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# Revealing genetic diversity and association mapping among Palaz hazelnut (Corylus avellana L.) clones in the East Black Sea region by morphological and ISSR/SRAP markers

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## Abstract

This study aimed to determine the yield, nut traits, and genetic diversity of promising and phenotypically distinct Palaz hazelnut clones, as well as the markers associated with these traits. Principal component analysis (PCA) showed that the yield and nut traits could effectively explain the variability among the Palaz clones, which exhibited wide variation in terms of morphological traits. Broad-sense heritability was determined to range from 0.18 (rotten kernel) to 0.99 (trunk cross-sectional area). Key agronomic traits, including husk length ($h^2$ = 0.87), yield efficiency ($h^2$ = 0.86), nut yield per plant ($h^2$ = 0.83) and nuts per cluster ($h^2$ = 0.73), revealed strong genetic control and high selection efficiency, independent of environmental factors. Molecular characterization using ISSR and SRAP markers identified 95 loci, 89 of which were polymorphic. Principal coordinate analysis (PCoA) effectively distinguished between the clones, especifically P-3 and P-20. These clones were genetically different from the standard Palaz cultivar and the other clones. This indicates that they probably have been originated from a seedling. Of these, P3 yielded notable results in terms of yield and nut traits. It can be used to develop new cultivars in hazelnut breeding programs. In the association mapping, 67 loci were related to investigated traits. Important traits for hazelnut breeding programs were associated with pleiotropic loci [(AG)7YC-900], which could help breeders to select superior accessions regarding yield and nut traits. In conclusion, the identified loci could be useful for breeders to select individuals with the desired traits in hazelnut breeding programs. The obtained data will be a valuable resource for future breeding efforts and contribute to the conservation and sustainability of hazelnut genetic resources.

## Introduction

The deciduous hazelnuts, *Corylus* ssp., are a diploid species (2n = 22) belonging to the Betulaceae family, monoecious, wind pollinated, and self-incompatible (Erdogan and Mehlenbacher 2000). It is grown since ancient times in most regions on the world including North Anatolia in Türkiye, Mediterranean coast of Europe, North America. There are between 9 and 25 species of Corylus found worldwide. Of these, *C. avellana* is the most commercially valuable. It is a small and multi-stemmed bush species, and propagates vegetatively with suckers. Although it grows naturally in a wide range of areas, it is commercially cultivated in regions with a Mediterranean climate (Ercisli 2004; Molnar 2011; Botta et al. 2019).

Türkiye, which has a climate suitable for hazelnut cultivation, is a leader in hazelnut production and trade. In Türkiye, 717,000 tons of hazelnuts are produced across an area of 750,000 hectares. Italy, USA, Azerbaijan, Chile and Georgia are other important hazelnut producer countries (FAO 2026). The Black Sea Region of Türkiye offers the most ideal ecological conditions for hazelnut cultivation. The region's major varieties, which have been widely cultivated for many years, include Tombul, Palaz, Çakıldak, Mincane and Foşa (Karadeniz et al. 2009; Karakaya et al. 2023). Among these, Palaz has a large nut size, a high kernel ratio, a round shape and a high blanching, and is suitable for industrial use.

Türkiye is one of the most important centers of origin for hazelnuts. The sporophytic incompatibility of hazelnuts has led to significant genetic and phenotypic diversity within population (Erdogan et al. 2005). Interestingly, genetic differences even exist among clones of the same variety. This has been demonstrated by many researchers (Balta et al. 1997; İslam 2003; Petriccione et al. 2010; Bostan and Karakaya 2024). As the long juvenile period of the hazelnut increases the time and cost required for long-term hybrid-based breeding programs, selecting promising individuals from the existing population through selection breeding is an important alternative method. Using various methods to identify selected individuals is important for enhancing the effectiveness of breeding programs (Petriccione et al. 2010; Karakaya et al. 2023; Uzun et al. 2023).

Phenotypic and molecular characterization methods are widely used for the identification and evaluation of genetic resources in hazelnuts (Boccacci et al. 2013; Demir 2014; Yılmaz et al. 2024). The best strategy for obtaining reliable results regarding the use and conservation of genetic resources is to appropriately integrate these methods. Phenotypic characterization based on morphological traits serves as a preliminary step in assessing genetic diversity (Kaur et al. 2022). However, as these traits are influenced by environmental factors, molecular methods are necessary to determine genetic differences among selected individuals. Assessing genetic diversity is highly beneficial for understanding the structure and constitution of the populations under study (Boccacci et al. 2009; Martins et al. 2015). In hazelnuts, SSR (Simple Sequence Repeats), ISSR (Inter-Simple Sequence Repeats), RAPD (Random Amplified Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphisms), iPBS (Inter-Primer Binding Site), and SNPs (Single Nucleotide Polymorphisms) are molecular characterization methods widely used to identify selected individuals (Kafkas et al. 2009; Mishchenko et al. 2025; Salardi Jost et al. 2025).

Many agronomic traits are influenced by the interaction of multiple genes, as well as environmental factors. This complicates the breeding process when attempting to improve undesirable traits. However, QTL studies can identify the genomic regions that control desired traits in hazelnut breeding. This can facilitate the development of new varieties within breeding programs. Furthermore, marker-assisted selection (MAS), which utilizes molecular markers for desired traits in breeding programs, is considered a promising approach for cultivar development (Beltramo et al. 2016; Ozturk et al. 2017; Salardi Jost et al. 2025).

Protecting and conserving individuals selected under breeding programs enables breeders and growers to develop varieties adapted to climate change and resistant to biotic and abiotic stress factors. Furthermore, the characterization and identification of genetic resources are considered the most important foundational activities for their more effective utilization (Martins et al. 2015; Güler et al. 2024; Arslan et al. 2026). This study aims to determine the yield, nut characteristics, genetic diversity, and markers associated with these traits in promising and phenotypically distinct Palaz hazelnut clones selected from the Fatsa (Ordu) region of the Eastern Black Sea Region, which is rich in hazelnut genetic resources.

## Materials and methods

# Study area and plant materials

The study was conducted on clones of the Palaz hazelnut cultivar grown in the Fatsa (Ordu) district of the Eastern Black Sea Region of Türkiye, in four consecutive years, 2015–2018. A total of 145 clones were evaluated in accordance with hazelnut breeding objectives. Detailed evaluations were performed

on the 20 selected clones and the standard Palaz cultivar. Promising clones were selected based on yield, kernel ratio, shell thickness, nut size, kernel size, internal cavity, and good kernel traits. Ten out of twenty clones were promising in terms of yield and nut traits (P-1, P-3, P-4, P-5, P-10, P-11, P-12, P-13, P-14 and P-20), while the remaining ten were phenotypically different clones (P-2, P-6, P-7, P-8, P-9, P-15, P-16, P-17, P-18 and P-19).

### Climate and soil characteristics of the study area

Long-term climatic records from the research area, covering the period from 1985 to 2018, indicate that the mean annual air temperature is 14.5°C. August has the highest average monthly temperature (23.3°C), while January is the coldest month with an average temperature of 7.3°C. The lowest minimum temperature observed during the study period was − 7.5°C in February, while the highest minimum temperature was 13.7°C in August. In terms of maximum temperatures, the lowest annual value was 23.1°C in January and the highest was 34.8°C in April. Precipitation varied considerably from month to month, with the lowest rainfall recorded in June (52.7 mm) and the highest in October (162.3 mm). The mean annual precipitation is 1131.2 mm, and the average relative humidity is around 70% (Fig. 1, Fig. 2, Fig. 3, Fig. 4) (TSMS 2026).

The soil in the study area consists mainly of clay and clay loam. It is rich in potassium, but low in nitrogen and phosphorus. The soil contains moderate levels of organic matter and lime, and its pH level ranges from 5.5 to 7.5 (Ayaz 2019).

### Morphological traits

In clones, the morphological traits of vigor, growth habit, suckering, and stem density were evaluated according to UPOV descriptors (UPOV 1979). Vigor was classified as very weak, weak, medium, strong and very strong; habitus as very upright, upright, semi-upright, spreading and very spreading; suckering as absent, very weak, weak, medium, strong and very strong; and stem density as sparse, medium and dense.

### Nut traits

Nut traits were examined in 50 nuts in each clone. The nut and kernel weight (g) were determined using a digital scale (Radwag, Poland) accurate to 0.01 g. The husk length (mm), internal cavity (mm), shell thickness, and nut and kernel dimensions (mm) (width, thickness, and length) were measured using a digital caliper (Mitutoyo, Japan) accurate to 0.01 mm. The kernel ratio was calculated as the ratio of kernel weight to nut weight, and expressed as a percentage. The nut and kernel shape indexes were calculated by dividing the length by the average of the width and thickness. The good kernels, blank nuts, and defective kernels were determined in the clones investigated. The good kernel ratio was determined by dividing the number of defect-free kernels that completely filled the shell by the total number of nuts examined. The blank nut ratio was calculated as the ratio of nuts with no seed formation to the total number of examined nuts. The defective kernel ratio was obtained by dividing the number of kernels with defects—such as abortive, shriveled, twin, moldy, and rotten kernels, excluding good kernels, and blank nuts—by the total number of examined nuts. These traits were examined in 100 nuts in each clone (Güler and Balta 2020; Bak et al. 2024).

### Yield traits

The traits of plant yield (g), yield efficiency (g $cm^{-2}$) and the number of nuts per cluster were examined for each clone. Plant yield was calculated by multiplying the total number of clusters on the plant by the number of nuts per cluster by the nut weight. The number of nuts per cluster was determined by counting all the clusters on each plant. Yield efficiency was calculated by dividing the plant yield by the trunk cross-sectional area. The trunk cross-sectional area (TCSA) was calculated using the formula $\pi r^2$, based on the average of trunk diameter measurements taken from two different directions at 20 cm above the ground using a digital caliper (Mitutoyo, CD-15CP, Japan).

## Molecular characterization

DNA was extracted from hazelnut leaf tissue using a modified Doyle and Doyle (1990) protocol. The molecular characterization of the hazelnut clones was performed using twelve ISSR primers [$(VHVG(TG)_7$, $(CA)_6AC$, $(AG)_7YC$, $DBD(ACA)_7$, $BDB(CA)_7C$, $(TCC)_5RY$, $HVH(TCC)_7$, $(CT)_8TG$, $(AG)_8T$, $(GAA)_6$, $(GA)_8YG$ and $(CAC)_3GC$] (Kafkas et al. 2009; Martins et al. 2009, Semiz 2016) and three SRAP primer combinations (em13–me11, em13–me7, and em8–me10). PCR amplification was carried out in a total volume of 15 µL, containing: 2 µL of DNA, 1.5 µL of 10× PCR buffer, 0.2 µL of Taq DNA polymerase, 1 µL of primer (for SRAP: 1 µL each of forward and reverse primers), 1 µL of a dNTP mixture, 1.5 µL of $MgCl_2$, and nuclease-free water to make up the final volume. For ISSR analysis, the amplification conditions included an initial denaturation step 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, and extension at 72°C for 2 min, concluding with a final extension step at 72°C for 5 min. For SRAP amplification, an initial denaturation step was performed at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 38°C for 45 s and extension at 72°C for 2 min. This was followed by a final extension step at 72°C for 10 min (Uzun et al. 2009). The PCR products were separated by electrophoresis in a 2% agarose gel, and the gel was then examined under UV light using an imaging system.

## Statistical analysis

The data obtained were analyzed using the JMP 16.0 (trial version) statistical software package. The minimum (Min.), maximum (Max.), mean, standard deviation (SD), and coefficient of variation (CV, %) values of the traits were determined. The coefficient of variation was calculated as the ratio of the standard deviation to the arithmetic mean. The LSD multiple comparison test was used to determine differences among clones.

Broad-sense heritability ($h^2$) for all traits was estimated using the REML (Restricted Maximum Likelihood) method according to the approach of Holland (2006). For each trait, broad-sense heritability was calculated as the ratio of genetic variance to phenotypic variance.

To determine the most important traits influencing the grouping of the clones, principal component analysis (PCA) was performed using JMP 16.0 software. The first and second principal components (PC1 and PC2) were used to construct a two-dimensional biplot, illustrating the distribution of clones and quantitative variables. To classify the clones and variables, a heatmap analysis based on Ward's method and Euclidean distance coefficients was utilized. Additionally, Pearson correlation coefficients (r) were calculated to determine the relationships among the examined traits.

For genetic analysis, each band observed in the gel was scored as either present (1) or absent (0). The size of the ISSR and SRAP bands was determined using a GENESTATM 100 bp DNA ladder. The performance of the ISSR and SRAP markers was evaluated based on total band count, number of polymorphic bands and polymorphism rate. Genetic similarity among clones was calculated using the Jaccard similarity coefficient. Principal Coordinate Analysis (PCoA) was performed using the PAST v4.03 software to visualize the relationships between genetic resources (Hammer et al. 2001). Marker-trait relationships were analyzed using a generalized linear model (GLM) implemented in TASSEL 5 software (Bradbury et al. 2007).

## Results and discussion

**Morphological traits**

Breeding efforts in hazelnuts have focused on varieties exhibiting moderate vigor (Thompson et al. 1996; Botta et al. 2019). Most of the investigated clones (55%) exhibit moderate vigor. Additionally, clones with weak, vigorous, and very vigorous vigor are also present (Table 1). Köksal (2018) reported that the standard Palaz variety has moderate vigor. Different researchers have reported weak, medium, and vigorous vigor in Palaz hazelnut clones, noting that the vast majority possess moderate vigor (İslam and Bostan 1999; İslam 2000).

An upright growth habit is a desirable breeding criterion for hazelnuts (Botta et al. 2019). The most of the investigated clones (65%) had a spreading growth habit. The remaining clones exhibited upright or semi-upright growth habits (Table 1). A spreading growth habit was reported for the Palaz variety (Köksal 2018). Relevant studies have reported that the majority of Palaz clones examined had a spreading growth habit, while a small number had an upright growth habit (İslam and Bostan 1999; İslam, 2000). The findings regarding vigor and habit were consistent with those of the researchers.

Hazelnut is a nut species with a high tendency to produce suckers. The development of varieties that do not produce suckers is one of the breeding objectives for hazelnuts and is very important for cultivation (Botta et al. 2019). Of the clones investigated, only P-1 was found to have a weak tendency to produce suckers. Most of the other clones exhibited a moderate to strong tendency to produce suckers (Table 1). In the Palaz clones studied in Ordu Province, a weak to moderate tendency to produce suckers was reported (İslam 2000). Similarly, a weak to moderate tendency to produce suckers was reported in the most of Tonda Gentile delle Langhe clones. Some clones were also found not to produce any suckers at all (Valentini et al. 2014). The Palaz clones investigated exhibited varying levels of suckering tendency, which was moderate to high in most clones. It is considered that clonal differences in the tendency to produce suckers are due to genetic structure.

**Table 1.** Frequency distribution for morphological characteristics of investigated Palaz hazelnut clones

| Morphological characteristics | Frequency (%) | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 3 | 5 | 7 | 9 | 11 |
| Vigor | Very weak (0) | Weak (10) | Medium (55) | Vigorous (25) | Very vigorous (10) | - |
| Habitus | Very upright (0) | Upright (5) | Semi-upright (30) | Spreading (65) | Very spreading (0) | - |
| Suckering | Absent (0) | Very weak (0) | Weak (5) | Medium (60) | Strong (30) | Very strong (5) |
| Stem density | Sparse (10) | Medium (30) | Dense (60) | - | - | - |

Significant differences in husk length were observed among the clones ($p<0.05$), ranging from 26.77 to 36.88 mm (Table 2). Different researchers reported husk length in Palaz clones ranging from 39.4 to 50.53 mm (İslam 2000; Demir and Beyhan 2000). Compared to the clones investigated by these researchers, the clones in this study had shorter husks. Husk length is a trait with a high heritability coefficient (0.82) (Mehlenbacher 2018). In this context, the observed differences are due to genetic variation.

**Nut traits**

Nut weight, kernel weight, kernel ratio, shell thickness, and internal cavity are important quality traits in hazelnuts. The heritability coefficients for nut weight, kernel weight, kernel ratio, and shell thickness are 0.63, 0.67, 0.87, and 0.77, respectively (Yao and Mehlenbacher 2000; Mehlenbacher 2018). A high kernel ratio, thin shell, and small or non-cavitated kernel are desirable traits in hazelnut breeding and in the hazelnut industry. Kernel ratio is influenced by nut weight, kernel weight, shell thickness, and internal cavity (Balta et al. 1997; İslam and Özgüven 2003; Karakaya et al. 2022). Significant differences were observed in nut weight, kernel weight, kernel ratio, shell thickness and internal cavity among the investigated clones ($p<0.05$). Nut weight, kernel weight, kernel ratio, shell thickness, and internal cavity ranged from 1.73–2.28 g, 0.92–1.19 g, 51.59–56.13%, 0.85–1.31 mm, and 2.70–4.14 mm, respectively (Table 2).

Different researchers reported the following values for Palaz clones: 1.79–2.50 g nut weight, 0.98–1.44 g kernel weight, 50.55–57.50% kernel ratio, 0.85–1.10 mm shell thickness, and 2.72–3.96 mm internal cavity (Balta et al. 1997; İslam 2000; Demir and Beyhan 2000; Bostan and Günay 2009). Among the

clones investigated, P-10 yielded notable results in terms of nut weight and kernel weight (2.28 g and 1.19 g). Most of the clones (55%) had a kernel ratio of 54% or higher, with the P-14 clone (56.13%) was considered promising in this regard. The P-12 clone was considered promising due to its thinner shell compared to many of the clones investigated by the researchers. These clones should be a proper parent for hazelnut breeding programs that aim to obtain high quality. Also, the findings regarding the investigated characteristics were generally consistent with those of the researchers. Some observed clonal differences may be attributed to genetic structure (Balta et al. 1997; Balık and Beyhan 2014), ecological conditions, nutritional status (İslam 2003; Turan and Beyhan 2009), technical and cultural practices (Serdar et al. 2005; Balta et al. 2021), and the fruit load on the plant (Milosevic and Milosevic 2012; Karakaya et al. 2023).

Small and medium-sized hazelnuts are important for the confectionery industry, whereas large hazelnuts are better suited to marketing in their shells (Solar and Stampar 2011). Significant clonal differences in nut and kernel size were observed ($p<0.05$). Nut size ranged from 16.15 to 17.89 mm. Kernel size was found to be between 12.22 and 13.52 mm (Table 2). Other researchers have reported nut sizes ranging from 16.06 to 19.22 mm and kernel sizes ranging from 11.36 to 14.68 mm in Palaz clones (İslam 2000; Bostan and Günay 2009; Balık and Beyhan 2014). The results obtained were generally similar to those reported by other researchers. Additionally, according to TSI's internal hazelnut classification, kernels sizes of 13–15 mm fall into the 'extra' group (TSI 2026). In terms of kernel size, 50% of the clones fall into the 'extra' group. Clones that stand out in terms of nut and kernel size, especially P-1, P-3 and P-10, can be used in breeding programmes to develop varieties with large nuts.

**Table 2.** Descriptive statistics of investigated Palaz hazelnut clones

| Traits | Abbreviation | Unit | Min. | Max. | Mean | SD | CV | Sig. | LSD (0.05) |
|---|---|---|---|---|---|---|---|---|---|
| Husk length | HL | mm | 26.77 | 36.88 | 31.44 | 2.83 | 0.09 | *** | 3.03 |
| Nuts per cluster | NPC | number | 2.33 | 3.65 | 2.81 | 0.32 | 0.11 | ** | 0.56 |
| Nut weight | NW | g | 1.73 | 2.28 | 1.94 | 0.12 | 0.06 | * | 0.29 |
| Kernel weight | KW | g | 0.92 | 1.19 | 1.05 | 0.06 | 0.06 | * | 0.17 |
| Kernel ratio | KR | % | 51.59 | 56.13 | 54.14 | 1.50 | 0.03 | * | 3.70 |
| Shell thickness | ST | mm | 0.85 | 1.31 | 1.02 | 0.12 | 0.11 | * | 0.31 |
| Internal cavity | IC | mm | 2.70 | 4.14 | 3.34 | 0.39 | 0.12 | * | 1.25 |
| Nut length | NL | mm | 14.36 | 16.98 | 15.75 | 0.53 | 0.03 | ** | 0.95 |
| Nut width | NWt | mm | 17.64 | 19.68 | 18.53 | 0.53 | 0.03 | ** | 0.92 |
| Nut thickness | NT | mm | 15.65 | 17.15 | 16.32 | 0.36 | 0.02 | * | 0.97 |
| Nut size | NS | mm | 16.15 | 17.89 | 16.82 | 0.41 | 0.02 | * | 0.84 |
| Kernel length | KL | mm | 11.23 | 12.56 | 12.01 | 0.38 | 0.03 | * | 0.98 |
| Kernel width | KWt | mm | 13.41 | 15.32 | 14.37 | 0.53 | 0.04 | * | 1.08 |
| Kernel thickness | KT | mm | 12.14 | 13.52 | 12.80 | 0.38 | 0.03 | * | 0.83 |
| Kernel size | KS | mm | 12.22 | 13.52 | 13.02 | 0.35 | 0.03 | * | 0.80 |
| Nut shape index | NSI | - | 0.83 | 0.93 | 0.90 | 0.02 | 0.02 | ** | 0.04 |
| Kernel shape index | KSI | - | 0.82 | 0.92 | 0.89 | 0.03 | 0.03 | * | 0.07 |
| Good kernel | GK | % | 73.12 | 88.99 | 83.62 | 3.94 | 0.05 | * | 8.87 |
| Blank nut | BN | % | 1.25 | 8.72 | 4.40 | 1.85 | 0.42 | * | 6.32 |
| Poor fill | PF | % | 5.67 | 22.28 | 11.97 | 3.75 | 0.31 | ** | 6.35 |
| Shriveled kernel | SK | % | 1.80 | 5.93 | 3.84 | 1.34 | 0.35 | ns | 4.65 |
| Abortive kernel | AK | % | 1.10 | 8.07 | 5.41 | 1.98 | 0.37 | * | 5.98 |
| Rotten kernel | RK | % | 0.0 | 3.70 | 0.86 | 1.31 | 1.52 | * | 3.62 |
| Moldy kernel | MK | % | 0.0 | 0.90 | 0.08 | 0.24 | 3.12 | * | 0.72 |
| Twin kernel | TK | % | 0.0 | 4.51 | 0.89 | 1.27 | 1.43 | * | 2.82 |
| Cracked nut | CN | % | 0.0 | 2.67 | 0.40 | 0.66 | 1.65 | * | 1.65 |
| Black tipped kernel | BTK | % | 0.0 | 2.67 | 0.49 | 0.66 | 1.35 | * | 1.84 |
| Kernel fiber [a] | F | - | 2 | 3 | 2.2 | 0.37 | 0.17 | - | - |

* significant at $p<0.05$, ** significant at $p<0.01$, *** significant at $p<0.001$ and ns: not significant ($p>0.05$).

[a] Fiber on pellicle rated from 1 (none) to 4 (much)

A round shape is a crucial character in hazelnut breeding and is a desired trait in the industry (Ozdemir and Akıncı 2004). Significant variations were observed in the nut and kernel shape index among the Palaz clones ($p<0.05$). The nut shape index ranged from 0.83 to 0.93, while the kernel shape index was found between 0.82 and 0.92 (Table 2). Different researchers have reported a nut shape index of 0.87–1.12 (Balta et al. 1997; Demir and Beyhan 2000) and a kernel shape index of 0.80–0.92 (İslam 2000; Yılmaz 2009) for Palaz clones. In addition, a nut shape index ranging from 0.81 to 1.19 is classified as round shape. In this context, all clones investigated possess a round shape, which is of significance for hazelnut breeding and industry.

A high good kernel percentage is a desirable trait in hazelnut breeding (Mehlenbacher 2018). Significant clonal differences were observed in terms of good kernel percentage and poor fill (shriveled, abortive, rotten, moldy, and twin kernels) ($p<0.05$). The good kernel ranged from 73.12 to 88.99%, while the poor fill was found between 5.67 and 22.28% (Table 2). Good kernel and poor fill were reported from 80.81-99.0% and 0.0-18.70% in Palaz clones selected from many different regions, respectively (Beyhan and Demir 2001; Balık and Beyhan 2014). The findings of good kernel and poor fill are generally consistent with those of previous studies. Some observed differences are due to genetic structure, ecological conditions, nutritional status, cultural practices, crop load, and a lack of pollination and fertilization (Serdar et al. 2005; Bostan 2019; Balta et al. 2021). Furthermore, the drought conditions experienced in June and July reduce the good kernel ratio while increasing the defective kernel ratio (Külahcılar et al. 2018; Bostan 2019).

Significant variations were observed in the blank nuts among the Palaz clones ($p<0.05$). The heritability of blank nuts is 0.51 (Mehlenbacher 2018). Blank nuts were determined between 1.25 and 8.72% (Table 2). Various researchers have reported blank nuts in Palaz clones ranging from 1.27% to 22.43% (Bostan and İslam 1999; İslam 2000; Balık and Beyhan 2014). However, the findings regarding blank nuts were lower than those reported by these researchers. The clones investigated showed promising results in this regard. In hazelnuts, blank nuts are influenced by genetic structure, climatic conditions during the pollination period, nutritional status, cultural and technical practices, and a lack of pollinator varieties (Fatahi et al. 2014; Bostan 2019; Balta et al. 2021).

**Yield traits**

The number of nuts per cluster is a trait with a high heritability coefficient (0.70) (Thompson et al. 1996), which has a direct effect on yield. Significant clonal differences in terms of nuts per cluster were observed ($p<0.05$). The number of nuts per cluster among the clones ranged from 2.33 to 3.65 (Table 3). Related studies have reported a range of 1.82–4.67 nuts per cluster in Palaz clones (Beyhan et al. 1998; İslam 2000). Furthermore, the number of nuts per cluster in newly developed foreign hazelnut varieties (Dorris, Hunterdon, Raritan and Monmouth) was reported to be between two and three (Mehlenbacher and Molnar 2021). The number of nuts per cluster in the clones investigated was consistent with the researchers' findings.

High yield is a desirable trait in the breeding and cultivation of hazelnuts. Therefore, it is important to select individuals with this trait from the existing population (Karakaya et al. 2025). Genetic structure, ecological conditions, cultural practices, number of stems per shrub, pollinator, altitude and direction are effective factors for hazelnut yield (Balta et al. 2021; Jenderek et al. 2022). Significant variations were observed in the nut yield per plant among the Palaz clones ($p<0.05$). According to the four-year average data, P-3 had the highest nut yield per plant (661.3 g). This was followed by P-5 (637.1 g) and P-10 (607.2 g). The lowest yield was recorded in P-8 (94.9 g) (Table 3). In previous studies conducted on the Palaz variety, nut yield per plant was reported to range from 182.5–204.9 g in the province of Ordu (Türkiye) (Bak and Karadeniz 2021) and from 244.6–595.4 g in the district of Çarşamba (Samsun, Türkiye) (Çalışkan et al. 2019). In addition, different researchers have determined the yield per plant to be 400.4–587.9 g in Tombul clones (Pekdemir 2019), 45.1–694. 8 g in Sivri clones (Şahin 2019) and 174.5–543.5 g (Karakaya et al. 2025) in Çakıldak clones. Compared with these findings, clones yielding 600 g or over (P-3, P-5 and P-10) produced significant results. These clones should be a proper parent for hazelnut breeding programs aimed at improving high-yielding cultivars.

There were significant differences among clones in terms of yield efficiency ($p<0.05$). It ranged from 1.38 to 11.22 g $cm^{-2}$ (Table 3). When different studies on the Palaz variety were compared, the yield efficiency values obtained were similar to those reported by Şen (2018) (5.38–7.80 g $cm^{-2}$), but lower than those reported by Çalışkan et al. (2019) (24.0–74.0 g $cm^{-2}$). These differences may be attributed to genetic structure, ecological conditions and technical and cultural practices.

**Table 3.** Nut yield per plant (g), trunk cross-sectional area ($cm^2$) and yield efficiency (g $cm^{-2}$) of investigated Palaz hazelnut clones

| Clones | Nut yield per plant (g) | | | | | Trunk cross-sectional area ($cm^{-2}$) | | | | | Yield efficiency (g $cm^{-2}$) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2015 | 2016 | 2017 | 2018 | Mean | 2015 | 2016 | 2017 | 2018 | Mean | 2015 | 2016 | 2017 | 2018 |
| P-1 | 585.4 | 423.0 | 503.8 | 319.4 | 457.9 a-d | 83.90 | 91.00 | 99.07 | 110.48 | 96.11 def | 6.98 | 4.65 | 5.09 | 2.89 |
| P-2 | 440.5 | 138.0 | 447.0 | 235.2 | 315.2 d-h | 96.23 | 104.24 | 114.71 | 128.92 | 111.02 d | 4.58 | 1.32 | 3.90 | 1.82 |
| P-3 | 1045.6 | 497.2 | 784.3 | 318.2 | 661.3 a | 332.74 | 350.02 | 371.06 | 399.62 | 363.36 a | 3.14 | 1.42 | 2.11 | 0.80 |
| P-4 | 547.9 | 243.4 | 615.8 | 213.0 | 405.0 a-e | 69.35 | 75.51 | 82.91 | 93.44 | 80.30 fg | 7.90 | 3.22 | 7.43 | 2.28 |
| P-5 | 1119.1 | 220.0 | 810.9 | 398.4 | 637.1 ab | 124.48 | 134.21 | 146.14 | 162.13 | 141.74 c | 8.99 | 1.64 | 5.55 | 2.46 |
| P-6 | 559.2 | 114.8 | 687.9 | 410.6 | 443.1 a-d | 50.61 | 56.94 | 63.30 | 72.55 | 60.85 hi | 11.05 | 2.02 | 10.87 | 5.66 |
| P-7 | 384.8 | 111.9 | 558.6 | 360.3 | 353.9 c-h | 35.42 | 38.99 | 42.10 | 47.21 | 40.93 j | 10.86 | 2.87 | 13.27 | 7.63 |
| P-8 | 174.5 | 39.4 | 115.1 | 50.4 | 94.9 h | 61.97 | 67.86 | 74.91 | 84.88 | 72.40 gh | 2.82 | 0.58 | 1.54 | 0.59 |
| P-9 | 276.3 | 233.1 | 356.3 | 621.2 | 371.7 c-g | 70.16 | 76.33 | 83.83 | 94.32 | 81.16 efg | 3.94 | 3.05 | 4.25 | 6.59 |
| P-10 | 1024.1 | 269.7 | 780.9 | 354.0 | 607.2 abc | 47.82 | 52.98 | 59.21 | 68.10 | 57.03 hij | 21.42 | 5.09 | 13.19 | 5.20 |
| P-11 | 530.1 | 346.8 | 493.9 | 265.3 | 409.0 a-e | 34.78 | 38.34 | 41.47 | 46.55 | 40.29 j | 15.24 | 9.04 | 11.91 | 5.70 |
| P-12 | 485.4 | 361.5 | 459.4 | 259.9 | 391.5 b-f | 84.33 | 91.81 | 101.65 | 115.13 | 98.23 de | 5.76 | 3.94 | 4.52 | 2.26 |
| P-13 | 424.8 | 554.2 | 428.8 | 552.8 | 490.2 a-d | 183.01 | 195.88 | 211.71 | 233.40 | 206.00 b | 2.32 | 2.83 | 2.03 | 2.37 |
| P-14 | 308.6 | 214.8 | 381.6 | 167.5 | 268.2 d-h | 40.89 | 45.58 | 48.96 | 54.49 | 47.48 ij | 7.55 | 4.71 | 7.79 | 3.07 |
| P-15 | 196.7 | 23.9 | 164.5 | 74.9 | 115.0 gh | 38.50 | 42.24 | 45.43 | 50.76 | 44.24 ij | 5.11 | 0.57 | 3.62 | 1.48 |
| P-16 | 195.6 | 62.5 | 116.8 | 128.5 | 125.9 gh | 39.38 | 44.08 | 49.72 | 57.92 | 47.77 ij | 4.97 | 1.42 | 2.35 | 2.22 |
| P-17 | 166.9 | 90.1 | 130.4 | 148.2 | 133.9 fgh | 42.15 | 46.06 | 49.44 | 55.02 | 48.17 ij | 3.96 | 1.96 | 2.64 | 2.69 |
| P-18 | 388.5 | 83.5 | 382.4 | 112.4 | 241.7 d-h | 59.34 | 66.11 | 73.11 | 82.92 | 70.37 gh | 6.55 | 1.26 | 5.23 | 1.36 |
| P-19 | 221.2 | 80.8 | 267.7 | 70.7 | 160.1 e-h | 36.47 | 42.02 | 47.58 | 55.64 | 45.43 ij | 6.07 | 1.92 | 5.63 | 1.27 |
| P-20 | 285.0 | 264.2 | 327.5 | 247.1 | 281.0 d-h | 85.61 | 93.17 | 103.05 | 116.59 | 99.61 d | 3.33 | 2.84 | 3.18 | 2.12 |
| Significance | | | | | *** | | | | | *** | | | | |
| LSD (0.05) | | | | | 263.1 | | | | | 17.61 | | | | |

**Principal components analysis (PCA)**

Thirty-five traits were used for PCA. Ten PCs had an eigenvalue greater than 1.0, accounted for 91.97% of variability. The first two PCs explained 45.6% of total variation. PC1 was mainly related to nut yield per plant, nut weight, kernel weight, nut length, nut thickness, nut size, kernel length, kernel width, kernel thickness, kernel size, husk length by explaining 30.4% of the total variation. Nut size (0.94) and nut length (0.93) had the highest effect on PC1. PC2, which explained 15.2% of variability, was related to kernel ratio, nut width, nut shape index, twin kernel, cracked kernel, black tipped kernel, rotten kernel, poor fill and good kernel. Poor fill (0.85) had the highest effect on PC2. PC3 was related to nut per cluster, yield efficiency, trunk cross-sectional area, internal cavity, kernel shape index and fiber, and explained 11.95% of total variation. Yield efficiency (0.78) had the highest on PC3 (Table 4; Figure 5). Similarly, Ozturk et al. (2017) stated high positive correlation nut weight, kernel weight, kernel percentage, nut size (length, width and thickness), good kernel and poor fill in the Slovenian hazelnut genetic resources, noted that the first two PCs explained 51.2% of total variation. Karakaya et al. (2022) reported also robust positive correlation between important nut traits in the Turkish hazelnut germplasm, and stated that the first two PCs explained 72.11% of variability.

**Table 4.** Principle components analysis of investigated Palaz hazelnut clones

| Traits | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 | PC7 | PC8 | PC9 | PC10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Vigor | 0.32554 | -0.05249 | -0.47680 | -0.11930 | **0.50405** | 0.14764 | 0.07411 | 0.42089 | -0.07718 | -0.23810 |
| Growth habit | -0.19622 | -0.03031 | 0.29564 | **0.55560** | 0.32683 | 0.46401 | 0.06787 | 0.25484 | -0.05561 | 0.05291 |
| Suckering | -0.17868 | -0.04884 | 0.31116 | **-0.68689** | -0.20717 | 0.31107 | 0.40678 | -0.01959 | -0.12072 | -0.01037 |
| Stem density | -0.19442 | 0.02695 | 0.33850 | 0.46951 | 0.03805 | 0.24883 | 0.20980 | -0.07228 | 0.18028 | **0.62785** |
| Nuts per cluster | 0.05777 | 0.33097 | **0.65476** | -0.07721 | 0.12465 | -0.13873 | -0.30478 | -0.14078 | -0.35579 | -0.31717 |
| Nut yield per plant | **0.73928** | 0.26191 | 0.04370 | -0.17956 | 0.37400 | 0.26663 | -0.00881 | 0.05108 | -0.02220 | -0.12685 |
| Yield efficiency | 0.38677 | 0.25553 | **0.77579** | -0.10209 | -0.06953 | -0.10734 | -0.13764 | 0.06020 | -0.06078 | -0.05370 |
| Trunk cross-sectional area | 0.39540 | 0.12228 | **-0.62061** | -0.25450 | 0.39312 | 0.37734 | 0.04971 | -0.04985 | -0.03364 | 0.00659 |
| Nut weight | **0.91550** | 0.24446 | 0.15630 | -0.01095 | -0.13424 | -0.05727 | -0.00645 | -0.04762 | -0.00200 | 0.15322 |
| Kernel weight | **0.91756** | -0.09805 | 0.00759 | 0.05352 | -0.27503 | 0.07135 | 0.01655 | 0.04882 | -0.05258 | 0.15243 |
| Kernel ratio | -0.05981 | **-0.78078** | -0.29449 | 0.11750 | -0.25060 | 0.28441 | 0.04407 | 0.21969 | -0.13010 | -0.02313 |
| Shell thickness | -0.12681 | -0.49145 | 0.08012 | 0.02032 | 0.25088 | 0.21324 | **-0.62826** | 0.15221 | 0.35366 | 0.00717 |
| Internal cavity | 0.30205 | 0.23024 | **-0.47760** | 0.22991 | 0.01169 | -0.45223 | -0.07311 | -0.05366 | 0.45185 | 0.04220 |
| Nut length | **0.93369** | -0.04532 | 0.19074 | -0.12801 | 0.04358 | -0.11610 | 0.06488 | -0.07162 | 0.05647 | 0.09690 |
| Nut width | 0.61228 | **0.67394** | -0.06255 | 0.04107 | -0.07651 | -0.03995 | -0.21078 | -0.07150 | 0.14968 | -0.08082 |
| Nut thickness | **0.87314** | 0.11565 | 0.06084 | -0.06772 | -0.06481 | -0.12308 | 0.21057 | 0.26482 | 0.02383 | 0.12175 |
| Nut size | **0.93528** | 0.27087 | 0.08129 | -0.06495 | -0.02663 | -0.10737 | 0.01223 | 0.01588 | 0.09119 | 0.04966 |
| Kernel length | **0.69673** | -0.23622 | 0.32995 | 0.37537 | -0.10616 | 0.23060 | 0.11524 | 0.11960 | -0.10090 | -0.13516 |
| Kernel width | **0.60274** | 0.47431 | -0.45192 | 0.14957 | -0.24284 | 0.20000 | -0.18444 | 0.13121 | 0.01065 | -0.10235 |
| Kernel thickness | **0.84274** | 0.02027 | -0.27910 | 0.08670 | -0.19830 | 0.24169 | 0.07128 | -0.13600 | -0.22535 | 0.11213 |
| Kernel size | **0.85805** | 0.12022 | -0.17136 | 0.24294 | -0.22299 | 0.26691 | -0.00766 | 0.05391 | -0.11913 | -0.05461 |
| Nut shape index | 0.55592 | **-0.58126** | 0.29231 | -0.18827 | 0.15372 | -0.08226 | 0.13861 | -0.18085 | -0.03234 | 0.14313 |
| Kernel shape index | -0.03702 | -0.51959 | **0.71279** | 0.22190 | 0.13443 | 0.01046 | 0.18846 | 0.09832 | 0.00100 | -0.11750 |
| Husk length | **0.47770** | -0.08315 | 0.30488 | 0.12822 | 0.44552 | 0.27498 | -0.13281 | -0.22981 | 0.40049 | -0.15969 |
| Fiber | 0.40228 | 0.15703 | **0.56547** | -0.40067 | -0.07519 | -0.26872 | -0.14991 | 0.40523 | 0.15934 | 0.06133 |
| Blank nut | 0.04940 | 0.04280 | 0.00608 | 0.29091 | -0.18133 | -0.29846 | **0.67451** | -0.06388 | 0.29647 | -0.40395 |
| Twin kernel | -0.10102 | **0.60338** | 0.45923 | 0.46623 | 0.05041 | 0.21241 | -0.13212 | -0.27087 | -0.09177 | -0.05871 |
| Shriveled kernel | -0.00769 | -0.17431 | 0.21386 | **-0.68627** | 0.03653 | 0.47492 | 0.18355 | -0.03878 | 0.38373 | 0.01964 |
| Abortive kernel | -0.14241 | 0.46693 | -0.11065 | -0.12119 | **0.52465** | -0.44273 | 0.01365 | 0.24342 | -0.26296 | 0.30514 |
| Cracked nut | -0.47707 | **0.53748** | -0.02335 | 0.06847 | -0.37454 | 0.15326 | 0.00476 | 0.42845 | 0.16401 | -0.03240 |
| Black tipped kernel | -0.45973 | **0.61255** | 0.23489 | 0.02533 | -0.35544 | 0.25645 | -0.06908 | 0.34835 | 0.05771 | -0.01287 |
| Rotten kernel | -0.23495 | **0.70503** | -0.24733 | -0.14693 | -0.04980 | 0.31533 | 0.01446 | -0.40296 | -0.04322 | 0.00367 |
| Moldy kernel | 0.15100 | 0.21726 | -0.04372 | 0.28994 | **0.56974** | -0.10432 | 0.39484 | 0.13501 | -0.05453 | -0.03193 |
| Poor fill | -0.34969 | **0.85111** | 0.12186 | -0.16612 | 0.19688 | 0.18338 | 0.04715 | 0.02869 | -0.01223 | 0.13901 |
| Good kernel | 0.31019 | **-0.83152** | -0.11903 | 0.02178 | -0.10255 | -0.03469 | -0.36165 | 0.00264 | -0.12754 | 0.05714 |
| Eigenvalue | 9.51 | 5.96 | 4.18 | 2.64 | 2.31 | 2.19 | 1.78 | 1.37 | 1.20 | 1.05 |
| % of variance | 30.40 | 15.20 | 11.95 | 7.55 | 6.61 | 6.25 | 5.08 | 3.92 | 3.42 | 3.00 |
| Cumulative % | 30.40 | 45.60 | 57.55 | 65.10 | 71.71 | 77.96 | 83.04 | 86.96 | 90.38 | 93.38 |

Factor loading ≥ |0.47| are marked.

Three main groups were formed between Palaz hazelnut clones on the PCA graph. The first group (green) consisted of therteen clones (P-4, P-6, P-7, P-8, P-9, P-11, P-12, P-13, P-14, P-15, P-16, P-17 and P-20), and these clones came to the fore by yield efficiency, nuts per cluster, kernel ratio, shell thickness, good kernel, shriveled kernel, nut and kernel shape index traits. The second group (red) included six clones (P-1, P-2, P-3, P-5, P-10 and P-18). Clones in this group

had the highest value in yield, nut weight, kernel weight, internal cavity, moldy kernel, nut and kernel size (except kernel width) traits. The third group (blue) consisted of one clones (P-19), and these clone came to the fore by kernel width, blank nut, poor fill, abortive kernel, rotten kernel, cracked nut, black tipped kernel, twin kernel traits (Figure 5).

**Fig. 5** Biplot of the first two principal components (PC1 and PC2) in the Palaz hazelnut clones

**Molecular characterization**

According to the results of 12 ISSR primers and 3 SRAP primer pairs that produce interpretable and clear bands, in the Palaz clones, the polymorphic band lengths were 150–1200 bp and 100–510 bp, the total number of bands was 4–10 and 2–6; the number of polymorphic bands was 4–10 and 2–6; and the polymorphism rate was 66.7–100% and 100%, respectively (Table 5).

Studies using ISSR primers to determine the genetic relationships among hazelnut germplasm found that the total number of bands, the number of polymorphic bands, and the polymorphism rate were 4–14, 3–14, and 28.57–100% in Turkish hazelnut varieties (Karakaya et al. 2025); 9–21, 8–21, and 71.43–100% in Iranian hazelnut genetic resources (Mohammadzadeh et al. 2024); 3–17, 2–17, and 45.5–100% in some hazelnut genetic resources from Ordu Province (Bostan and Karakaya 2024); and 4–10, 3–10, and 5% in Çakıldak hazelnut genetic resources.0–100% (Karakaya et al. 2025). Furthermore, the total number of bands, the number of polymorphic bands, and the polymorphism rate were reported as 2–6, 2–5, and 83.33–100%, respectively, in Çakıldak hazelnut clones characterized using SRAP primers (Karakaya et al. 2025). The results of the ISSR and SRAP analyses generally consistent with those of other researchers. However, the total number of bands and the number of polymorphic bands obtained from ISSR primers were lower than those reported by some researchers (Mohammadzedeh et al. 2014; Bostan and Karakaya 2024). These differences may be attributed to genetic structure and the primers used (Yılmaz et al. 2024; Karakaya et al. 2025).

The genetic similarity coefficient for the Palaz clones ranged from 0.59 to 0.96. In the UPGM, the Palaz clones were divided into two main groups (A and B). Group A included eighteen Palaz clones and the standard Palaz cultivar, and was further divided into two sub-groups. Sub-group A-1 consisted of two clones (P-16 and P-20). Subgroup A-2 included the standard Palaz cultivar and 16 Palaz clones (P-1, P-2, P-4, P-5, P-6, P-7, P-8, P-9, P-10, P-11, P-12, P-13, P-14, P-15, P-17 and P-18). Group B, on the other hand, consisted of two clones (P-3 and P-19). While clones P-9 and P-17 were adjacent to one another, clones P-3 and P-19 were distant from the standard Palaz variety and most of the Palaz clones (Figure 6). Consistent with the results of the present study, the genetic similarity coefficient in Çakıldak hazelnut clones identified using ISSR and SRAP primers was reported to range from 0.59 to 0.96 (Karakaya et al. 2025). In addition, the genetic similarity coefficient for different hazelnut genetic resources identified using ISSR primers ranges from 0.73 to 0.96 in Türkiye (Kafkas et al. 2009); from 0.50 to 0.98 in Spain (Ferreira et al., 2010); from 0.18 to 0.74 in Iran (Mohammadzedeh et al. 2014); and from 0.58 to 0.98 at Gürgenetepe in Ordu, Türkiye (Bostan and Karakaya 2024). The genetic similarity ratio of the clones was consistent with the findings of other researchers, except for those by Mohammadzedeh et al. (2014). The fact that the individuals investigated in the present study were clones of the same variety is an important factor in explaining the high genetic similarity ratio. Indeed, individuals resulting from cross-pollination in hazelnuts possess high genetic diversity (Öztürk et al. 2017). Similarly, higher genetic diversity has been reported in wild hazelnut genetic resources than in cultivated varieties (Campa et al. 2011; Martins et al. 2015).

According to the results of the ISSR and SRAP analyses, the most of individuals examined exhibited genetic differences from one another and from the standard 'Palaz' cultivar. These results once again confirmed that individuals propagated clonally via suckers may exhibit genetic variation. Similar results have indeed been recorded in numerous studies in which clones of different hazelnut varieties were characterized using molecular techniques (Valentini et al. 2001; Petriccione et al. 2010; Karakaya et al. 2025). Furthermore, many researchers have reported that Turkish hazelnut varieties consist of a group of clones with similar characteristics, rather than a single clone (Balta et al. 1997; Mehlenbacher 2018; Karakaya et al. 2025).

**Table 5.** ISSR and SRAP primers studied, polymorphic band length, total number of bands, number of polymorphic band and polymorphism rate

| Primers | Polymorphic band length (bp) | Total number of bands | Number of polymorphic band | Polymorphism rate (%) |
|---|---|---|---|---|
| ISSR | | | | |
| VHVG$(TG)_7$ | 490-900 | 7 | 7 | 100 |
| DBD$(ACA)_7$ | 390-900 | 6 | 6 | 100 |
| $(CA)_6$AC | 400-900 | 5 | 5 | 100 |
| $(CAC)_3$GC | 250-900 | 7 | 7 | 100 |
| BDB$(CA)_7$C | 490-1200 | 7 | 6 | 85.7 |
| HVH$(TCC)_7$ | 350-1150 | 9 | 8 | 88.9 |
| $(TCC)_5$RY | 350-1150 | 8 | 8 | 100 |
| $(AG)_7$YC | 150-1100 | 10 | 10 | 100 |
| $(AG)_8$T | 300-1000 | 7 | 6 | 85.7 |
| $(CT)_8$TG | 400-1000 | 4 | 4 | 100 |
| $(GAA)_6$ | 350-1200 | 6 | 5 | 83.3 |
| $(GA)_8$YG | 300-800 | 6 | 4 | 66.7 |
| Total | - | 82 | 76 | - |
| Mean | - | 6.8 | 6.3 | 92.5 |
| SRAP | | | | |
| em13 x me7 | 110-250 | 2 | 2 | 100 |
| em13 x me11 | 100-510 | 6 | 6 | 100 |
| em8 x me10 | 100-500 | 5 | 5 | 100 |
| Total | - | 13 | 13 | - |
| Mean | - | 4.3 | 4.3 | 100 |

**Fig. 6** UPGM dendrogram obtained from ISSR and SRAP primers in Palaz hazelnut clones

**Association between important nut traits and ISSR/SRAP markers**

GLM was used to determine the correlations between the loci identified by ISSR and SRAP markers and important nut characteristics. Notably, some significant correlations were identified. Nut yield per plant was associated with 3 ISSR loci and had a moderate correlation with them. 3 ISSR loci were associated with yield efficiency and had a moderately correlated with the $(TCC)_5$RY-900 locus. Nuts per cluster was moderately correlation with $(AG)_7$YC-900 and $(TCC)_5$RY-900 loci. Nut weight was associated with 2 ISSR and 1 SRAP loci, exhibiting a moderate correlation with them. 3 ISSR and 1 SRAP loci were associated with kernel weight and had a moderate correlation with them. Kernel ratio was associated with 8 ISSR loci, possessing moderate correlation with most loci. Shell thickness was associated with VHVG$(TG)_7$-710 locus. Nut size was associated with 1 ISSR and 2 SRAP loci and had a moderate correlation with them. Kernel size was associated with 3 ISSR loci and displayed a moderate correlation with them. Good kernel was associated with 8 ISSR loci, with a moderate correlation with the $(GAA)_6$-350 locus. Internal cavity was associated with 2 ISSR and 1 SRAP loci (Figure 7; Table S1).

Moldy kernel was associated with 3 ISSR and 3 SRAP loci and had a highly correlation with BDB$(CA)_7$C-490. Nut shape index was associated with 9 ISSR loci and had a moderate correlation with them. Kernel shape index was associated with 6 ISSR and 1 SRAP loci. Nut length was associated with 5 ISSR and 1 SRAP loci, exhibiting a moderate correlation with them. 5 ISSR and 3 SRAP loci were associated with nut width. Nut thickness was associated with 3 ISSR loci. 4 ISSR loci were associated with kernel length. Kernel width was associated with 2 ISSR and 1 SRAP loci. 5 ISSR loci were associated with kernel thickness. Abortive kernel was associated with 5 ISSR and 2 SRAP loci. Blank nut was associated with $(AG)_8$T-500 locus. Black tipped kernel was associated with 12 ISSR loci. 11 ISSR loci were associated with cracked nut. Kernel fiber was associated with 7 ISSR and 2 SRAP loci. 6 ISSR loci were associated with poor fill. Rotten kernel was associated with 4 ISSR and 1 SRAP loci. 3 ISSR loci were associated with shriveled kernel. Twin kernel was associated with 5 ISSR loci. Husk length was associated with VHVG$(TG)_7$-490 locus. em13/me11-510 locus was associated with habitus. Suckering was associated with 2 ISSR loci. 7 ISSR and 1 SRAP loci were associated with trunk cross-sectional area. Stem density was associated with 2 ISSR loci. Vigor was associated with 3 ISSR loci (Figure 7; Table S1).

The present study reported many important marker-traits associations. Ozturk et al. (2017) determined SSR loci linked to several traits of Slovenian hazelnut genetic resources, including nut length, thickness, caliber, shell thickness, blank nut, kernel weight, kernel shape uniformity, and moldy kernel. Frary et al. (2019) stated SSR loci associated with plant habit, suckering, vigor, blank nut, nut shape, shell thickness, nut size, twin kernel, and kernel size in 390 hazelnut

accessions that were selected from different areas in Türkiye. Salardi-Jost et al. (2025) identified ten SNPs associated with nut characteristics, such as size, calibre, and perimeter. Also, Karakaya et al. (2025) stated that the yield and nut traits of Çakıldak hazelnut clones were associated with 65 ISSR/SRAP loci, and that some of these traits were linked to pleotropic loci. Nut weight, kernel weight, and nut size, which are fundamental traits of nuts, were linked to a single locus [$(AG)_7$YC-390], and these traits had a moderate correlation with this locus. As well, the parameters of yield per plant and nuts per cluster, which have a direct effect on plant productivity, were also linked to a single genetic locus [$(AG)_7$YC-900]. These loci exhibit the pleiotropic effect, meaning that one gene influencing two or more traits. These loci will assist breeders to select individuals with high potential in terms of yield and nut traits.

## Conclusion

This study investigated the morphological, yield and nut traits of clones selected from the Palaz hazelnut population in the Eastern Black Sea Region, where hazelnut cultivation has been ongoing for centuries, and determined the genetic diversity among them. Significant variation was observed among the clones in terms of the investigated traits. The P-3, P-5 and P-10 clones stood out for their yield characteristics, while the P-3, P-10, P-12 and P-14 clones stood out for their nut traits, such as nut and kernel weight, kernel ratio, shell thickness, nut and kernel size and good kernel. The most of clones were genetically distinct from the standard Palaz cultivar and from each other. This confirmed the conclusion that Turkish hazelnut varieties constitute a group of clones with similar characteristics. P-3 and P-19 clones were genetically different from the standard Palaz variety and the individuals investigated. This indicates that they are probably have been originated from a seedling. Association mapping revealed that loci [$(AG)_7$YC-900] with pleiotropic effect will help breeders to select superior accessions with regard to yield and nut traits. Consequently, these individuals can contribute to the development of high-quality, high-yielding hazelnut varieties that are adaptable to climate change and resistant to abiotic and biotic stress factors within breeding programs. These results are also significant for the conservation and sustainability of hazelnut genetic resources, constituting an important genetic pool for future breeding programs.

## Declarations

**Author contributions**

**OK:** Investigation, methodology, conceptualization, formal analysis, writing – original draft, validation, visualization. **MFB:** Supervision, methodology, conceptualization, writing – review editing, validation. **AU:** Co-supervision, methodology, validation. **FB:** methodology, conceptualization, validation, writing – review editing. **SU:** formal analysis, visualization.

**Conflict interest**

The authors declare no conflict interest.

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**Ethical approval**

Not applicable.

**Data availability**

No dataset were generated or analyzed during the current study.

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## Figures

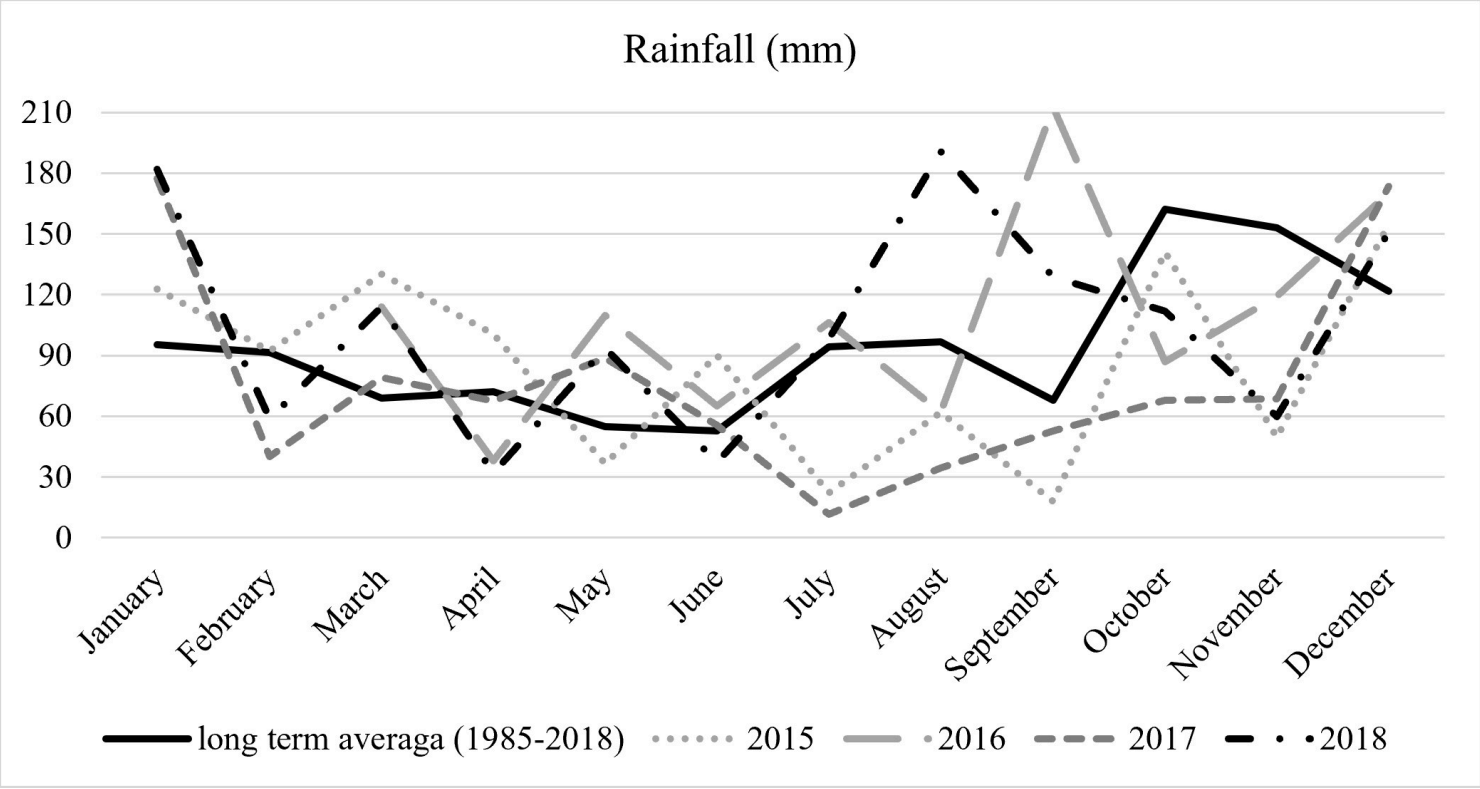


**Figure 1**

Rainfall (mm) between January and December

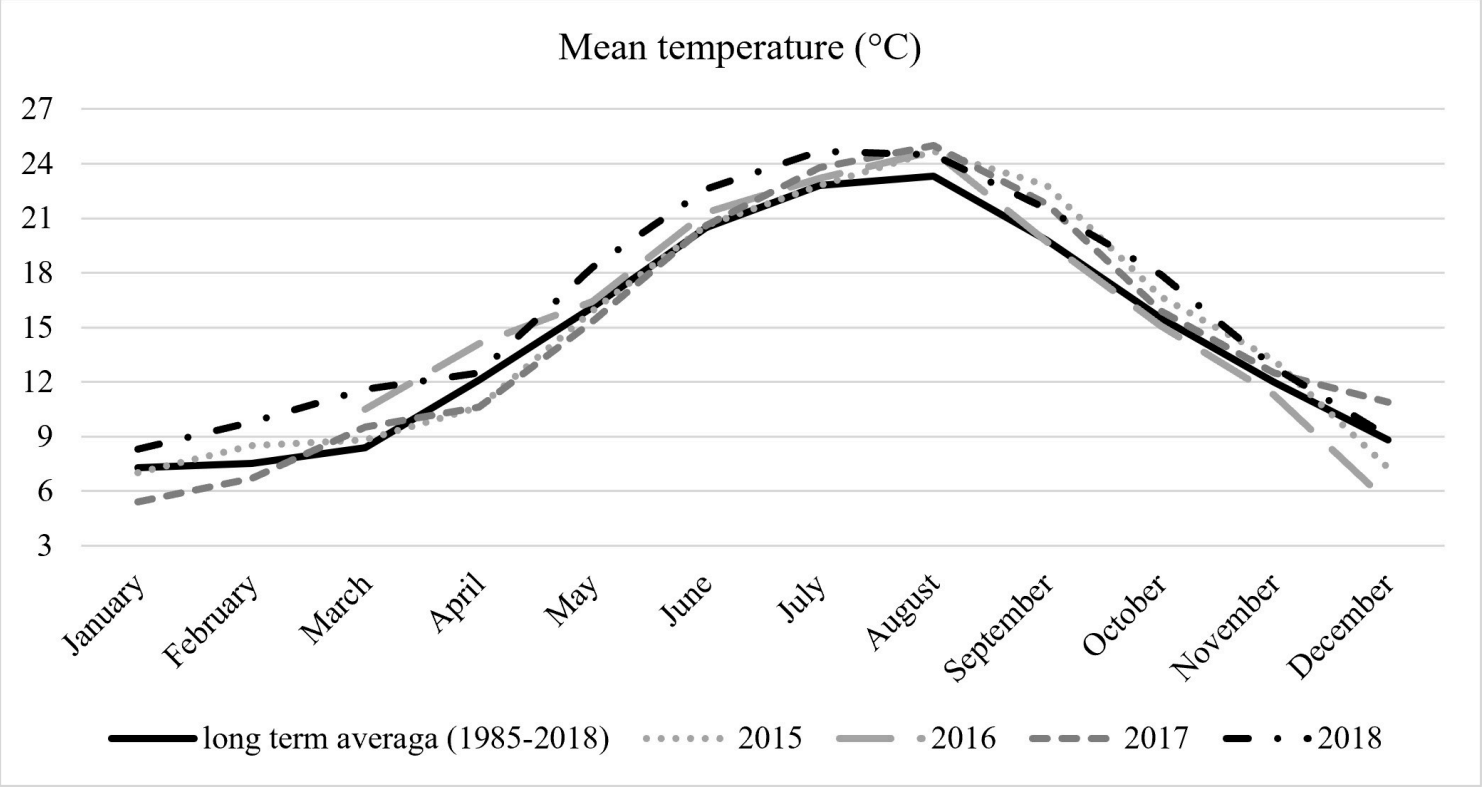


**Figure 2**

Mean temperature (°C) between January and December

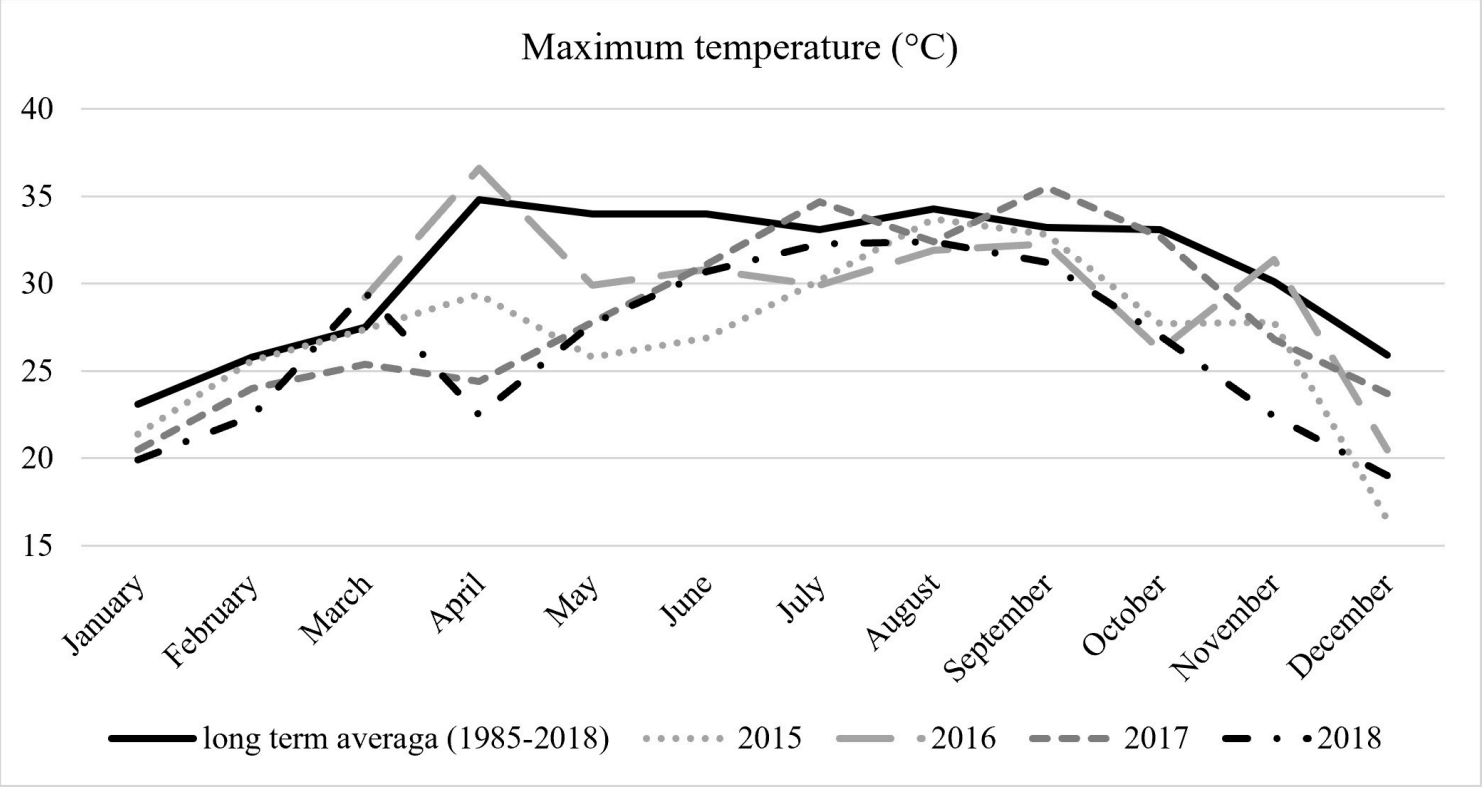


**Figure 3**

Maximum temperature (°C) between January and December

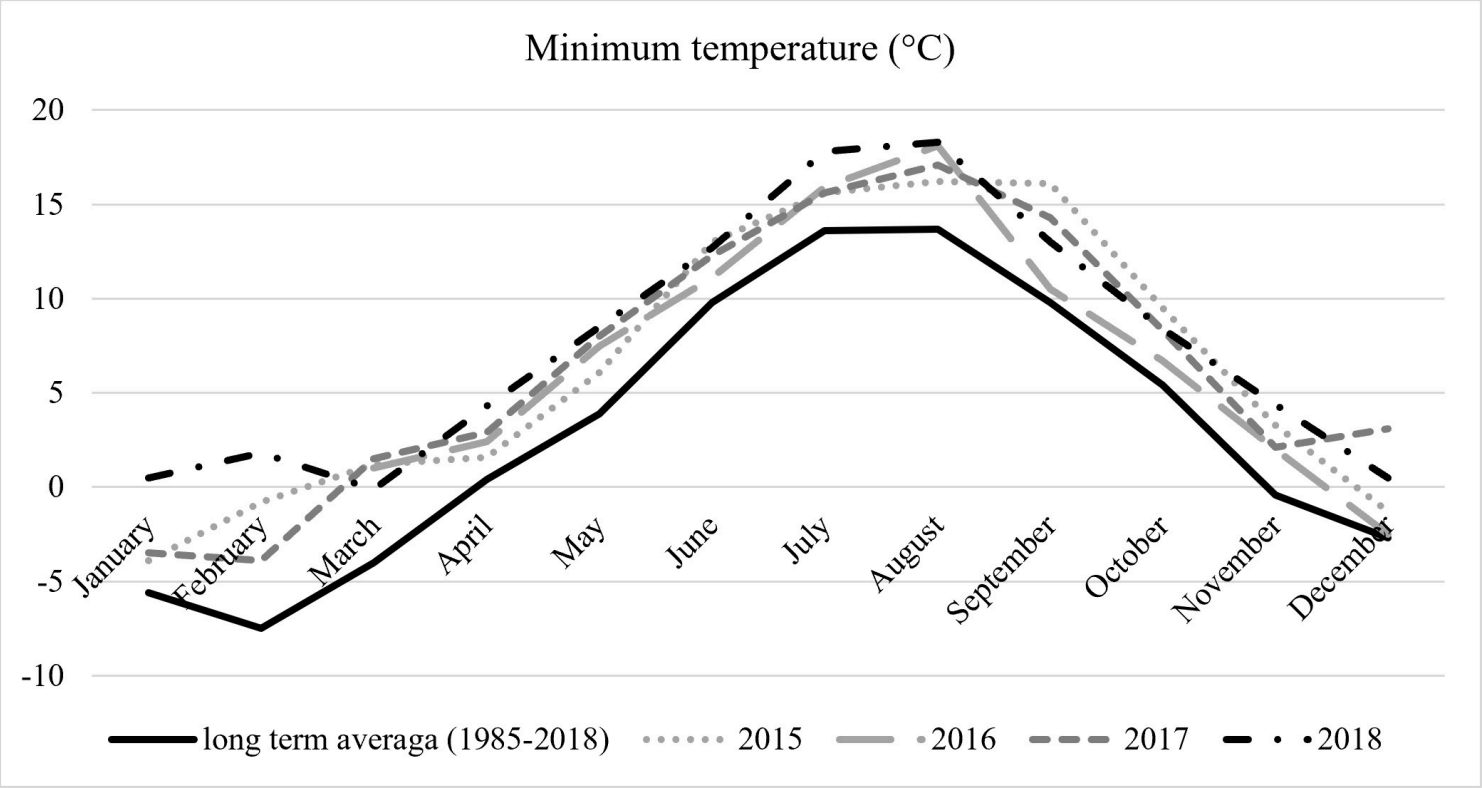


**Figure 4**

Minimum temperature (°C) between January and December

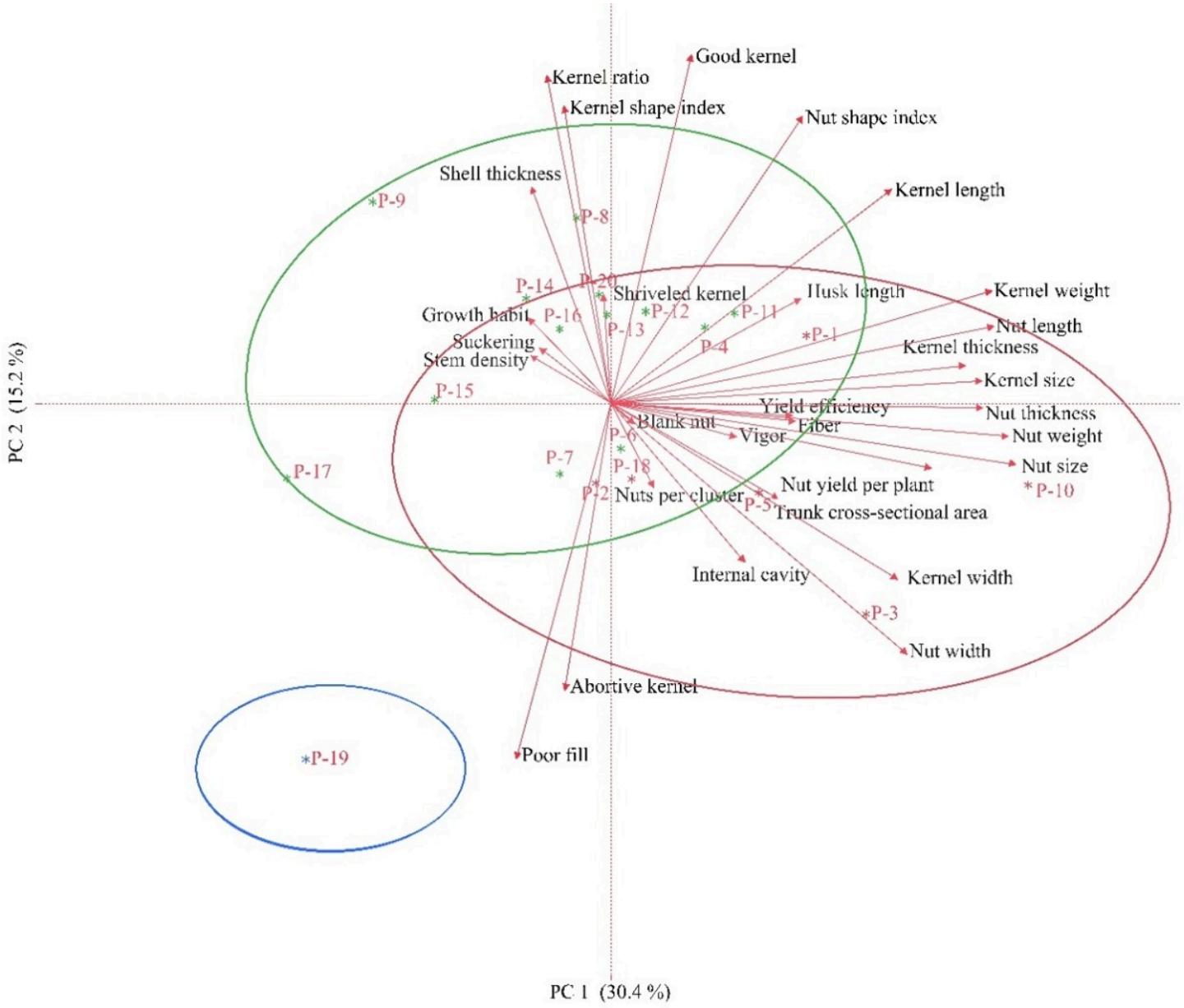


**Figure 5**

Biplot of the first two principal components (PC1 and PC2) in the Palaz hazelnut clones

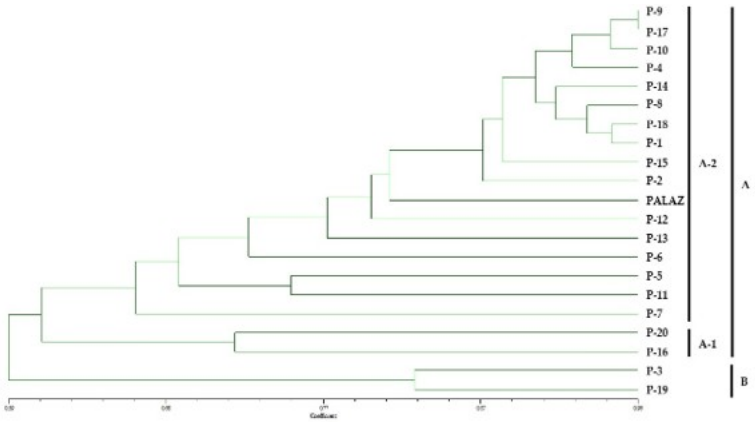


**Figure 6**

UPGM dendrogram obtained from ISSR and SRAP primers in Palaz hazelnut clones

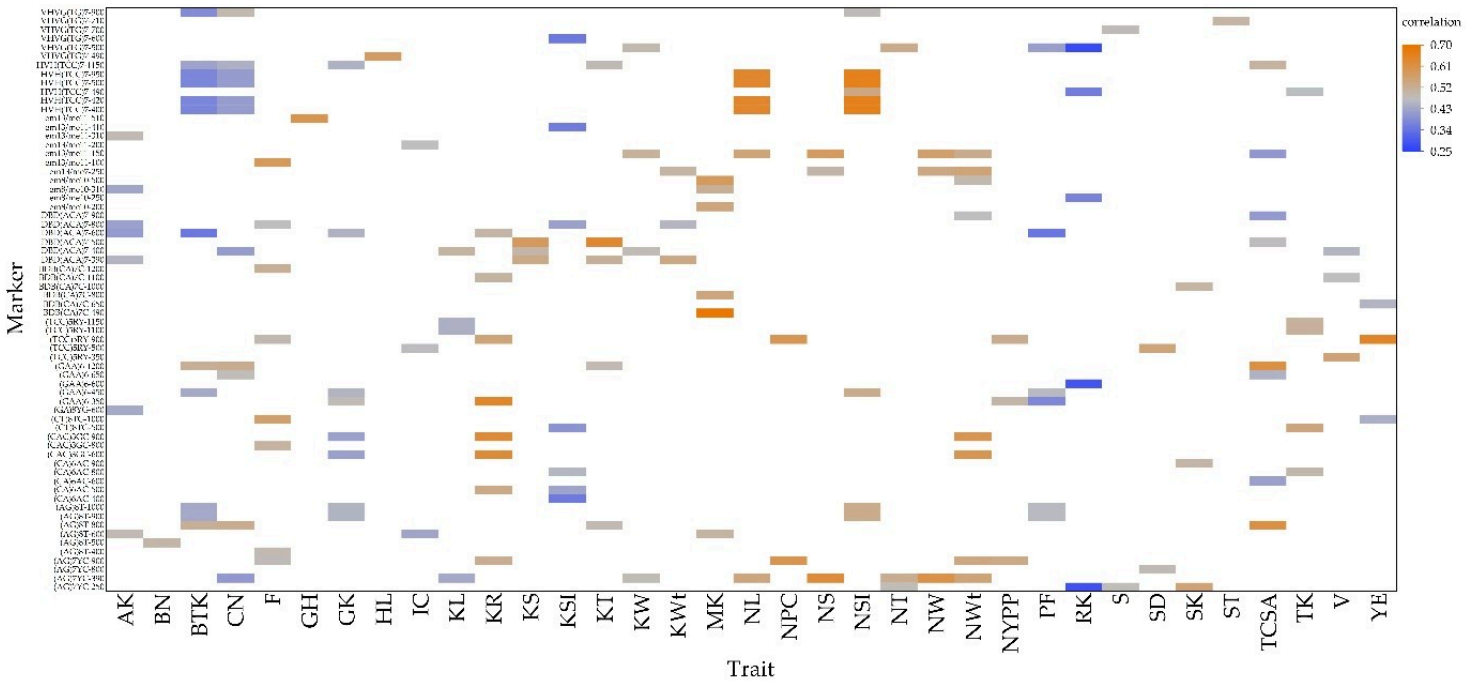


**Figure 7**

Trait loci associations determined by GLM

## Supplementary Files

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