

Pomological and Biochemical Characterization of Local Walnut (*Juglans regia* L.) Genotypes from Kırşehir for Breeding and Functional Food Applications

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Walnut (*Juglans regia* L.) plays an important role due to its health-promoting properties. In this study, local walnut genotypes collected from Kırşehir were characterized in terms of fruit morphology, pomological characteristics, and antioxidant capacity. The genotypes were compared with standard cultivars ('Kaman 5' and 'Chandler') and showed significant differences in the characteristics examined. Nut weight ranged from 9.79-16.60 g, and kernel ratio from 46.42-59.44%. Total phenolic content ranged between 3386-5861 mg GAE 100 g⁻¹, and DPPH activity between 56.98-91.22 µmol TE g⁻¹. 'Chandler' showed the highest DPPH activity and the lowest IC₅₀ value. A very strong positive correlation ($r = 0.90$) was found between nut weight and kernel weight. A negative correlation was observed between kernel-related traits and antioxidant activity. Cluster analysis grouped the genotypes into two categories: small-fruited with high phenolic content and antioxidant activity (Cluster A1), and large-fruited with high nut weight and kernel weight (Cluster B). G5 and G8 were prominent for nut weight and kernel weight, G3, G4, and G8 for kernel ratio, and 'Chandler' for antioxidant activity. Genotypes G1 and G2 combined high phenolic content with moderate kernel weight, showing potential for functional food applications. Multi-year evaluation under different ecological conditions is recommended to confirm genotype performance.

Keywords: Phenolic content, DPPH activity, multivariate analysis, fruit breeding

INTRODUCTION

Walnut (*Juglans regia* L.) is a type of nut widely cultivated in temperate zones and commonly used in both the food industry and human nutrition. Walnuts have a rich phenolic compound content compared to other nuts, as well as polyunsaturated fatty acids, tocopherols, and other bioactive components (Vinson and Cai, 2012; Trandafir *et al.*, 2016; Gao *et al.*, 2019; Sharma and Sharma, 2024). Regular walnut consumption is known to have beneficial effects on plasma lipid profile, oxidative stress biomarkers, and cardiometabolic risk (Ros, 2009). Components such as flavonoids and phenolic acids found in walnuts provide a protective effect against fat oxidation due to their strong free radical scavenging capacity. Furthermore, it plays a role in lowering LDL cholesterol, improving endothelial function, and regulating inflammation (Jarai and Koller, 2024; Hodoşan *et al.*, 2025). In addition, walnuts are preferred in various fields such as confectionery, snacks, functional foods, and the

vegetable oil industry due to their long shelf life and ease of storage (Şen *et al.*, 2011).

The long-standing generative propagation of *J. regia*, its anemophilous flower structure, and its adaptation to wide ecological areas have resulted in high heterozygosity and phenotypic variation in populations (McGranahan and Leslie, 2012; Ding *et al.*, 2022). These variations constitute an important genetic resource in terms of phenological events, such as yield, fruit size, and flowering time, which are crucial in breeding studies, as well as resistance to biotic and abiotic stress factors (Vahdati *et al.*, 2020). Türkiye, considered one of the gene centers for many fruit species and cultivars, has numerous local genotypes and populations grown from seed in regions with different ecological conditions. The Kırşehir location, where the research was conducted, is important for the evaluation of walnut genotypes due to its ecological conditions, altitude, and topographical features.

Modern walnut breeding methods consider high yield as well as adaptation characteristics and biological and functional

Aygün, A., E. Turan, A. Şimşek, F.E.Ş. Saka and L. Kırca. 2026. Pomological and Biochemical Characterization of Local Walnut (*Juglans regia* L.) Genotypes from Kırşehir for Breeding and Functional food applications. Pakistan Journal of Agricultural Sciences 63:525-537.

[Received 03 Apr 2026; Accepted 03 Jun 2026; Published (online) 27 Jun 2026]



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components together (Mirmahdi and Khadivi, 2021; Ahmed *et al.*, 2019; Tian *et al.*, 2021). In walnut breeding studies, factors such as large fruit size, kernel ratio of more than 50%, light and homogeneous kernel color, easily crackable shell structure, high oil and protein content, and biochemical characteristics are included (McGranahan and Leslie, 2012; Martínez *et al.*, 2010). Among traditional breeding methods, identifying seed-derived local populations and selecting genotypes with superior characteristics provides significant advantages in terms of increasing local adaptation and conserving genetic resources (Guzzon *et al.*, 2021; Allier *et al.*, 2020). Recent studies have shown that identifying superior genotypes in terms of morphological and biochemical characteristics is important for functional food development and cultivar breeding (Kırca *et al.*, 2014; Kırca, 2025a; Kırca, 2025b; Bak *et al.*, 2025; Kırca and Aygün, 2025; Mumtaz *et al.*, 2025; Al Aboud *et al.*, 2025; Rezk *et al.*, 2026; Aygün *et al.*, 2026).

The aim of this study is to characterize local walnut genotypes collected from Kırşehir province, a semi-arid continental environment at 1350–1550 m altitude where systematic walnut evaluation is lacking, along with two standard cultivars, in terms of morphological and biochemical quality parameters. A key contribution of this work is the joint multivariate analysis of pomological and antioxidant traits — an approach that enables direct matching of genotypes to specific end-use profiles (functional food ingredient versus fresh-market kernel production) and identifies the trade-off between fruit yield and phenolic density within a geographically defined germplasm pool.

MATERIALS AND METHODS

Plant material: The plant material consists of local walnut (*J. regia* L.) genotypes of seed origin obtained from Başköy town in Kaman district of Kırşehir province. These genotypes, preferred by producers in the region, stand out with their climate adaptation and yield stability, high kernel ratio, thin shell structure, intact kernel, light-colored and delicious fruits. These genotypes, which have been cultivated in the region for many years and selected for their superior characteristics, are still used today in the establishment of new orchards by propagation through grafting. In order to compare the 10 selected genotypes, the standard cultivars ‘Kaman 5’ and ‘Chandler’ were also examined in the study (Fig. 1). All trees were between 16 and 25 years old and were grown under the same ecology and orchard conditions. The walnut orchard where the study was conducted is located in the Başköy region of Kaman district, Kırşehir province (39°21’N, 33°44’E), at an altitude of 1350–1550 m above sea level. The region has a semi-arid continental climate with cold winters and dry summers, mean annual precipitation of approximately 350–400 mm, and mean annual temperature of around 11°C. Samples were collected during the 2023 growing season, and

the results should be interpreted as a preliminary characterization of the germplasm pending multi-year evaluation.

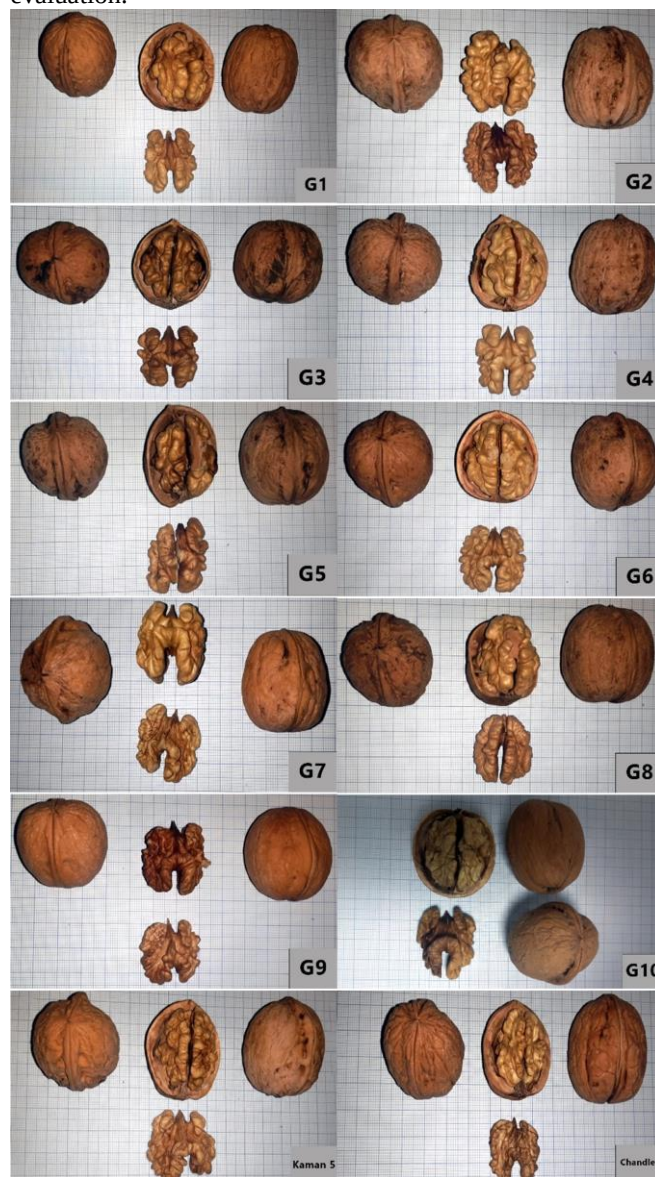


Figure 1. Images of the walnut genotypes and cultivars evaluated.

Morphological and pomological analyses: Pomological analyses were performed on 25 shade-dried fruit samples randomly selected from each genotype and cultivar, a sample size consistent with standard practice in walnut pomological evaluation (Muradoğlu and Balta, 2010; Kırca, 2025). The width, length, and thickness of the shell and kernel, as well as the shell thickness, were measured using a digital caliper with a precision of 0.01 mm. Kernel weight was recorded using a balance with a precision of 0.01 g. Kernel percentage was calculated by dividing the kernel weight by the nut weight and

multiplying by 100. Shell color (light, brown, dark), shell roughness (smooth, slightly rough, rough), and kernel color (light, yellow, dark) were visually evaluated according to the scales of Muradoğlu and Balta (2010). Shell breakage ease (easy, medium, difficult), kernel shriveling (assessed on a five-point percentage scale), and kernel removal ease (good, medium, poor) were classified qualitatively. Shell seal (good, medium, poor), kernel fill (good, medium, poor), and kernel veining (smooth, slight, high) were determined using the same approach. Kernel shriveling and kernel rot were assessed on 10 fruits per genotype. Each kernel was divided into four parts, and the percentage of affected parts was calculated separately for each trait using the following formulas:

$$\text{Kernel shriveling (\%)} = \frac{\text{Number of shriveled kernel parts in 10 walnuts}}{40}$$

$$\text{Kernel rot (\%)} = \frac{\text{Number of rotted kernel parts in 10 walnuts}}{40}$$

(Muradoğlu and Balta, 2010).

Chemical analyses

Sample preparation: For biochemical analyses, 25 shade-dried fruits from each genotype were pooled and ground to obtain a homogeneous sample. Each genotype was represented by a single pooled sample, from which three independent extractions (biological triplicates) were prepared. Each extract was then measured in triplicate (technical triplicates), and the mean of three technical readings per extraction was used for statistical analysis. Results are therefore based on $n = 3$ extraction replicates per genotype. All results are expressed on a defatted dry weight basis. Both methanol and ethanol extracts were prepared from the same homogenized bulk sample per genotype.

Total phenolic content and antioxidant activity were determined from methanol (80:20 methanol:distilled water + 0.1% HCl) and ethanol (80:20 ethanol:distilled water + 0.1% HCl) extracts of defatted walnut samples, from which oil had been removed with *n*-hexane. One gram of walnut kernel was weighed into each solvent (10 mL) and extracted on a shaker at room temperature for 6 hours. The mixtures were centrifuged at 4500 rpm for 10 minutes, and the supernatants were retained as the methanol and ethanol extracts.

Total phenolic content analysis: Total phenolic content was determined using the Folin-Ciocalteu method of Singleton and Rossi (1965). 0.02 mL of each extract was pipetted into a test tube. It was then mixed with 0.755 mL of distilled water, 0.075 mL of Folin-Ciocalteu reagent, and 0.75 mL of Na_2CO_3 solution. After incubation in the dark for 90 minutes, the absorbance was read against a blank sample at 725 nm. The obtained results were recorded as mg gallic acid equivalent (GAE) per 100 g, according to a prepared gallic acid standard curve.

DPPH radical scavenging activity analysis: DPPH activity was determined according to the method of Brand-Williams

et al. (1995). 100 μL of each extract was added to 2.9 mL of DPPH solution, vortexed, and incubated in the dark at 30°C for 30 minutes. Absorbance was read at 517 nm, and results were recorded as μmol Trolox equivalent (TE) per gram. IC_{50} values were obtained from linear regression plots of DPPH inhibition percentage against extract concentration. In the results, lower values indicate higher antioxidant activity. A Trolox standard curve was prepared over a concentration range of 10–50 $\mu\text{mol/L}$ ($y = 1.3878x - 3.8709$, $R^2 \geq 0.99$). Extract concentrations used for IC_{50} calculation ranged from 75 to 375 mg/L. Both methanol and ethanol extracts were prepared separately from the same homogenized sample per genotype, each in triplicate.

Data analysis: Quantitative characteristics were analyzed using the JMP Pro 17.0.0 (SAS Institute Inc., Cary, NC, USA) statistical program. A one-way ANOVA model was applied with genotype as the fixed effect. Genotype and cultivar means were compared using Tukey's HSD test at a $p < 0.05$ level. The results were recorded as mean $\pm$ standard deviation. Multivariate analyses included correlation matrix analysis (CMA), principal component analysis (PCA), hierarchical clustering (HMA), and heatmap analysis. Pearson correlation analysis was performed to determine the relationships between the examined characteristics. A PCA biplot plot was created to visualize the distribution of the data. HCA and heatmap analysis were performed using the Ward method with Euclidean distance to group genotypes and variables.

In all analyses, the genotype was treated as the experimental unit. For pomological traits, 25 fruits per genotype were used as the measurement sample, and the genotype mean was calculated for statistical comparisons; individual fruits were not treated as independent biological replicates. For biochemical analyses, three independent extractions per genotype were used as biological replicates ($n = 3$), and each extraction was measured in triplicate. ANOVA assumptions of normality and homogeneity of variance were verified prior to analysis using the Shapiro–Wilk test and Levene's test, respectively. PCA and Pearson correlation analyses were performed on genotype means ($n = 12$, including two standard cultivars). Since a single tree per genotype was sampled, the results represent a descriptive characterization of the collected material rather than a population-level inference.

RESULTS

Morphological and pomological traits: The genotypes and standard cultivars 'Kaman 5' and 'Chandler' differed significantly in fruit quality characteristics (Table 1). G6 and G9 had smooth shells, G1, G3, and G8 had rough shells, and smooth shell texture was also characteristic of 'Chandler'. Most genotypes and both reference cultivars had light-colored shells. G3, G5, G6, and G7 genotypes were found to have a brown shell color. In terms of shell seal, G2, G4, G5, and 'Chandler' showed medium seal, while the other genotypes

Table 1. Morphological characteristics of the walnut genotypes and cultivars examined.

Genotypes	Shell Roughness	Shell Color	Shell Seal	Kernel Fill	Kernel Veining	Ease of Removal	Breakage Ease	Kernel Shriveling	Kernel Color	Kernel Rot
G1	Rough	Light	Good	Good	Slightly veined	Good	Poor	Low	Brown	None
G2	Medium	Light	Medium	Good	Highly veined	Medium	Good	None	Yellow	None
G3	Rough	Brown	Good	Good	Slightly veined	Good	Good	None	Light	None
G4	Medium	Light	Medium	Medium	Slightly veined	Good	Good	None	Brown	None
G5	Medium	Brown	Medium	Good	Highly veined	Medium	Medium	Low	Brown	None
G6	Smooth	Brown	Good	Medium	Slightly veined	Medium	Good	None	Light	None
G7	Medium	Brown	Good	Good	Slightly veined	Good	Good	None	Light	None
G8	Rough	Light	Good	Medium	Highly veined	Good	Medium	None	Yellow	None
G9	Smooth	Light	Good	Poor	Slightly veined	Medium	Good	Low	Light	None
G10	Medium	Light	Good	Medium	Highly veined	Good	Good	None	Light	None
Kaman 5	Rough	Light	Good	Medium	Highly veined	Medium	Medium	None	Yellow	None
Chandler	Smooth	Light	Medium	Good	Slightly veined	Medium	Medium	Low	Yellow	None

Table 2. Pomological characteristics of the walnut genotypes and cultivars examined.

Genotypes / Cultivars	Nut weight	Nut length	Nut width	Nut thickness	Shell thickness	Kernel weight	Kernel ratio
G1	11.45±0.63 ^{de}	39.15±2.98 ^c	35.23±2.11 ^{a-c}	34.16±2.84 ^{ab}	0.97±0.03 ^f	6.48±0.29 ^c	56.73±3.98 ^{ab}
G2	13.58±0.58 ^b	37.53±3.54 ^c	35.38±0.82 ^{a-c}	31.03±1.32 ^{ab}	1.15±0.05 ^{d-f}	7.78±0.45 ^b	57.32±3.74 ^{ab}
G3	9.79±0.24 ^f	38.95±3.02 ^c	33.66±1.29 ^{a-c}	29.68±1.39 ^b	1.28±0.05 ^{c-e}	5.82±0.13 ^{c-e}	59.44±2.60 ^a
G4	13.06±0.36 ^{b-d}	38.07±3.63 ^c	32.82±0.86 ^{bc}	35.98±3.32 ^a	0.96±0.05 ^f	7.69±0.38 ^b	58.89±2.28 ^a
G5	16.60±0.67 ^a	46.08±1.42 ^{ab}	37.51±0.72 ^a	34.67±2.00 ^{ab}	1.98±0.16 ^a	8.86±0.63 ^a	53.43±4.36 ^{a-c}
G6	11.88±1.00 ^{c-e}	37.94±1.74 ^c	31.54±2.01 ^c	32.84±1.85 ^{ab}	1.22±0.08 ^{de}	6.16±0.40 ^{cd}	52.33±7.69 ^{a-c}
G7	13.96±0.75 ^b	48.21±2.01 ^a	37.91±1.69 ^a	35.86±2.29 ^a	1.07±0.05 ^{ef}	7.58±0.47 ^b	54.40±3.91 ^{a-c}
G8	15.73±1.26 ^a	40.67±1.92 ^{bc}	35.19±0.79 ^{a-c}	34.92±1.06 ^a	1.56±0.01 ^b	9.20±0.55 ^a	58.62±3.20 ^a
G9	11.43±0.07 ^{de}	39.17±2.75 ^c	33.53±1.74 ^{a-c}	33.27±0.65 ^{ab}	1.36±0.07 ^{b-d}	5.31±0.48 ^{de}	46.42±4.19 ^c
G10	13.32±0.36 ^{bc}	34.92±2.27 ^c	36.01±2.93 ^{ab}	33.81±1.59 ^{ab}	1.48±0.11 ^{bc}	6.37±0.45 ^c	47.84±3.84 ^{bc}
Kaman 5	13.15±0.46 ^{bc}	35.75±1.65 ^c	35.99±2.77 ^{ab}	35.71±3.17 ^a	1.35±0.16 ^{b-d}	6.53±0.34 ^c	49.63±2.30 ^{a-c}
Chandler	10.46±0.60 ^{ef}	37.75±0.89 ^c	35.31±1.85 ^{a-c}	34.72±1.70 ^{ab}	1.32±0.04 ^{cd}	5.02±0.07 ^e	48.08±2.74 ^{bc}

Different letters in the same column are statistically different ($p < 0.05$).

and ‘Kaman 5’ showed good seal. Kernel fill was good in G1, G2, G3, G5, G7, and ‘Chandler’; poor in G9; and medium in the remaining genotypes and ‘Kaman 5’. Kernel shriveling occurred at a low level in G1, G5, G9, and ‘Chandler’ and was absent in the remaining genotypes. G1, G3, G4, G6, G7, G9, and ‘Chandler’ had a slightly veined structure, while G2, G5, G8, G10, and ‘Kaman 5’ were highly veined. Ease of kernel removal was good in G1, G3, G4, G7, G8, and G10, and medium in the remaining genotypes and standard cultivars. In terms of breakage ease, G2, G3, G4, G6, G7, G9, and G10 were good; G5, G8, ‘Kaman 5’, and ‘Chandler’ were medium; and G1 was poor. Kernel shriveling was observed at a low level in G1, G5, G9, and ‘Chandler’, and was none in other genotypes. Regarding kernel color, genotypes G1, G4, and G5 were brown; G2, G8, ‘Kaman 5’, and ‘Chandler’ were yellow; and other genotypes were light-colored. No kernel rot was detected in any genotype.

The genotypes and standard cultivars differed significantly in pomological characteristics (Table 2). G5 (16.60 g) and G8 (15.73 g) had the highest nut weight and belonged to the same statistical group, while G3 (9.79 g) and ‘Chandler’ (10.46 g) had the lowest values. G7 (48.21 mm) and G5 (46.08 mm) had the greatest nut length, while G10 (34.92 mm) and

‘Kaman 5’ (35.75 mm) recorded the lowest values. G5, G7, and G8 formed the highest statistical group for nut width, while G6 (31.54 mm) had the lowest value. In terms of nut thickness, G4 (35.98 mm), G7 (35.86 mm), and ‘Kaman 5’ (35.71 mm) had the highest value, while G3 (29.68 mm) had the lowest value. Shell thickness was greatest in G5 (1.98 mm) and G8 (1.56 mm) and lowest in G1 (0.97 mm) and G4 (0.96 mm). G8 (9.20 g) and G5 (8.86 g) had the highest kernel weight, while ‘Chandler’ (5.02 g) and G9 (5.31 g) had the lowest. Kernel ratio was highest in G3 (59.44%) and G4 (58.89%), and lowest in G9 (46.42%) and G10 (47.84%). Both standard cultivars, ‘Chandler’ (48.08%) and ‘Kaman 5’ (49.63%), fell in the lower range.

Total phenolic content and antioxidant activity: The genotypes and standard cultivars differed significantly in total phenolic content and antioxidant activity (Table 3). Total phenolic content in ethanol extract was highest in G5 (5861.16 mg GAE 100g⁻¹), followed by G2 (5459.23 mg GAE/100 g) and G1 (5220.77 mg GAE 100g⁻¹), and lowest in G6 (3386.15 mg GAE 100g⁻¹). In methanol extract, G5 (4759.23 mg GAE 100g⁻¹) and G2 (4688.08 mg GAE 100g⁻¹) had the highest total phenolic content, while G7 (3084.23 mg GAE 100g⁻¹) and ‘Chandler’ (3143.85 mg GAE 100g⁻¹) had

Table 3. Total phenolic content and antioxidant activity values of walnut samples

Genotypes / Cultivars	Total Phenolic Content (mg GAE/100 g)		DPPH Radical Scavenging Activity (μmol TE/g)		IC ₅₀ (mg/mL)	
	Ethanol Extract	Methanol Extract	Ethanol Extract	Methanol Extract	Ethanol Extract	Methanol Extract
G1	5220.77±285.56 ^b	4220.77±67.99 ^{a-c}	69.98±0.09 ^f	70.48±2.15 ^b	0.23±0.01 ^b	0.23±0.02 ^d
G2	5459.23±144.14 ^{ab}	4688.08±125.10 ^{ab}	76.53±0.42 ^{c-e}	59.23±1.78 ^{ef}	0.20±0.02 ^{c-e}	0.25±0.01 ^{b-d}
G3	5109.23±116.94 ^{bc}	4636.16±258.37 ^{ab}	56.98±3.78 ^g	65.89±1.75 ^c	0.25±0.01 ^a	0.24±0.01 ^{cd}
G4	4055.39±367.15 ^{d-f}	4451.54±345.39 ^{ab}	78.39±2.66 ^{cd}	55.14±1.58 ^g	0.18±0.02 ^{def}	0.28±0.01 ^a
G5	5861.16±179.50 ^a	4759.23±122.39 ^a	81.99±3.50 ^{bc}	66.12±1.81 ^c	0.16±0.01 ^{fg}	0.24±0.02 ^{cd}
G6	3386.15±78.87 ^f	4134.23±313.39 ^{bc}	71.58±1.10 ^{ef}	60.07±1.10 ^e	0.22±0.01 ^{bc}	0.25±0.01 ^{cd}
G7	3745.77±331.80 ^{ef}	3084.23±291.62 ^e	75.89±0.14 ^{de}	55.94±1.58 ^{fg}	0.20±0.01 ^{b-d}	0.27±0.02 ^{a-c}
G8	4538.08±329.70 ^{c-e}	3820.77±280.13 ^{cd}	72.46±1.21 ^{ef}	55.16±1.61 ^g	0.21±0.02 ^{bc}	0.28±0.01 ^{ab}
G9	4572.70±50.44 ^{cd}	3582.31±84.31 ^{de}	85.30±3.68 ^b	63.87±0.20 ^{cd}	0.16±0.01 ^{fg}	0.24±0.01 ^{cd}
G10	5066.92±361.71 ^{bc}	4324.62±247.49 ^{a-c}	78.89±3.03 ^{cd}	71.38±1.90 ^b	0.18±0.01 ^{ef}	0.23±0.01 ^d
Kaman 5	3963.08±242.05 ^{d-f}	3299.62±309.19 ^{de}	73.84±0.95 ^{d-f}	60.63±1.16 ^{de}	0.21±0.01 ^{b-d}	0.25±0.02 ^{b-d}
Chandler	3534.24±261.09 ^{ef}	3143.85±138.70 ^e	91.22±2.54 ^a	80.81±0.71 ^a	0.15±0.01 ^g	0.17±0.01 ^e

Different letters in the same column are statistically different ($p < 0.05$).

Table 4. Descriptive statistics for morphological and biochemical characteristics of walnut genotypes

Variable (abbreviation, unit)	Min	Max	Mean	SD	CV (%)
Nut Weight (NuWe, g)	9.79	16.60	12.87	2.01	15.62
Nut Length (NuLe, mm)	34.92	48.21	39.52	3.90	9.88
Nut Width (NuWi, mm)	31.54	37.91	35.01	1.85	5.29
Nut Thickness (NuTh, mm)	29.68	35.98	33.89	1.94	5.72
Shell Thickness (ShTh, mm)	0.96	1.98	1.31	0.28	21.61
Kernel Weight (KeWe, g)	5.02	9.20	6.90	1.33	19.24
Kernel Ratio (KeRa, %)	46.42	59.44	53.59	4.71	8.79
Total Phenolic Content in Ethanol Extract (TPCEE, mg GAE/100 g)	3386.15	5861.15	4542.72	808.93	17.81
Total Phenolic Content in Methanol Extract (TPCME, mg GAE/100 g)	3084.23	4759.23	4012.12	610.34	15.21
DPPH Radical Scavenging Activity in Ethanol Extract (DPPHEE, μmol TE/g)	56.98	91.22	76.09	8.54	11.23
DPPH Radical Scavenging Activity in Methanol Extract (DPPME, μmol TE/g)	55.14	80.81	63.72	7.73	12.13
DPPH Radical Scavenging Activity (IC ₅₀) in Ethanol Extract (DPPHEEIC50, mg/mL)	0.15	0.25	0.20	0.03	15.91
DPPH Radical Scavenging Activity (IC ₅₀) in Methanol Extract (DPPHMEIC50, mg/mL)	0.17	0.28	0.24	0.03	11.77

Min – minimum, Max – maximum, SD – standard deviation, CV – coefficient of variation.

the lowest.

DPPH radical scavenging activity in ethanol extract was highest in ‘Chandler’ (91.22 μmol TE/g), followed by G9 (85.30 μmol TE/g) and G5 (81.99 μmol TE/g), and lowest in G3 (56.98 μmol TE/g). In methanol extract, ‘Chandler’ (80.81 μmol TE/g) and G10 (71.38 μmol TE/g) had the highest DPPH activity, while G4 (55.14 μmol TE/g) and G8 (55.16 μmol TE/g) had the lowest.

IC₅₀ in ethanol extract was lowest in ‘Chandler’ (0.15 mg/mL) and G5 and G9 (0.16 mg/mL), and highest in G3 (0.25 mg/mL). In methanol extract, ‘Chandler’ (0.17 mg/mL) had the lowest IC₅₀, while G4 and G8 (both 0.28 mg/mL) had the highest.

Descriptive analysis of quantitative traits: Descriptive statistics are presented in Table 4. Shell thickness showed the greatest coefficient of variation (CV 21.61%), followed by kernel weight (CV 19.24%) and the biochemical traits (CV 15–18%). Fruit dimensions were more uniform across genotypes, with CV values below 6%. The population mean for kernel percentage was 53.59%, a value that meets the general requirements for commercial walnut production.

Correlation matrix analysis (CMA): Pearson correlation analysis revealed statistically significant relationships among pomological and biochemical characteristics of the walnut genotypes and cultivars (Fig. 2). Among pomological characteristics, a positive, very high-level statistically significant relationship was found between nut weight and kernel weight ($r = 0.90$, $p < 0.001$). The correlations between nut weight and nut width ($r = 0.54$, $p = 0.068$), nut weight and shell thickness ($r = 0.55$, $p = 0.064$), and nut width and nut length ($r = 0.53$, $p = 0.076$) did not reach the threshold of statistical significance ($p < 0.05$) and are therefore not discussed further.

Among biochemical characteristics, a positive high-level statistically significant relationship was determined between total phenolic content in ethanol extract and total phenolic content in methanol extract ($r = 0.72$, $p = 0.008$). A negative, very high-level significant relationship was detected between DPPH radical scavenging activity in ethanol extract and IC₅₀ value in ethanol extract ($r = -0.97$, $p < 0.001$). Similarly, a negative, very high-level significant relationship was also

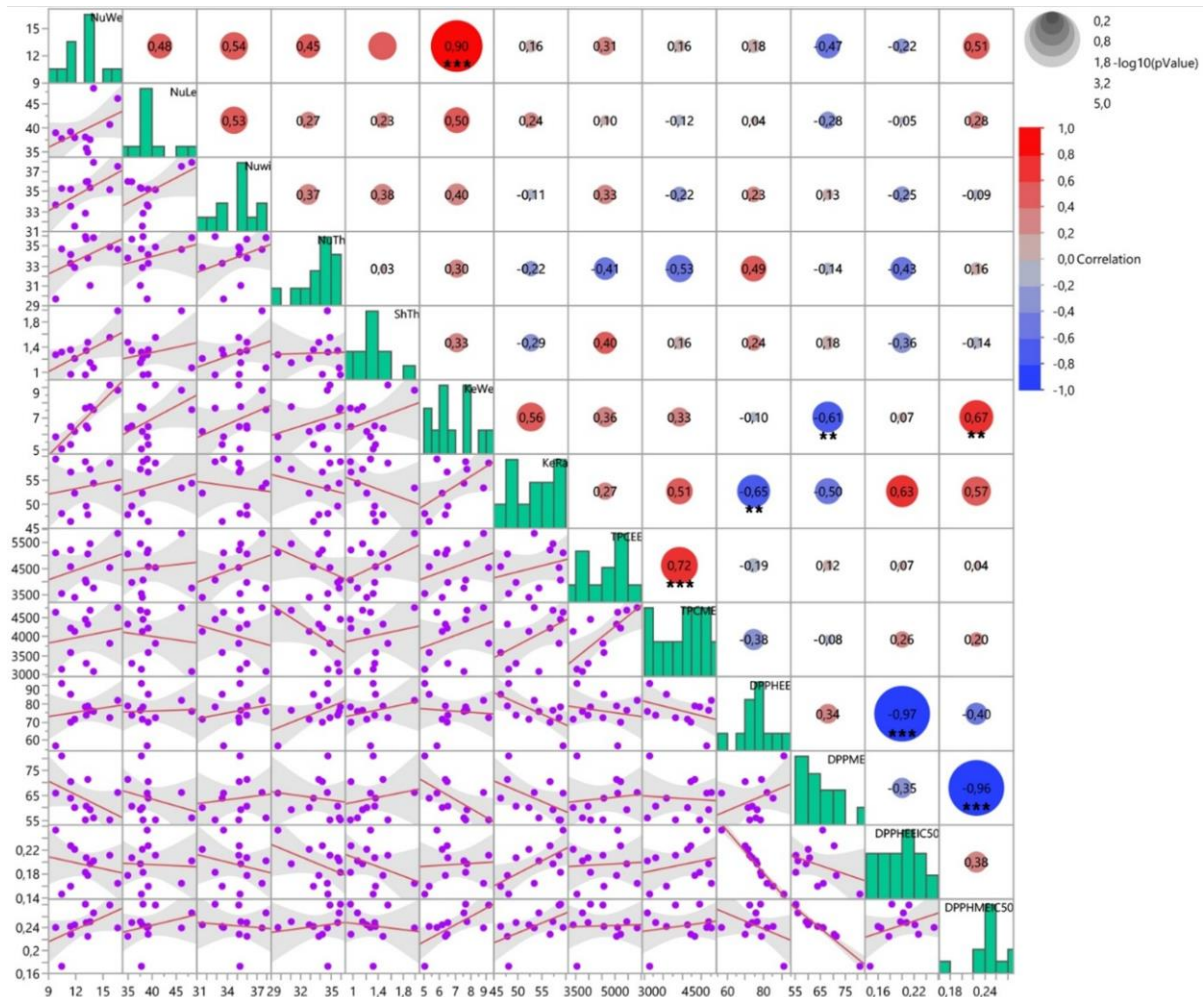


Figure 2. Correlation matrix and scatter plots of morphological and biochemical traits in walnut genotypes and cultivars. Circle size and colour intensity indicate the magnitude of the Pearson correlation coefficient (r); blue indicates positive and red indicates negative correlations. Only statistically significant correlations ($p < 0.05$) are shown with filled circles; non-significant correlations ($p \geq 0.05$) are displayed without fill. For variable abbreviations and units, refer to Table 4.

found between DPPH radical scavenging activity in methanol extract and IC_{50} value in methanol extract ($r = -0.96$, $p < 0.001$). Regarding the relationships between pomological and biochemical characteristics, a negative significant relationship was detected between kernel weight and DPPH radical scavenging activity in ethanol extract ($r = -0.61$, $p = 0.04$). A positive significant relationship was determined between kernel weight and IC_{50} value in methanol extract ($r = 0.67$, $p = 0.02$). A negative significant relationship was observed between DPPH radical scavenging activity in ethanol extract and kernel ratio ($r = -0.65$, $p = 0.02$), while a positive significant relationship was detected between kernel ratio and IC_{50} value in ethanol extract ($r = 0.63$, $p = 0.03$). The correlation between IC_{50} value in methanol extract and kernel ratio ($r = 0.57$, $p = 0.052$) did not reach statistical significance

and is not interpreted.

Principal component analysis (PCA): The pomological and biochemical characteristics of walnut genotypes and cultivars were evaluated by principal component analysis (PCA) (Fig. 3, Table 5). PC1 explained 32.4% and PC2 explained 27.3% of the total variance; together, the four principal components accounted for 85.44%.

PC1 separated genotypes primarily along a kernel size and antioxidant activity axis. Kernel weight, kernel ratio, and IC_{50} in methanol extract loaded positively, while DPPH radical scavenging activity in both extracts loaded negatively, indicating that genotypes with larger kernels and higher kernel ratio tended to show lower radical scavenging capacity. PC2 reflected fruit physical dimensions, with nut weight, nut width, nut thickness, and shell thickness all loading

Table 5. Principal component loadings (Pearson correlations between variables and principal components) obtained from PCA of traits in the studied walnut genotypes and cultivars.

Principal Component Loadings	Component			
	1	2	3	4
Nut Weight	0.62^a	0.79^a	0.07	-0.17
Nut Length	0.42^a	0.55^a	-0.09	0.53^a
Nut Width	0.11	0.74^a	0.22	0.58^a
Nut Thickness	-0.07	0.72^a	-0.57^a	-0.02
Shell Thickness	0.05	0.60^a	0.59^a	-0.11
Kernel Weight	0.86^a	0.52^a	0.03	-0.11
Kernel Ratio	0.85^a	-0.35^a	-0.02	0.13
Total Phenolic Content in Ethanol Extract	0.42^a	0.06	0.88^a	0.02
Total Phenolic Content in Methanol Extract	0.53^a	-0.31^a	0.69^a	-0.36^a
DPPH Radical Scavenging Activity in Ethanol Extract	-0.61^a	0.71^a	-0.04	-0.33^a
DPPH Radical Scavenging Activity in Methanol Extract	-0.80^a	-0.02	0.54^a	0.25^a
DPPH Radical Scavenging Activity (IC ₅₀) in Ethanol Extract	0.56^a	-0.72^a	-0.10	0.38^a
DPPH Radical Scavenging Activity (IC ₅₀) in Methanol Extract	0.88^a	0.03	-0.43^a	-0.25^a
Eigenvalue	4.21	3.55	2.26	1.09
Variance	32.36	27.27	17.42	8.39
Σvariance	32.36	59.63	77.05	85.44

^aBold values indicate variables with high loadings ($|\text{loading}| \geq 0.25$) that most strongly contribute to each PC.

positively, separating large-fruited genotypes from smaller ones. PC3 captured phenolic content variation independent of fruit size and kernel-related traits, with total phenolic content in both extracts dominating this component. PC4 accounted for an additional 8.39% of the variance and primarily reflected minor dimensional differences among genotypes.

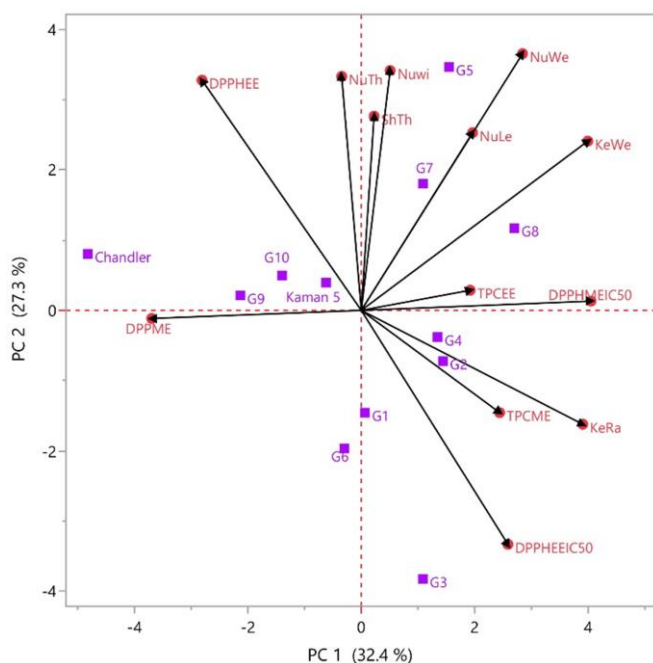


Figure 3. Biplot for variables based on PC1/PC2 of the examined walnut genotypes and cultivars. For variable abbreviations and units, refer to Table 4.

In the biplot, G5 and G8 clustered toward large fruit size along

the PC2 axis, while ‘Chandler’ and G9 were positioned toward high antioxidant activity on the negative PC1 axis. G3 clustered near the kernel ratio variable on the positive PC1 axis. The remaining genotypes, including G4, G2, G7, ‘Kaman 5’, and G10, occupied central positions without clear separation.

Heat map analysis (HMA): The pomological and biochemical characteristics of walnut genotypes and cultivars were evaluated by hierarchical cluster analysis (HCA) and heat map analysis (Fig. 4). Variables were standardized (z-score) prior to clustering to account for differences in measurement units. The genotypes and cultivars were grouped under two main clusters (A and B).

Cluster A comprised G1, G2, G3, G6, G9, G10, ‘Kaman 5’, and ‘Chandler’, and was divided into two subclusters. Subcluster A1 included G1, G2, and G3. These genotypes generally showed low values for nut weight, kernel weight, and IC₅₀ in methanol extract, and relatively high values for total phenolic content in ethanol and methanol extracts. However, within A1, G3 was an exception: it recorded the lowest DPPH radical scavenging activity in ethanol extract (56.98 $\mu\text{mol TE/g}$) and the highest IC₅₀ in ethanol extract (0.25 mg/mL) in the entire dataset, indicating that high total phenolic content in this genotype did not correspond to high radical scavenging activity. Subcluster A2 comprised G6, G9, G10, ‘Kaman 5’, and ‘Chandler’. Within this subcluster, ‘Chandler’ showed distinctly high DPPH radical scavenging activity in both ethanol (91.22 $\mu\text{mol TE/g}$) and methanol (80.81 $\mu\text{mol TE/g}$) extracts and the lowest IC₅₀ values in the dataset. G6 showed the lowest total phenolic content in ethanol extract (3386.15 mg GAE/100 g) among all genotypes and cultivars.

Cluster B comprised G4, G8, G7, and G5, and was divided into two subclusters. Subcluster B1 included G4, G8, and G7.

Subcluster B2 consisted of G5 alone. According to the heat map, genotypes in Cluster B generally showed high values for nut weight, kernel weight, and IC₅₀ in methanol extract, and low values for DPPH radical scavenging activity. G5 recorded the highest nut weight (16.60 g) and one of the highest kernel weights (8.86 g) in the dataset.

The variable dendrogram grouped all variables into two main groups, X and Y. Group X was divided into subgroups X1 (nut thickness) and X2 (DPPH radical scavenging activity in methanol extract). Group Y was divided into subgroups Y1 (nut weight, kernel weight, IC₅₀ in methanol extract, nut length, nut width, and shell thickness) and Y2 (kernel ratio, IC₅₀ in ethanol extract, total phenolic content in ethanol extract, and total phenolic content in methanol extract). The clustering of DPPH radical scavenging activity in ethanol extract with nut thickness in group X suggests that these variables share a common pattern of variation across genotypes that is independent of the fruit size and phenolic content dimensions captured by group Y.

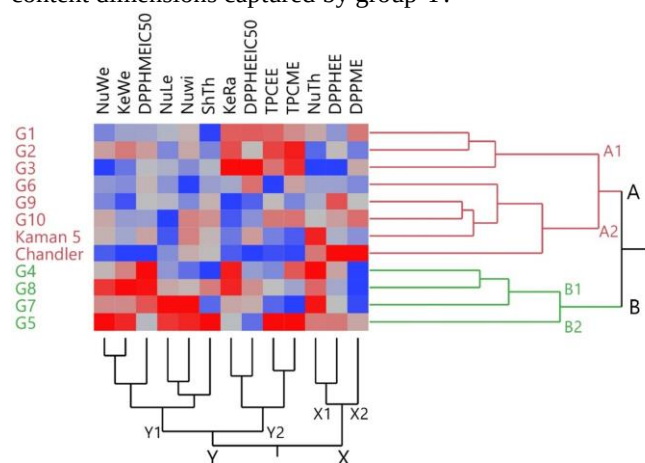


Figure 4. Heatmap hierarchical clustering analysis dendrogram of walnut genotypes and cultivars. Red indicates values above the genotype mean; blue indicates values below the mean. Variables were standardized (z-score) prior to clustering using the Ward method with Euclidean distance. For variable abbreviations and units, refer to Table 4.

DISCUSSION

Morphological and pomological traits: Regarding shell roughness, G6 and G9 genotypes and the standard cultivar ‘Chandler’ exhibited smooth shell structure. Smooth-shelled genotypes reduce irregular fracture and kernel damage in mechanical processing lines due to homogeneous cracking property (Ortiz *et al.*, 2019). Therefore, G6 and G9 genotypes can be evaluated as advantageous for industrial cracking operations. Genotypes showed differences in terms of shell seal at medium and good levels. While tight seal limits

oxygen entry and lipid oxidation (Benelli *et al.*, 2013; Garrido-Miranda *et al.*, 2025), it should be noted that very tight seal may cause kernel damage during cracking (Jeong *et al.*, 2022). This finding is parallel with the view of Altuntaş and Erkol (2009) that structurally sound shells require higher force but increase whole-kernel recovery. This situation constitutes an important balance factor for industrial operations.

When visual quality parameters were examined, it was determined that most genotypes had light shell and kernel color. Light color is an important criterion in market preference because it shows better stability against pellicle browning during storage (Grilo and Wang, 2021; Ampofo *et al.*, 2022). The combination of light color, low kernel shriveling and absence of kernel rot shows that the evaluated genotypes are suitable for long-term storage and premium markets when appropriate postharvest conditions are provided (Mitcham *et al.*, 2022). In terms of kernel veining, the dominance of slightly veined structure is a positive characteristic. Increased vein prominence can accelerate darkening by expanding surface area for oxidation (Żurek *et al.*, 2022). Therefore, the prevalence of slightly veined phenotype may help delay visual quality losses during distribution.

In terms of processing efficiency, kernel extractability and breakage behavior are determinants of commercial grade (Ortiz *et al.*, 2019). G1, G3 and G8 genotypes are in the “good” class in terms of kernel extractability. These genotypes are promising candidates for high-value whole-kernel production. The dominance of flawless and light-colored kernels reveals that this population provides raw material with sensory properties meeting consumer demands for premium walnut products (Zaini *et al.*, 2020; Jahanban-Esfahlan *et al.*, 2019).

The marked variation in fruit size and kernel weight carries practical consequences. G5 and G8 genotypes having the highest nut weight and dimensions are attractive for in-shell marketing. Compact structured walnuts such as G3 may provide advantage in terms of mechanical handling in high-throughput processing lines (Bernard *et al.*, 2020). The observed value ranges show consistency with those reported for superior walnut genotypes in other geographical regions (Cosmulescu and Ionescu, 2021; Zarinkolah *et al.*, 2025). This situation supports the view that Kırşehir population can provide competitive material for local and broader markets. Shell thickness range (0.96–1.98 mm) shows the technological diversity of the examined material. Thin shells generally reduce cracking force and improve kernel recovery (Han *et al.*, 2023). Thick shells provide mechanical durability for long-term storage and transportation (Kabiri *et al.*, 2018; Sideli *et al.*, 2020). In terms of kernel characteristics, while G5 and G8 showed superior performance in kernel weight, G3, G4 and G8 had the highest kernel ratios. High kernel ratio is the primary determinant of economic return and industrial

processing efficiency (Sütyemez *et al.*, 2021). Consequently, G3, G4 and G8 are prominent candidates for shelled-kernel industry. Large-fruited G5 is particularly suitable for table-consumption market (Bernard *et al.*, 2020; Ampofo *et al.*, 2022).

Total phenolic content and antioxidant activity: Ethanol extracts showed higher total phenolic content values compared with methanol extracts. This situation is consistent with solvent-dependent selectivity for phenolic subclasses of differing polarity (Pereira *et al.*, 2007). These differences have practical relevance: extraction conditions influence the oxidative stability and color retention of walnut products (Jahanban-Esfahlan *et al.*, 2019; Gao *et al.*, 2019).

In the antioxidant assays, ‘Chandler’ showed the highest DPPH radical scavenging activity and the lowest IC₅₀ value in both ethanol and methanol extracts. G9 and G5 ranked close behind in ethanol extract, while G10 followed in methanol extract. The strong inverse relationship between DPPH activity and IC₅₀ across the population confirms the consistency of the evaluation (Shahidi and Ambigaipalan, 2015). In the literature, radical scavenging capacity has been consistently linked to resistance against lipid oxidation under storage conditions, particularly through the inhibition of free radical chain reactions in unsaturated fatty acids (Elouafy *et al.*, 2022; Dordoni *et al.*, 2019; Shahidi and Ambigaipalan, 2015). The proximity of several local genotypes to ‘Chandler’ — the reference cultivar with the highest DPPH activity — in both ethanol and methanol extracts indicates that Kırşehir germplasm carries antioxidant potential at a level relevant for industrial applications where oxidative stability is a selection criterion.

G3 showed high total phenolic content in ethanol extract alongside relatively low DPPH antioxidant activity and a high IC₅₀ value — a pattern worth examining. The Folin–Ciocalteu method captures the total reducing capacity of an extract, which may include non-phenolic compounds alongside phenolics (Muflihah *et al.*, 2021). The divergence observed in G3 — high total phenolic content alongside low DPPH activity — is consistent with a compositional profile in which quantitatively dominant compounds contribute less to radical scavenging than minor but structurally active fractions. This pattern has been documented in other *Juglans* genotypes where ellagitannins and flavonoids differ markedly in their per-unit radical quenching efficiency (Amaral *et al.*, 2003; Bolling *et al.*, 2011). Lipophilic antioxidants such as tocopherols also contribute independently to overall capacity, and their recovery varies with solvent polarity, which can shift apparent rankings between genotypes (Miraliakbari and Shahidi, 2008; Gao *et al.*, 2022). Profiling individual phenolic compounds in future investigations would help clarify the biochemical basis of this divergence.

Descriptive analysis of quantitative traits: The moderate variability in nut weight alongside the relatively narrow variation in fruit dimensions suggests that fruit mass

differences are driven primarily by structural factors such as shell thickness and kernel cavity ratio (Sharma *et al.*, 2024). Shell thickness had the highest coefficient of variation in the dataset (CV 21.61%), indicating a broad genetic base for selecting thin-shelled types for easy cracking or thicker-shelled types for storage durability (Soveili and Khadivi, 2023).

Biochemical traits showed similarly useful diversity (CV ~15–18%), providing a basis for selecting genotypes that combine desirable physical traits with enhanced phenolic profiles. The mean kernel percentage of 53.59% aligns with commercial thresholds reported in the literature (Mir *et al.*, 2025), confirming that the material meets the basic requirements for economically viable kernel production. The observed variation also permits the identification of genotypes with simultaneously high antioxidant capacity and high kernel percentage — a trait combination with direct relevance to functional food development. The relative uniformity of dimensional metrics is practical for processing standardization, while the wider spread in shell thickness and kernel weight supports differentiated selection for specific market segments (Simoes Grilo *et al.*, 2020).

Correlation matrix analysis (CMA) and scatter plot: The very strong positive correlation between nut weight and kernel weight confirms that fruit mass is the primary determinant of kernel output, consistent with findings reported for other walnut populations (Amaral *et al.*, 2003). The absence of significant correlations among dimensional traits indicates that fruit mass and physical dimensions vary along partially independent axes in this population.

The strong agreement between ethanol and methanol total phenolic content suggests that the two solvents capture overlapping phenolic pools. The very strong inverse relationships between DPPH radical scavenging activity and IC₅₀ in both extracts confirm full consistency between the two antioxidant measures across genotypes (Shahidi and Ambigaipalan, 2015).

The negative correlations between kernel-related traits and DPPH radical scavenging activity are consistent with a dilution effect: as kernel mass increases, phenolic density per gram decreases, because phenolics are concentrated primarily in the pellicle (Bolling *et al.*, 2011). The positive correlations between kernel weight and kernel ratio with IC₅₀ values reflect the same pattern expressed in the opposite direction. Selecting exclusively for large kernel size and high kernel ratio may therefore reduce phenolic density per gram of kernel, a trade-off relevant to breeding programs targeting antioxidant quality.

Principal component analysis (PCA): Four principal components accounted for 85.44% of total variance, suggesting that a small number of underlying dimensions are sufficient to describe most of the quality-related diversity in this population (Tošić *et al.*, 2015). PC1 (32.4%) loaded positively on IC₅₀ value in methanol extract, kernel weight,

kernel ratio, and total phenolic content in methanol extract, while loading negatively on DPPH radical scavenging activity in methanol extract and DPPH radical scavenging activity in ethanol extract, capturing the kernel-size and kernel-ratio versus antioxidant-activity pattern noted in earlier walnut studies (Martínez *et al.*, 2010; Gao *et al.*, 2022; Shah *et al.*, 2018; Trandafir *et al.*, 2016).

PC2 (27.3%) reflected fruit size and shape, with high positive loadings on nut weight, nut length, nut width, nut thickness, shell thickness, and kernel weight — separating the larger-fruited genotypes such as G5 and G8 from the rest, a distinction with practical relevance for mechanical cracking (Ni *et al.*, 2022). PC3 (17.42%) was dominated by total phenolic content and shell thickness, accounting for an additional 17.42% of the total variance (Bourais *et al.*, 2023). Most PCA-based walnut studies have focused on fatty acid or mineral profiles (Li *et al.*, 2017; Fallah *et al.*, 2022; Kömür *et al.*, 2024; Aygün *et al.*, 2026). Integrating pomological and antioxidant traits, as done here, allows genotypes to be matched more directly to functional food applications (Lal *et al.*, 2022; Vu *et al.*, 2018).

Heat map analysis (HMA): The distinct trait clusters in the HMA mirror the multivariate structure observed in PCA. The grouping of total phenolic content with DPPH radical scavenging activity in ethanol and methanol extracts has practical relevance, since ethanol is a preferred food-grade solvent for phenolic extraction (Tabaraki and Rastgoo, 2014). The very strong inverse correlations between DPPH radical scavenging activity and IC₅₀ in both ethanol and methanol extracts confirm that the two metrics are highly consistent: higher radical scavenging activity corresponds to lower IC₅₀. Although expressed in opposite directions, both measures converged on the same genotype ranking for antioxidant capacity (Shahidi and Ambigaipalan, 2015).

The genotype clusters map reasonably well onto distinct end-use profiles. Subcluster A1 (G1, G2, and G3), defined by relatively high phenolic content and small fruit size, contains genotypes that could be useful as sources of concentrated phenolic extracts or nutraceutical ingredients (Medic *et al.*, 2021). Subcluster A2 (G6, G9, G10, ‘Kaman 5’, and ‘Chandler’) showed distinctly high DPPH radical scavenging activity in ethanol extract — a pattern in which ‘Chandler’ combined high DPPH activity with relatively modest total phenolic content in ethanol extract, suggesting that radical scavenging efficiency in this cultivar may not be driven solely by bulk phenolic quantity (Temizyürek *et al.*, 2025). Clusters B1 (G4, G8, and G7) and B2 (G5) combined large kernel size and high kernel ratio with preserved antioxidant capacity, a pairing that is attractive from both an economic and an oxidative stability standpoint (Okatan, 2022). These findings are consistent with recent multivariate work arguing for the joint consideration of agronomic and biochemical traits in nut genotype evaluation (Einollahi and Khadivi, 2024; Martínez *et al.*, 2010).

Conclusion: In the Kırşehir walnut population, significant differences were detected among genotypes in both pomological and biochemical characteristics. The very strong positive correlation between nut weight and kernel weight confirmed that fruit mass is the primary determinant of kernel output. Multivariate analyses revealed distinct profiles within the material: G5 and G8 were prominent for nut weight and kernel weight; G3, G4, and G8 showed the highest kernel ratios; and ‘Chandler’ exhibited the highest DPPH radical scavenging activity and the lowest IC₅₀ values in both extracts. Genotypes G1 and G2 combined relatively high total phenolic content with moderate kernel weight. These findings provide a preliminary basis for prioritizing genotypes in regional breeding and selection programs, with different genotypes showing potential for distinct end uses such as kernel production, and with some genotypes warranting further investigation for functional food applications based on their phenolic profiles. Since the data are based on a single growing season, multi-year evaluation across different ecological conditions is necessary before stable performance rankings can be established. Characterization of genotype-environment interactions will add further resolution to this picture.

Acknowledgment: Not applicable.

Conflict of interests: No potential conflict of interest was reported by the authors

Authors’ contribution statements: AA: Supervision, experimental design, manuscript review and editing. ET: Chemical analyses, data interpretation. AŞ: Chemical analyses, data interpretation. FEŞS: Sample collection and preparation. LK: Conceptualization, experimental design, data collection, statistical analysis, manuscript preparation, manuscript review and editing.

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