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RESEARCH ARTICLE

Morphological, chemical, and molecular characterization of a new late-leafing and high fruit quality hazelnut (*Corylus avellana* L.) genotype

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Abstract Hazelnuts are widely employed in various dietary practices, making them one of the most frequently utilized nuts. This study morphologically, chemically, and molecularly characterized a new hazelnut genotype throughout the 2018 to 2020 growing seasons. The variety burst leaf buds between 10th and 15th April and reached harvest maturity between 5th and 15th September. The genotype bore a mean of 2.33 nuts per cluster, with the majority of clusters consisting of double and triple nuts. The means of the nut weight was 2.38 g, the kernel weight was 1.35 g, the kernel ratio was 56.81%, the shell thickness was 0.96 mm, the nut size was 18.32 mm, the kernel size was 14.77 mm, the good kernel ratio was 91.7%, the protein content was 13.5%, the oil content was 55.8%, the oleic acid ratio was 81.43%, and the linoleic acid ratio was 10.68%. The genetic similarity rate between the new hazelnut genotype and the standard Çakıldak, Palaz, Tombul cultivars, as well as randomly selected Çakıldak hazelnut clones from the region, ranged from 0.58 to 0.98. Additionally, the polymorphism rate varied from 45.5 to 100%. On average, the investigated hazelnut genotype exhibited a genetic distinctiveness of 38% compared to randomly selected Çakıldak clones from the same region, and 47% compared to standard hazelnut cultivars. Consequently, this genotype could serve as valuable genetic material for hazelnut breeding programs through genetic distinctiveness and promising nut quality, and could potentially be registered as a new cultivar.

Keywords Hazelnut · Breeding · Kernel ratio · Oleic acid · ISSR

Introduction

Hazelnut (*Corylus avellana* L.) is a monoecious, wind pollinating, and multi-stemmed shrub (Boccacci et al. 2013; Leinemann et al. 2013), having high economic and nutritional value. Hazelnuts belong to the *Corylus* genus of the *Betulaceae* family. There are 13 species within the *Corylus* genus, among which *C. avellana* and *C. colurna* hold commercial significance (Molnar 2021; Mehlenbacher 2018). *C. avellana* is extensively cultivated in Türkiye, as well as in Italy, Azerbaijan, United States of America, Chile, Georgia, Spain, France, and Iran. Türkiye is the largest producer and exporter of hazelnuts, covering 64% (765.000 tons) of global hazelnut production. Following Türkiye, Italy (98.670 tons), Azerbaijan (72.104 tons), the

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USA (70.310 tons), Chile (62.557 tons), and Georgia (33.400 tons) are the leading hazelnut-producing countries (FAO 2024).

Hazelnuts are renowned for their high nutritional value and widespread consumption, owing to their composition of various beneficial fatty acids. (Di Nunzio 2019). Hazelnut oil boasts high levels of monounsaturated fatty acids, notably oleic and linoleic acids, with low levels of saturated fatty acids such as palmitic, palmitoleic, and stearic acids (Balta et al. 2006; Karakaya et al. 2023a). The abundance of unsaturated fatty acids in hazelnuts plays a crucial role in promoting human health and aiding in the prevention of various diseases (Yücesan et al. 2010; Di Nunzio 2019). Moreover, the fatty acid composition, particularly the ratio of oleic to linoleic acid, is deemed essential in determining kernel quality. Hazelnuts with low linoleic acid content offer advantages in processing, storage, and transportation, whereas those rich in oleic acid are nutritionally significant (Rezaei et al. 2014; Karaosmanoglu 2022). Recent research has focused on the biochemical characteristics and fatty acid composition of hazelnut genetic resources (Krol et al. 2020; Yaman et al. 2023).

Türkiye, along with Europe, Asia, Iran, and North America, possesses rich hazelnut genetic resources and is an important region for commercial hazelnut cultivation (Erdoğan and Mehlenbacher 2000). Molecular characterization research indicates that Turkey, the Mediterranean region, and Iran serve as gene centers for hazelnuts (Boccacci and Botta 2009). In Türkiye, specifically Northern Anatolia (Black Sea Region), which harbors a rich potential in terms of hazelnut germplasm, is among the oldest regions where hazelnuts have been cultivated for many years. Numerous investigations have been conducted in the region to identify and characterize hazelnut germplasm through selection breeding (Bostan and İslam 1999a; İslam 2003; Turan and Beyhan 2009; Karadeniz et al. 2020; Güler and Balta 2020; Karakaya 2021; Uzun 2021). These investigations revealed genetic variations even among clones of the same cultivar and suggested that Turkish hazelnut cultivars are a group of clones with similar characteristics (Balta et al. 1997; İslam 2003; Mehlenbacher 2018).

Selecting individuals with high yield, early maturity, round shape, high good kernel, thin shell, high kernel percentage, large nut, high kernel blanching, low defect kernel, late leafing, and resistance to diseases and pests is crucial in hazelnut breeding (Mehlenbacher 2018; Botta et al. 2019). High genetic diversity presents a significant advantage for plant breeders and breeding programs, as it allows for the selection of individuals with desired traits (Guo et al. 2023; Güler and Karadeniz 2023; Güler et. al. 2024). Due to the extended juvenile period of hazelnut plants, determining individuals with desired traits through selection breeding is critical for hazelnut breeding programs (Ochi-Ardabili et al. 2023). Determining the genetic relationships among selected individuals enhances the effectiveness of hazelnut breeding programs and is essential for preserving and managing genetic diversity and resources (Martins et al. 2015).

Molecular markers, being independent of environmental interactions, are excellent tools for revealing genetic diversity and relationships among individuals (Yang et al. 2023). Various molecular markers such as random amplified polymorphic DNA (RAPD) (Erdoğan et al. 2010; Demir 2014), simple sequence repeats (SSR) (Yang et al. 2023), inter simple sequence repeats (ISSR) (Karakaya et al. 2023b), restriction fragment length polymorphism (RFLP) (Kafkas et al. 2009), and start codon targeted (SCoT) (Ochi-Ardabili et al. 2023) have been widely utilized for determining genetic diversity, genetic mapping, and managing genetic resources in hazelnuts. This study aimed to characterize a new late-leafing, thin-shelled, round-shaped, high kernel percentage with high kernel blanching and large nuts hazelnut genotype in morphological, chemical, and molecular aspects.

Materials and method

Materials

The study was conducted in the Gürgentepe district (Ordu, Turkey) throughout the 2018–2020 seasons. The study consisted of morphologically different hazelnut genotype (G-1), four randomly selected Çakıldak hazelnut clones (Ç-1, Ç-2, Ç-3, and Ç-4) from the orchard where G-1 was located, along with standard Çakıldak, Tombul, and Palaz cultivars those are extensively cultivated in the region. The Çakıldak clones and standard hazelnut cultivars were used in

molecular characterization. The selection of these clones and cultivars as materials took into account the widely cultivated hazelnut cultivars in both the Gürgentepe district where the research was conducted and the broader Ordu province to which the district belongs.

Climate conditions

The climate of the region is characterized by mild and rainy summers and cold winters. The coldest month is January by a mean of − 3 ℃, while the hottest month is August with an average of 22 ℃. The annual average temperature is 11.0 ℃. The total annual precipitation is 634 mm, with a relative humidity of 76.6%. The elevation of the region is 1260 m (Anonim 2024a, 2024b).

Method

Plant characteristics, leaf features, phenological traits, and fruit properties were examined for morphological characterization in the investigated hazelnut. For chemical characterization, ash, protein, oil, and fatty acid contents were evaluated. Morphological and chemical characterization was conducted solely on the investigated hazelnut genotype. In addition, for molecular characterization, leaf samples were collected in the first week of May from the investigated hazelnut genotype, four Çakıldak hazelnut clones, and Çakıldak, Palaz, and Tombul culitvars. Care was taken to ensure that the leaf samples were free from diseases and pests, and the samples were promptly transported to the laboratory in a cold chain transport bag without delay.

Plant characteristics

Yield fluctuations, self-fertility, vigor, habitus, suckering, and shoot density were determined according to UPOV criteria (Bioversity and CIHEAM 2008).

Phenological characteristics

The period at which leaf buds burst and the first two leaves become visible, reaching 90% of the stage, was considered the leaf emergence period. Harvest maturity was determined based on the period when the hard shell turns reddish by ¾ and the husk turns yellow. The leaf shedding period was determined based on the time when 50% of the leaves on the plant have fallen (Şen-Dülger 2023).

Leaf characteristics

Leaf length (cm), leaf width (cm), and petiole length (cm) were measured using a strip meter. Petiole thickness (mm) was determined using a digital caliper (Mitutoyo, Japan) with a precision of 0.01 mm (Beyhan and Demir 2001).

Nut traits

Nut traits were determined in the 100 nuts. Nut and kernel weight (g) were measured with a digital scale (Radwag, Poland) with a precision of 0.01 g. The kernel ratio was determined by relating the kernel weight to the nut weight and expressed as a percentage. Shell thickness (mm), nut and kernel dimension (width, length, and thickness) (mm), central cavity width and length (mm) were measured using a digital caliper (Mitutoyo, Japan) with a precision of 0.01 mm. The nut and kernel size was calculated by taking the geometric mean of the nut and kernel dimensions. Defective kernel ratio (blank, black-tipped, twin, shriveled, abortive, moldy, rotten kernel ratios) was determined through observation and expressed as a percentage. Husk length (mm) was measured with a digital caliper (Mitutoyo, Japan) with a precision of 0.01 mm. The number of nuts per cluster was determined by counting the clusters on the plant in singles, doubles, triples, etc. (Bostan and Günay 2009; Balta et al. 2018; Bostan and Bozkurt 2019). Kernel taste and aroma were determined by a team of five experts. The blanching rate was determined by keeping the hazelnuts in the oven at 175 °C for 15 min, then the testa was rubbed by hand, and the ratio of the blanched hazelnuts to the total number of kernels (Bostan and İslam 1999b).

Ash, protein, and oil ratio (%)

The ash content was determined by burning 3 g of hazelnut sample at 550 °C in an ash furnace (Kacar and İnal 2008). Protein ratio was determined using the *Kjeldahl* method (Venktachalam and Sathe 2006). Oil ratio was determined using the Soxhlet extraction method (Firestone 1989).

Fatty acid composition (%)

0.1 g of hazelnut oil was weighed, and 1 mL of potassium methoxide and 4 mL of hexane were added. The prepared mixture was shaken for 30 s. The resulting upper phase was taken, diluted with hexane, and filtered through a 0.45-micron filter (AOAC 1990). The fatty acid composition was determined using gas chromatography (GC-2010 Plus, Shimadzu, Japan) with a flame ionization detector (FID) and a thin capillary column (100 m × 0.25 mm × 0.2 µm, Teknokroma TR-CN100, Spain). After setting the column temperature to 140 °C, it was increased by 4 °C per minute until it reached 240 °C, where it was held for 15 min. Detector and injector temperatures were set at 250 °C. Nitrogen was used as the carrier gas, with a flow rate of 1 mL/min. The split ratio was 1:100. Fatty acid composition was detected according to fatty acid standards and compared with the retention times of the standards. The results were expressed as relative area percentages of fatty acids (Altun et al. 2013).

Molecular characterization

The DNA for molecular characterization was extracted from approximately 100 mg of frozen hazelnut leaves using the method reported by Doyle and Doyle (1990). DNA quality and quantity were determined by spectrophotometer (NanoDrop Technologies Inc. Wilmington, USA) at 260 and 280 nm wavelengths. DNAs were detected on a 2% agarose gel with electrophoresis using a 100 bp DNA ladder. The obtained DNAs were diluted to working concentrations and stored at − 20 °C.

To molecularly identify the investigated hazelnut genotype, 9 ISSR primers [DBDA$(CA)_7$, $(AG)_7$YC, $(AGC)_6$G, VHV$(GTG)_7$, $(CT)_8$TG, $(GT)_6$GG, HVH$(TCC)_7$, $(AG)_8$ T, $(GA)_8$YG] were employed (Kafkas et al. 2009; Martins et al. 2009; Karakaya et al. 2023b). The PCR mixture had a total volume of 15 µL, including 2 µL DNA, 1.5 µL 10 × PCR buffer, 1 µL ISSR primer, 1 µL dNTP, 1.5 µL $MgCl_2$, 0.2 µL Taq-DNA polymerase enzyme, and 7.8 µL ddH_2O. PCR conditions involved an initial denaturation at 94 °C for 2 min, followed by 45 cycles of denaturation at 94 °C for 1 min, annealing at 53 °C for 1 min, extension at 72 °C for 2 min, and a final extension at 72 °C for 5 min (Uzun et al. 2009). After PCR, the obtained products were subjected to electrophoresis using a 2% agarose gel and visualized under UV light using the Major Science SmartView Pro 1100 imaging system. The bands generated from the primers were scored as either present (1) or absent (0). The data were analyzed using NTSYS—pc version 2.11 (Numerical Taxonomy and Multivariate Analysis System) software (Rohlf 2000). Similarity data were calculated using the Dice method (1945) and constructed the UPGMA (Unweighted Pair Group Method with Arithmetic Average) dendrogram using NTSYS—pc version 2.11 software. The dendrogram was constructed by bootstrap analysis using 1000 replications. Also, the total number of bands, polymorphic bands, and polymorphism ratio were calculated for used markers. The polymorphism ratio was calculated using the following formula: [(Number of Polymorphic Bands × 100) / Total Number of Bands].

Statistical analysis

The standard deviation values of the obtained morphological and chemical data were calculated. Histogram graphs was constructed using by the JMP 16 (trial version) statistical package program. Data of molecular characterization analysis were evaluated using by NTSYS—pc 2.11 (Numerical Taxonomy and Multivariate Analysis System) software.

Results and discussion

Plant characteristics

The new hazelnut genotype showed no yield fluctuations and was found to be self-fertile (Table 1). It is critical in hazelnut breeding to develop high-yielding cultivars with a little or no yield fluctuation (Botta et al. 2019). Hazelnut yield varies depending on many factors. Especially late spring frosts and drought events in summer cause significant yield fluctuations in hazelnuts (Beyhan et al. 2007; Bostan and Tonkaz 2013). In this regard, the genotype investigated gave remarkable results in terms of yield character.

In hazelnut breeding, it is critical to select cultivars with medium growth, an upright growth habit, and little or no tendency to produce suckers (Botta et al. 2019). The investigated hazelnut genotype had a semi-erect habitus, strong growth, a medium

Table 1 Plant characteristics of the new hazelnut genotype

Plant characteristics	Values
Yield fluctuation	None
Self-fertile	Fertile
Habitus	Semi-erect
Vigor	Strong
Suckering tendency	Medium
Shoot density	Frequent
Leaf bud burst	10–15 April (very late)
Leaf fall	End of November (late)
Harvest maturity	5–15 September (late)
Leaf length (cm)	11.6 ± 1.30
Leaf width (cm)	9.9 ± 1.13
Petiole length (cm)	1.9 ± 0.42
Petiole thickness (mm)	1.21 ± 0.14
Nuts per cluster	2.33 ± 0.04
Distributon of nuts per cluster (%)	

Single	Double	Triple	Quart	Quintet	Senary
17.3	44.9	27.4	8.9	1.4	0.1

tendency to produce suckers, and frequent shoot density (Table 1). According to various researchers, the majority of Turkish hazelnut genetic resources are in medium to strong development. They also stated that these individuals had a spreading and semi-erect habitus, as well as a high sucker production (Bostan and İslam 1999a; İslam 2000; Semiz 2016; Karakaya 2021). In terms of growth and habitus, the genotype investigated was similar to the individuals studied by the researchers. It was also found to be remarkable for its ability to produce moderate suckers.

The leaf bud burst time for the new hazelnut genotype was between April 10th and 15th, the harvest time was between September 5th and 15th, and the leaf fall time was the end of November. In Turkish hazelnut cultivars grown in Giresun ecological conditions, the earliest leafing is in Palaz (March 5th and 10th) and the latest in Çakıldak (April 1st and 5th). The earliest harvest is in Mincane, Yassıbadem, and Acı cultivars (Augut 5th and 10th), and the latest harvest is in Çakıldak (August 20th and 25th). Leaf fall is recorded in the middle and end of November in many cultivars, and in the first week of December in a few cultivars (Balık et al. 2016). Late leafing is a desirable trait for hazelnut breeding. The investigated hazelnut genotype leafed out later than the Çakıldak cultivar (which leaves later than other Turkish hazelnut cultivars) located in the same orchard. These findings indicate that the investigated genotype has a high potential for developing new cultivars and improving the characteristics of existing cultivars in hazelnut breeding programs, especially in terms of late leafing trait.

In the hazelnut genotype investigated, the leaf length, leaf width, petiole length and petiole thickness were determined as 11.6 cm, 9.9 cm, 1.9 cm and 1.21 mm (Table 1), respectively. Local and standard hazelnut cultivars grown in Çarşamba district, leaf length, leaf width and petiole length were reported as 8.81–12.50 cm, 7.39–10.70 cm and 1.18–1.72 cm (Beyhan and Demir 2001), respectively. Again, in some hazelnut genotypes grown in the same ecology, leaf length, leaf width and petiole length was determined between 10.26–12.80 cm, 9.23–12.30 cm, and 1.60–2.91 cm (Semiz 2016), respectively. The findings regarding the leaf characteristics of the genotype investigated were among the reference values reported by the researchers.

The heritability of the number of nuts per cluster is high (0.70) (Thompson et al. 1996). The new hazelnut genotype had 2.33 nuts per cluster, with the most of them double or triple clusters (Table 1). In related

research, the number of nuts per cluster is 1.82–3.72 in local and standard hazelnut cultivars grown in Çarşamba district (Beyhan and Demir 2001), 1.5–3.4 in Çakıldak hazelnut clones selected from Gürgentepe (Ordu) district (İslam and Çayan 2019), 1.12–5.35 in the hazelnut population of Taşkesti (Mudurnu/Bolu) region (Güler and Balta 2020) and 1.90–2.72 in Çakıldak clones grown in the Fatsa (Ordu) region (Karakaya 2021). It was also determined between 2.0 (Yassıbadem) and 4.5 (Çetiner) in the standard Turkish hazelnut cultivars (Köksal 2018; Bektaş and Çil 2023). The number of fruits per cluster of the genotype examined was consistent with the reference values reported by researchers. In addition, the hazelnut genotype examined was similar to the Turkish hazelnut cultivars Çakıldak, Foşa, İncekara and Yuvarlakbadem in terms of nuts per cluster and characteristics.

Nut traits

Nut weight, kernel weight, kernel ratio, and shell thickness are important quality characteristics in the hazelnut. High kernel ratio and thin shell are desirable traits in hazelnut breeding. Nut weight, kernel weight, kernel ratio and shell thickness of new hazelnut genotype were determined as 2.38 g, 1.35 g, 56.81% and 0.96 mm (Fig. 1; Table S1), respectively. In related research, nut weight, kernel weight, kernel ratio and shell thickness were 1.42–3.60 g, 0.78–1.43 g, 39.83–59.50% and 0.67–1.16 mm in local and standard hazelnut cultivars grown in Çarşamba district (Beyhan and Demir 2001); 1.27–2.47 g, 0.79–1.99 g, 42.89–61.76% and 0.74–1.29 mm in some hazelnut genotypes grown in the same ecology (Semiz 2016); 1.50–2.17 g, 0.82–1.20 g, 52.9–59.6% and 0.66–0.91 mm in promising Çakıldak hazelnut clones selected in Gürgentepe district (İslam and Çayan 2019); 1.63–2.40 g, 0.90–1.18 g, 44.91–56.27% and 1.12–1.52 mm in wild hazelnut genotypes growing in the Tirebolu (Giresun) region (Karadeniz et al. 2020); 1.68–2.92 g, 0.92–1.44 g, 49.3–61.7% and 0.77–1.38 mm in the hazelnut genetic resources of the Black Sea region (Karakaya et al. 2023b), respectively. When evaluated in general, the nut weight, kernel weight, kernel ratio and shell thickness values of the genotype investigated were among the reference values reported by the researchers. Furthermore, the genotype tested outperformed many of the individuals investigated by the researchers in terms of the thin shell and high kernel ratio traits desired in hazelnut breeding.

In the investigated hazelnut genotype, nut width, thickness and length were determined as 18.12 mm, 16.56 mm and 20.55 mm. Kernel width, thickness and length were measured as 13.88 mm, 12.98 mm and 16.15 mm. Nut and kernel size were determined as 18.32 mm and 14.77 mm (Fig. 1; Table S1), respectively. In the individuals investigated in the

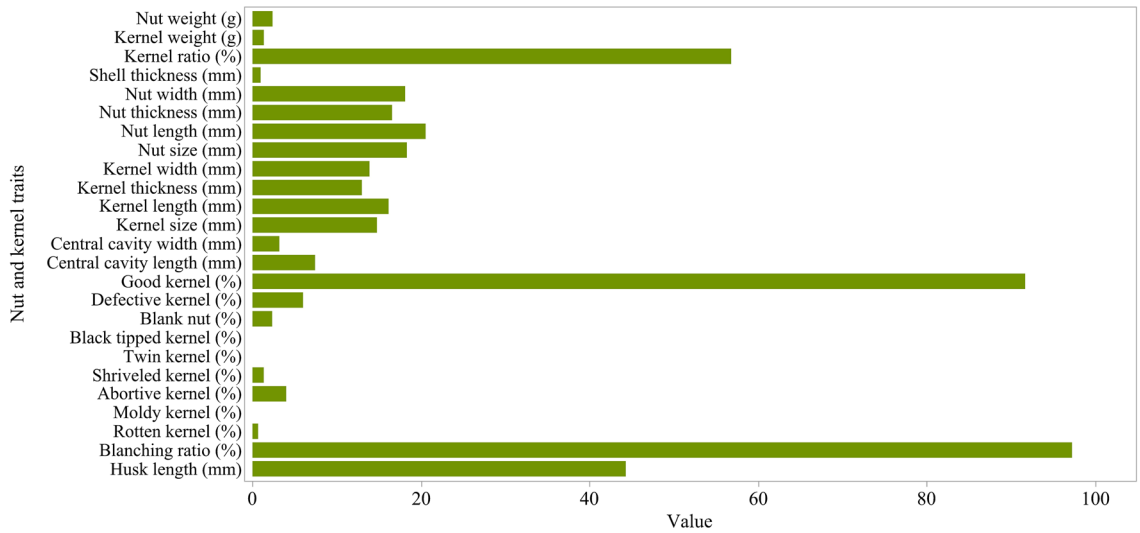

Fig. 1 Nut and kernel traits of the new hazelnut genotype

selection breeding research conducted on hazelnut genetic resources, the nut and kernel size were 16.18–22.16 mm and 12.05–16.07 mm in Çarşamba district (Beyhan and Demir 2001); 15.08–18.62 mm and 11.89–15.86 mm in the same ecology (Semiz 2016); 15.80–18.54 mm and 11.95–14.14 mm in Çakıldak hazelnut clones grown in Ulubey, Kabadüz and Gölköy districts (Bilgen et al. 2017), 16.64–17.29 mm and 12.52–13.60 mm in the Tirebolu region (Karadeniz et al. 2020); 16.2–18.9 mm and 12.4–14.5 mm in different locations of the Black Sea region (Karakaya et al. 2023b), respectively. The findings obtained in terms of nut and kernel size were consistent with the findings of the researchers. Furthermore, the investigated hazelnut genotype is classified as 'extra' (13–15 mm) by the TSE kernel size classification system.

Small central cavity is a desired trait in hazelnut breeding (İşbakan and Bostan 2020). The central cavity width was measured as 3.19 mm and the central cavity length was 7.43 mm (Fig. 1; Table S1). In related research, the central cavity width is 1.8–3.6 mm in the promising Çakıldak hazelnut clones selected in the Gürgentepe district (İslam and Çayan 2019), 0.53–3.28 mm in the wild hazelnut genotypes grown in the Tirebolu region (Karadeniz et al. 2020), and 1.47–3.45 mm in the Çakıldak hazelnut clones grown in the Fatsa region (Karakaya 2021). The central cavity values of the new hazelnut genotype were among the reference values reported by the researchers.

Genetic structure, ecological conditions, technical and cultural practices, lack of pollination and fertilization, technical-cultural practices, diseases, and pests all affect the ratio of good and defective nuts in hazelnuts (Serdar and Demir 2005; Beyhan and Marangoz 2007; Bostan 2019; Balta et al. 2021). High good kernel rate and low defective kernel rate are desirable traits in hazelnut breeding (Mehlenbacher 2018). In the hazelnut genotype investigated, the good kernel rate was 91.7%, the defective kernel rate was 6%, the blank nut rate was 2.3%, the shriveled kernel rate was 1.3%, the abortive kernel rate was 4.0% and the rotten kernel rate was 0.7%. Black-tipped, twin, and moldy kernel were not observed (Fig. 1; Table S1). The fibrousness status was determined as 'medium' (Table 2). Beyhan and Demir (2001) were reported from 78 to 95% for good kernel, 1 to 15% for blank nut, 1 to 8.5% for shriveled kernel, 0.5 to 3.5% for rotten kernel in local and standard hazelnut cultivars grown in Çarşamba district (Beyhan and Demir 2001). In different hazelnut cultivars grown under the same ecological conditions, Semiz (2016) was noted from 97.5 to 100% for good kernel, 0 to 1.8% for blank nut, 0.9 to 1.0% for shriveled kernel, 0 to 2.0% for twin kernel, 0% for rotten kernel in different hazelnut cultivars. Karadeniz et al. (2020) were reported as 100% for good kernel, 0% for blank nut, 0% for shriveled kernel, 0 to 20.0% for twin kernel in wild hazelnuts grown in the Tirebolu region. İslam and Çayan (2019) were noted from 70.9–96.0% for good kernel, 4.3–22.2% for defective kernel in the promising Çakıldak hazelnut clones selected in Gürgentepe district. The good and defective kernel ratios of new hazelnut genotype were consistent with the findings of the researchers. Furthermore, the new hazelnut genotype gave better results than many of the clones and genotypes studied by researchers in terms of good and defective kernel ratio.

Table 2 Kernel traits of the new hazelnut genotype

Kernel traits	Values
Fibrousness	Medium
Kernel taste and aroma	Medium

The husk length was measured as 44.3 mm (Fig. 1; Table S1). It was reported from 31.16 to 51.2 mm in local and standard hazelnut cultivars grown in Çarşamba district (Beyhan and Demir 2001), 25.0–52.0 mm in Çakıldak hazelnut clones grown in Gürgentepe district (İslam and Çayan 2019) and 26.57–42.0 mm in Çakıldak clones grown in Fatsa region (Karakaya 2021). The husk length values of the investigated hazelnut genotype were among the reference values reported by the researchers, and it was determined that the husk length was long when compared to the researchers' results.

The heritability of the blanching rate is 0.64 (Yao and Mehlenbacher 2000), and a high blanching rate is a desirable trait for both hazelnut breeding and the industry (Mehlenbacher 2018). The blanching rate in the new hazelnut genotype was found to be 97.26% (Fig. 1; Table S1). Different researchers reported the blanching rate as 34.5–97.5% in some hazelnut cultivars grown under Çarşamba ecological conditions (Beyhan and Demir 2001), 10–90% in some hazelnut cultivars grown in Slovenia (Solar and Stampar 2011)

and 88.24–95.37% in different hazelnut cultivars grown in Sakarya province (Bostan 2023). The new hazelnut genotype gave remarkable results in terms of blanching rate.

The taste and aroma of the fruit are important factors for consumer appeal. The kernel taste and aroma of the new hazelnut genotype investigated were found to be moderately delicious (Table 2). The kernel taste of hazelnut genetic resources from the Eastern Anatolia Region was described as highly and moderately delicious (Balta et al. 2006). Similar findings are reported in the Çakıldak, Palaz, and Tombul cultivars (Koç-Güler et al. 2017; Sali et al. 2022). When compared to the researchers' findings, it is clear that the kernel taste of the investigated genotype is acceptable.

Ash, protein and oil ratio

In the hazelnut genotype investigated, the ash, protein and oil rate were determined as 2.24%, 13.5% and 55.80% (Fig. 2; Table S2), respectively. In related research, ash, protein and oil ratios ranged from 0.79 to 3.21%, 10.7 to 19.2% and 57.5 to 74.1% in hazelnut genetic resources of the Eastern Anatolia region according to Balta et al. (2006), 2.62 to 4.13%, 14.64 to 24.61% and 43.22 to 68.44% in some hazelnut cultivars grown in Iran as indicated by Hosseinpour et al. (2013), 1.89 to 2.70%, 13.63 to 22.53% and 57.25 to 63.25% in local hazelnut cultivars grown in Turkey by Çetin et al. (2020), 2.14 to 2.35%, 11.80 to 13.03% and 64.54 to 64.89% in Spanish hazelnut genetic resources as determined by Negrillo et al. (2021). Ash, protein and oil content values of the genotype investigated were among the values reported by the researchers.

Fatty acid composition

In the new hazelnut genotype, the means of the palmitic acid was 5.02%, palmitoleic acid was 0.15%, linoleic acid was 10.68%, oleic acid was 81.43%, stearic acid was 2.22%, arachidic acid was 0.11%, heptadeconoic acid was 0.06%, eicosanoic acid was 0.14%, saturated fatty acids were 7.41%, monounsaturated fatty acids were 81.72%, polyunsaturated fatty acids were 10.68% and O/L ratio was 7.62% (Fig. 2; Table S2). In previous research, Balta et al. (2006) reported that in the genetic resources of hazelnuts grown in the Eastern Anatolia Region of Türkiye had a range of 5.06–8.85% for palmitic acid, 10.46–15.61% for linoleic acid, 73.48–81.57% for oleic acid and 1.74–3.18% for stearic acid. Granata et al. (2017) recorded a range of 4.8–10.9% for linoleic acid and 81–84% for oleic acid in wild hazelnuts grown in Italy. Krol et al. (2021) determined that in some hazelnut cultivars grown in Poland had a range of 4.66–6.01% for palmitic acid, 10.29–12.67% for linoleic acid, 78.61–82.01% for oleic acid,

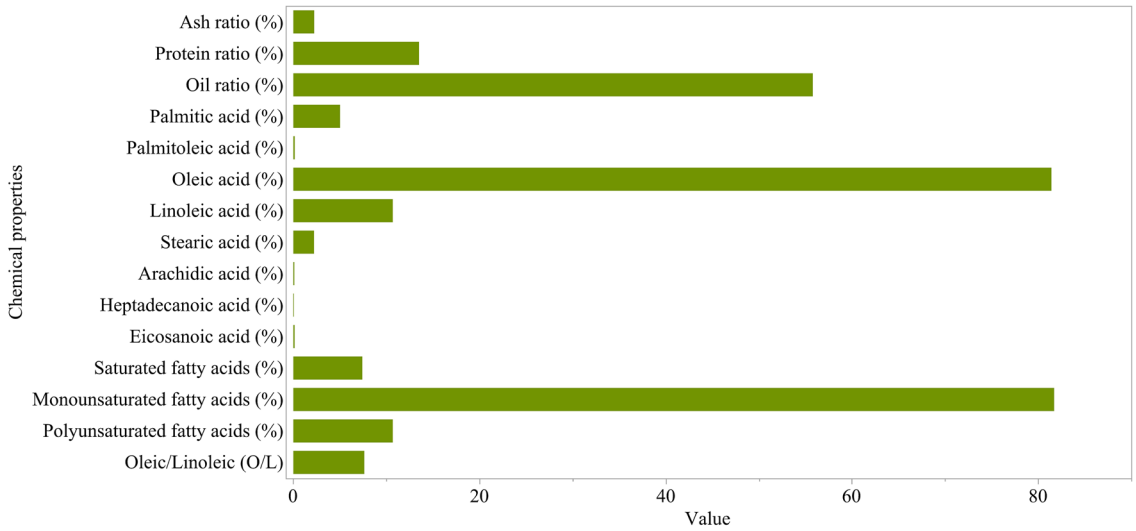

Fig. 2 Ash, protein, oil and fatty acid composition of the new hazelnut genotype

1.65–2.27% for stearic acid, 6.69–8.18% for saturated fatty acids, 78.96–82.22% for monounsaturated fatty acids, and 10.43–12.82% for polyunsaturated fatty acids. Çetin et al. (2020) recorded a range of 5.01–7.07% for palmitic acid, 0.12–0.28% for palmitoleic, 6.04–12.17% for linoleic acid, 80.36–85.11% for oleic acid and 1.76–6.33% for stearic acid in some local and standard hazelnuts grown in Turkey cultivars. Yaman et al. (2023) reported that in hazelnut genetic resources from Eastern Black Sea Region had a range of 4.68–5.75% for palmitic acid, 0.04–0.06% for palmitoleic acid, 4.35–18.06% for linoleic acid, 71.24–87.16% for oleic acid, 0.76–3.26% for stearic acid, 5.96–8.64% for saturated fatty acids, 70.52–87.21% for monounsaturated fatty acids, and 4.43–21.08% for polyunsaturated fatty acids. The findings obtained in terms of fatty acids were among the reference values reported by the researchers.

In the food industry, the product's low linoleic acid content indicates a high storage capacity (Bonvehi and Coll 1997). Furthermore, a high oleic/linoleic acid ratio is an important criterion for increasing hazelnut oxidation resistance during processing, storage, and transportation, as well as evaluating hazelnut kernel quality (Rezaei et al. 2014; Krol et al. 2021; Karaosmanoglu 2022). Compared to the researchers' findings, the hazelnut genotype investigated had lower linoleic acid content than many other genotypes and cultivars, as well as significant oleic acid content results. In this regard, the genotype investigated can be used as genetic material in breeding programs to develop cultivars with high oleic acid and low linoleic acid content.

Molecular characterization

The genetic relationship between the hazelnut genotype investigated and the standard Çakıldak, Palaz, Tombul hazelnut cultivars (the Ordu region, where the research area is located, constitutes 85–90% of the hazelnut population), as well asclones of the Çakıldak cultivar (the only dominant hazelnut cultivar in the region where the research was conducted) randomly selected from different orchards close to the location of the genotype was determined. 9 ISSR primers were used to determine the genetic relationship among the investigated individuals. 72 bands were obtained, 56 of which were polymorphic. The band length ranged from 100 to 700 bp, with 3–17 bands, 2–17 polymorphic bands, and 45.5–100% polymorphism rate (Table 3).

In research conducted using ISSR primers, the number of bands, the number of polymorphic bands and the polymorphism rate were 4–14, 3–14 and 60–100% in Portuguese hazelnut genetic resources (Martins et al. 2009), 4–9, mean 3.97 and 28.57–100% in Turkish hazelnut cultivars (Kafkas et al. 2009), 9–21, 8–21 and 71.43–100% in hazelnut cultivars and genotypes grown in Iran (Mohammadzedeh et al. 2014), 4–24, 4–24 ve % 66.7–100 in hazelnut genetic resources of the Eastern Black Sea Region (Karakaya et al. 2023b). The results in terms

Table 3 Base length (bp), total number of bands, total number of polymorphic bands ve polymorphism ratio (%)

Primers	Base length (bp)	Total number of bands	Total number of polymorphic bands	Polymorphism ratio (%)
$DBDA(CA)_7$	100–500	12	12	100.0
$(AG)_7YC$	280–450	3	2	66.7
$(AGC)_6G$	150–700	17	17	100.0
$VHV(GTG)_7$	120–220	5	3	60.0
$(CT)_8TG$	120–200	3	2	66.7
$(GT)_6GG$	200–450	5	3	60.0
$HVH(TCC)_7$	230–440	5	4	80.0
$(AG)_8$ T	120–500	11	8	72.7
$(GA)_8YG$	180–490	11	5	45.5
Total	–	72	56	–
Mean	100–700	8	6	72.4

of band numbers were consistent with the researchers' reported reference values.

According to the ISSR analysis results, two main group were formed in the dendogram obtained. The first group (A) consisted of only the new hazelnut genotype (G-1). The second group (B) was divided into two sub-group. First sub-group (B-1) included standard Çakıldak and Palaz cultivars, as well as Çakıldak clones (Ç-1, Ç-2, Ç-3 and Ç-4). The second sub-group (B-2) consisted of standard Tombul cultivar. First sub-group divided into two clusters. The first cluster included only standard Palaz cultivar. The second cluster included both the standard Çakıldak cultivar and its clones. (Fig. 3).

The similarity ratio ranged from 0.58 to 0.98 (Fig. 1). In research conducted using ISSR primers, the similarity rate was 0.73–0.96 in Turkish hazelnut cultivars (Kafkas et al. 2009), 0.50–0.98 in hazelnut genetic resources in the Asturias (Spain) region (Ferreira et al. 2010), 0.1–0.74 in hazelnut cultiv ars and genotypes grown in Iran (Mohammadzedeh et al. 2014), and 0.46–0.88 in hazelnut genetic resources of the Eastern Black Sea Region (Karakaya et al. 2023b). The similarity rate between the hazelnut genotype and cultivars investigated was consistent with the researchers' findings.

The new hazelnut genotype was genetically distinct from Çakıldak clones and standard hazelnut cultivars. It differed genetically 38.1% from the Ç-1 Çakıldak

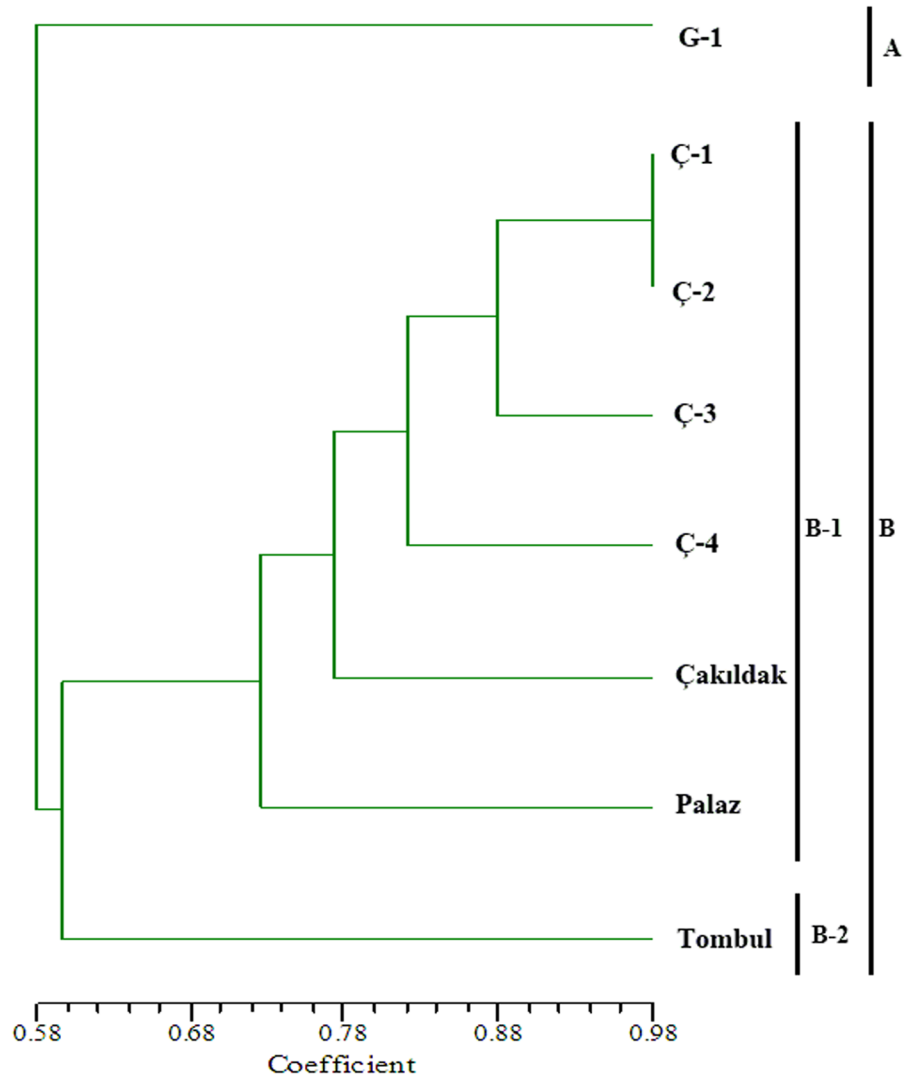

Fig. 3 Dendrogram of the new hazelnut genotype and other investigated hazelnut genetic resources (cultivars and clones) resulting from the unweighted paig-roup method of arithmetic avarage cluster analysis based on Dice similarity coeefficient obtained from 72 inter simple sequence repeat (ISSR) markers

Table 4 Dice similarity coefficient to genotype, cultivars and clones investigated

Clones/cultivars	G-1	Ç-1	Ç-2	Ç-3	Ç-4	Çakıldak	Tombul	Palaz
G-1	1							
Ç-1	0.6190	1						
Ç-2	0.6122	0.9803	1					
Ç-3	0.6875	0.8679	0.8928	1				
Ç-4	0.5833	0.8000	0.8085	0.8571	1			
Çakıldak	0.5769	0.8172	0.8041	0.8275	0.6500	1		
Tombul	0.5294	0.6666	0.6500	0.5714	0.4166	0.6976	1	
Palaz	0.4782	0.7272	0.7766	0.7500	0.6382	0.7021	0.5945	1

clone in the same orchard, 37.2% from the Ç-2, Ç-3, and Ç-4 Çakıldak clones randomly selected from different orchards in the same region, 42.3% from the Çakıldak cultivar, 52.2% from the Palaz cultivar, and 47.1% from the Tombul cultivar (Table 4). In molecular research to genetically identify the clones of Turkish hazelnut cultivars, genetic differences were reported among clones of the same cultivar. Furthermore, many researchers stated that Turkish hazelnut cultivars are not a single clone, but a group of clones with similar characteristics (Balta et al. 1997). In this regard, this genotype can be an accidental seedling grown from seeds.

Conclusion

In this study, a new hazelnut genotype was thoroughly investigated at both morphological and molecular levels. The new hazelnut genotype exhibited noteworthy results in terms of important traits for hazelnut breeding, including late leafing, round shape, high kernel percentage, thin shell, large nut, and high blanching rate. Molecular analyses revealed that this genotype is genetically distinct from randomly selected Çakıldak clones from the same orchard and from predominant cultivars in the Ordu province, namely Çakıldak, Palaz, and Tombul. Therefore, this genotype stands out for important traits for hazelnut breeding (late leafing, thin shell, rounded shape, high kernel percentage, large fruit size, and high blanching rate), as well as genetically distinct.. It has the potential to be registered as a new cultivar. Also, the investigated genotype could be considered valuable genetic material for hazelnut breeding programs, offering the development of new cultivars as well as the improvement of existing ones.

Author contributions SZB: Investigation, methodology, conceptualization, writing—review editing, validation, visualization. OK: Investigation, methodology, conceptualization, formal analysis, writing—original draft, visualization.

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Declarations

Conflict of interest The authors declare no conflict interest.

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