-l	nd	91.53± 0.49np	8.47± 0.49h-j
KW17	72.29± 1.61a-c	5.59± 0.23n-p	0.78± 0.10q-t	29.3± 0.81hi	52.82± 0.53q-t	11.52± 0.05op	nd	93.63± 0.33c-f	6.37± 0.33r-u
KW18	64.82± 0.44o-s	6.69± 0.37b-e	1.03± 0.18o-s	28.79± 0.33i	53.48± 0.50p-r	10.00± 0.28q-t	nd	92.28± 0.55lm	7.72± 0.55kl
KW19	68.9± 1.03d-k	6.62± 0.22c-f	2.59± 0.36g-i	26.21± 0.27jk	50.71± 0.88vw	13.87± 0.58f-i	nd	90.79± 0.58ru	9.21± 0.58c-f
KW20	68.07± 1.04g-o	6.12± 0.23i-k	1.19± 0.25m-p	25.3± 0.53k-m	57.82± 1.79g-j	9.56± 0.78r-t	nd	92.69± 0.48i-l	7.31± 0.48l-o
KW21	69.33± 1.24b-j	6.12± 0.23i-k	0.69± 0.14t	25.93± 0.92kl	56.89± 0.76j-l	10.37± 0.25qr	nd	93.19± 0.08f-i	6.81± 0.08o-r
KW22	66.18± 1.40j-s	4.71± 0.27st	6.66± 0.46a	22.8± 0.43q-t	52.3± 0.38r-u	13.54± 0.63h-k	nd	88.63± 0.19w	11.37± 0.19a
KW23	64.15± 0.70q-t	5.37± 0.33o-q	0.68± 0.15t	22.93± 1.02p-s	53.74± 1.25o-q	17.28± 0.70a	nd	93.94± 0.47c	6.06± 0.47u
KW24	70.16± 1.24b-g	6.71± 0.37b-e	5.09± 0.90b	21.97± 1.03s-u	54.92± 1.06m-o	11.31± 0.82op	nd	88.20± 1.27w	11.80± 1.27a
KW25	74.00± 0.21a	5.89± 0.18j-m	2.82± 0.21e-g	31.31± 0.54ef	46.98± 1.14za*	13.00± 0.56j-l	nd	91.29± 0.03o-s	8.71± 0.03e-i
KW26	67.76± 0.33g-o	5.38± 0.22o-q	3.13± 0.37e	28.26± 0.28i	50.22± 0.47wx	13.01± 0.16j-l	nd	91.49± 0.59np	8.51± 0.59h-j
KW27	69.17± 0.06c-j	6.12± 0.35i-k	2.30± 0.26ij	25.94± 0.42kl	55.83± 0.50k-m	9.80± 0.82r-t	nd	91.57± 0.09np	8.43± 0.09h-j
KW28	68.55± 0.67e-m	5.22± 0.22p-r	1.15± 0.16n-q	24.00± 0.58no	54.36± 0.53n-p	15.28± 1.18bc	nd	93.63± 0.07c-f	6.37± 0.07r-u
KW29	65.44± 0.31m-s	7.01± 0.29b	1.56± 0.12lm	17.08± 0.84y	59.00± 0.43e-g	15.35± 0.86bc	nd	91.42± 0.42np	8.58± 0.42h-j

KW30	65.72± 1.21k-s	5.70± 0.27l-o	0.7± 0.11t	23.74± 0.91o-q	56.10± 1.63k-m	13.75± 0.34g-j	nd	93.60± 0.38cf	6.40± 0.38r-u
KW31	72.43± 1.06a	6.51± 0.20d-h	2.69± 0.26f-h	23.86± 0.16op	56.90± 0.45j-l	10.05± 0.16q-s	nd	90.80± 0.45qu	9.20± 0.45c-g
KW32	67.31± 0.95h-r	2.33± 0.26uv	1.51± 0.17l-n	20.26± 0.37v-x	61.08± 0.69cd	14.82± 0.41c-e	nd	96.16± 0.09a	3.84± 0.09w
KW33	67.4± 0.21h-q	6.51± 0.22d-h	2.39± 0.12h-j	23.59± 0.37o-r	53.11± 1.16q-s	14.4± 0.45d-g	nd	91.10± 0.34qt	8.90± 0.34d-h
KW34	67.68± 1.05g-p	2.04± 0.10v	2.82± 0.08e-g	30.49± 0.33e-g	51.89± 0.08s-v	12.76± 0.43kl	nd	95.14± 0.02b	4.86± 0.02v
KW35	69.44± 0.61b-i	5.33± 0.20o-q	2.98± 0.28ef	28.48± 0.46i	51.83± 0.26t-v	11.39± 0.24op	nd	91.69± 0.48no	8.31± 0.48i-j
KW36	61.22± 1.24t	5.79± 0.14l-n	0.91± 0.09o-t	13.14± 0.88b*	65.16± 1.41a	15.00± 0.76b-d	nd	93.30± 0.23e-h	6.70± 0.23ps
KW37	68.16± 1.57g-n	6.78± 0.09b-d	2.56± 0.16g-i	16.94± 0.64y	59.06± 0.43ef	14.66± 0.81c-f	nd	90.66± 0.26tu	9.34± 0.26cd
KW38	63.41± 6.94st	2.50± 0.23u	5.22± 0.04b	30.94± 0.44e-g	51.65± 1.32t-v	9.69± 0.69r-t	nd	92.28± 0.19lm	7.72± 0.19kl
KW39	68.61± 0.74e-m	4.5± 0.251t	4.14± 0.30d	47.28± 0.58a	32.52± 1.84d*	11.55± 0.71op	nd	91.35± 0.55oq	8.65± 0.55g-i
KW40	67.12± 2.62h-r	5.17± 0.13qr	0.95± 0.04o-t	33.45± 1.23d	46.25± 0.10a*	14.18± 1.24d-h	nd	93.88± 0.09cd	6.12± 0.09tu
KW41	70.96± 1.90a-f	6.25± 0.19f-j	0.62± 0.09t	25.01± 0.49l-n	55.53± 0.57mn	12.60± 0.97l-n	nd	93.13± 0.09f-i	6.87± 0.09n-r
KW42	69.72± 0.70b-i	6.08± 0.15i-l	2.59± 0.15g-i	21.01± 0.48u-w	57.71± 0.64h-j	12.62± 0.45l-n	nd	91.34± 0.29or	8.66± 0.29f-i
KW43	64.5± 1.36p-s	6.35± 0.25e-i	1.83± 0.20kl	21.81± 0.88u	57.05± 0.14i-k	12.98± 0.57j-l	nd	91.83± 0.45m-o	8.17± 0.45i-k
KW44	65.62± 4.81l-s	7.00± 0.18bc	2.92± 0.20e-g	24.96± 0.62l-n	53.26± 0.11p-r	11.86± 0.12m-o	nd	90.08± 0.38v	9.92± 0.38b
KW45	66.69± 1.19i-r	6.65± 0.27b-e	0.73± 0.05rt	27.01± 0.75j	55.79± 0.51lm	9.51± 0.09st	0.32± 0.11a	92.63± 0.22j-l	7.37± 0.22l-n
KW46	67.28±0. 06h-r	5.54± 0.18n-q	0.66± 0.05t	22.67± 0.17rt	58.25± 0.18f-i	12.54± 0.11l-n	0.34± 0.10a	93.79± 0.13c-e	6.21± 0.13s-u
KW47	66.64± 0.47i-s	7.83± 0.27a	1.69± 0.15l	14.14± 0.81ab*	63.35± 0.44b	12.99± 0.04j-l	nd	90.48± 0.41uv	9.52± 0.41bc
KW48	65.53± 0.67l-s	7.00± 0.26bc	0.72± 0.09rt	19.88± 0.29x	61.01± 0.30cd	11.40± 0.18op	nd	92.29± 0.16lm	7.71± 0.16kl
KW49	69.41± 1.11b-j	5.52± 0.24n-q	2.56± 0.19g-i	46.12± 0.20b	38.42± 0.11c*	7.08± 0.30v	0.30± 0.04a	91.92± 0.06mn	8.08± 0.06jk
KW50	67.23± 0.15h-r	2.19± 0.19uv	4.78± 0.26c	29.93± 0.52gh	49.00± 0.87xy	14.10± 0.27e-h	nd	93.03± 0.07g-j	6.97± 0.07n-q
Means	67.88	5.71	2.12	25.44	54.4	12.3	0.03	92.17	7.83
LSD	3.25	0.38	0.38	1.05	1.23	0.84	0.05	0.56	0.56
Sig. Dg.	**	**	**	**	**	**	**	**	**

[+]The small letters in each column show the significant difference among the genotypes. CO: crude oil; PA: palmitic acid; SA: stearic acid; OA: oleic acid; LA: linoleic acid; α-LA: α-linolenic acid; DA: docosanoic acid; TUSFA: total unsaturated fatty acids; TSFA: total saturated fatty acids; Sig. Dg.: significant degree; LSD: least significant difference; **: P≤0.01, *Since all the lettering between a-z was completed in the first set, the second set was started.

Table 2. Correlations among crude oil and fatty acids of walnut genotypes

	CO	PA	SA	OA	LA	*α*-LA	DA
CO	1						
PA	0.131	1					
SA	-0.081	**-0.425**	1				
OA	0.140	**-0.360**	**0.319**	1			
LA	-0.109	**0.315**	**-0.454**	**-0.947**	1		
aLA	-0.172	-0.025	-0.143	**-0.561**	**0.341**	1	
DA	0.053	0.084	-0.212	0.043	0.032	-0.185	1

In bold: significant values (except diagonal) at the level of significance alpha=0.050 (two-tailed test); CO: crude oil; PA: palmitic acid; SA: stearic acid; OA: oleic acid; LA: linoleic acid; α-LA: a-linolenic acid; DA: docosanoic acid.

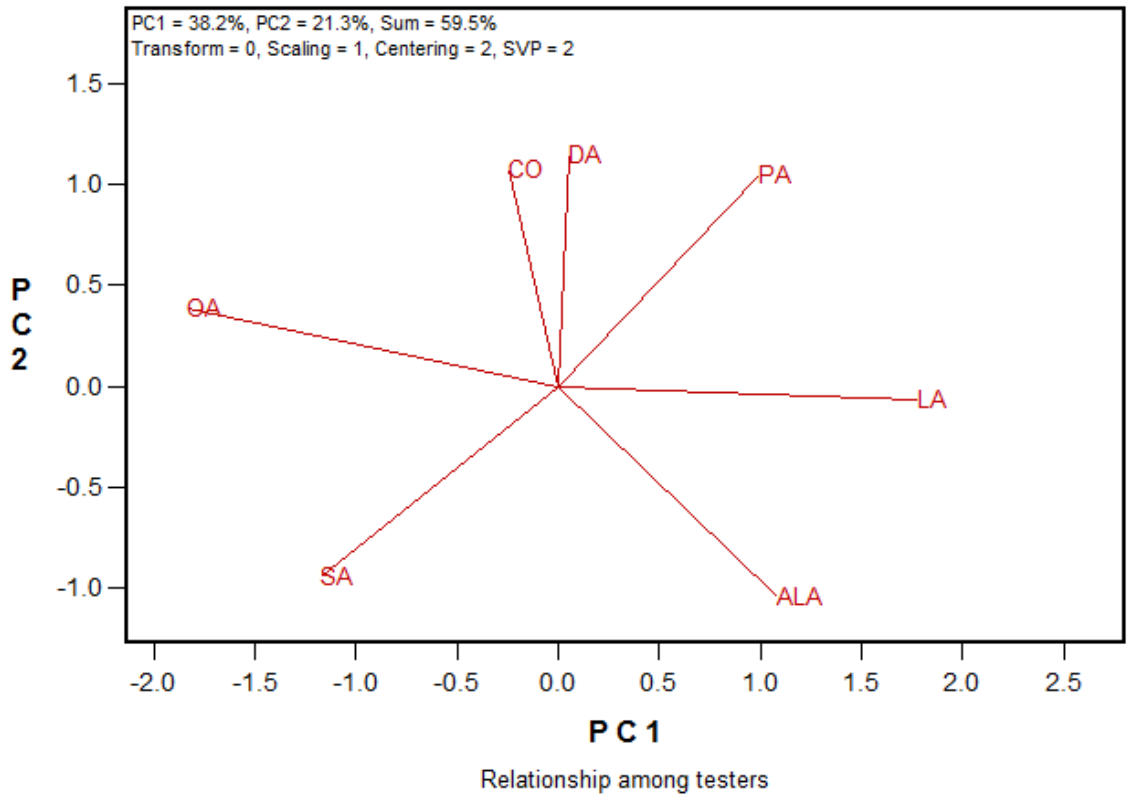

Fig. 1. GT-biplot based on oil content and fatty acid composition focus scaling.

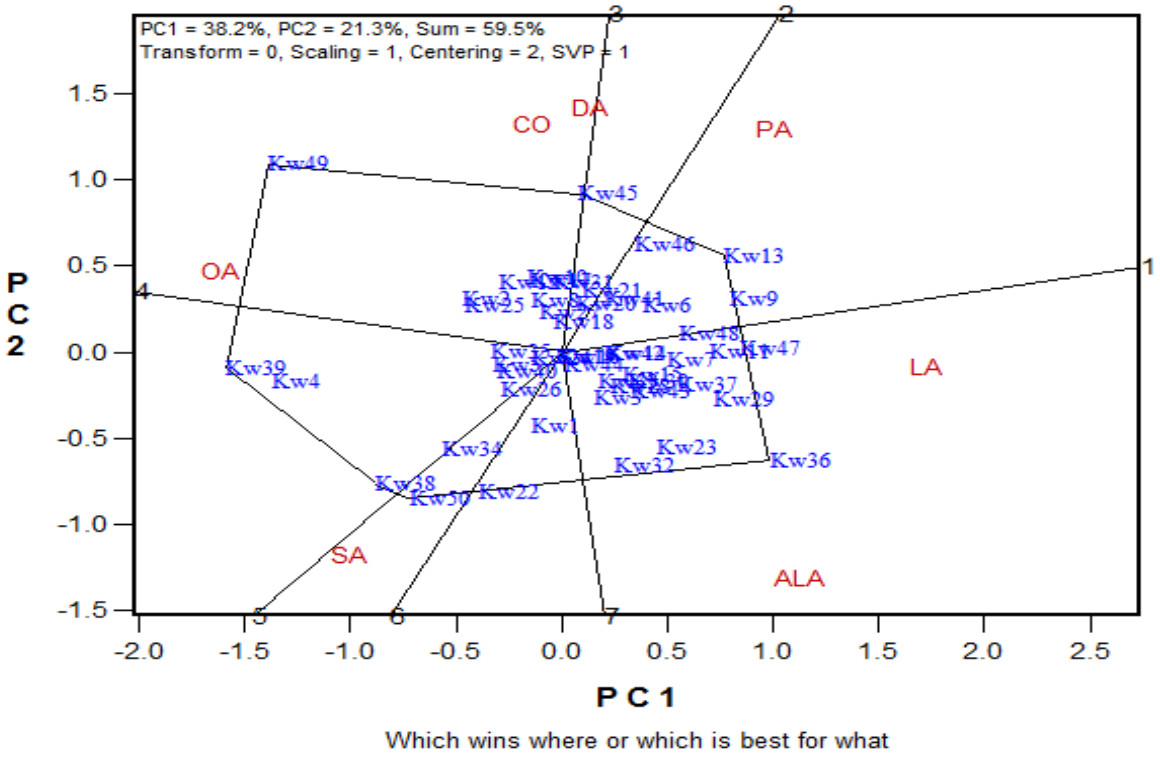

Fig. 2. Polygon views of the GT-biplot based on symmetrical scaling for the which-won-what pattern for genotypes and oil and fatty acids composition. Details of genotypes are presented in Table 1.

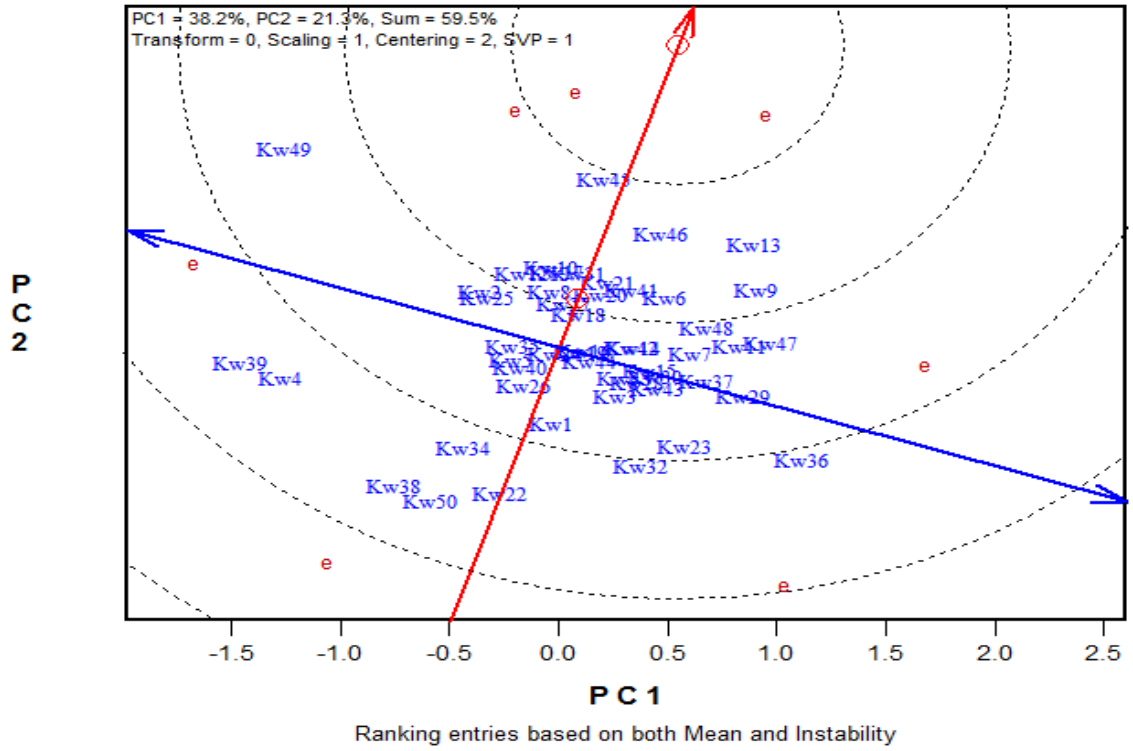

Fig. 3. GT-biplot based on genotype-focused scaling for comparison the genotypes with the ideal genotype

A polygon was created through connecting vector markers far from the origin of the biplot as to include all the genotypes within the polygon [42]. The genotypes with the greatest values for the investigated parameters were placed at the outermost section of the polygon.

Oil and fatty acid components of the selected 50 walnut genotypes were determined and prominent genotypes were identified. While the

genotypes KW24, KW10, KW41, KW2, KW6, KW17, KW31 and KW25 were found to be prominent with their crude oil contents, the genotypes KW32 and KW34 were found to be prominent with unsaturated fatty acids. Present findings revealed that GT biplot method could be used efficiently for selection of walnut genotypes prominent with oil content and fatty acid components.

CONCLUSIONS

Present findings revealed significant information about the selection of walnut genotypes with significant health benefits. It was observed that the fatty acid composition differed depending on the genotypes and it was concluded that the walnuts were quite rich in total unsaturated fatty acids. Because of richness in unsaturated fatty acid quantities and in linoleic acid which is an important fatty acid for the health, the walnuts have a highly significant potential to be a functional food. Present genotypes can also be used as parent materials in further walnut breeding studies.

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