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The Persian (English) walnut (*Juglans regia* L.) assortment of Hungary: Nut characteristics and origin

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ABSTRACT

Persian walnut growing has a long-term tradition in Hungary. The walnut breeding started with the domestication of French varieties propagated by seeds in the 1910s, which was not successful. From the 1950s the Hungarian breeding research used selections from the local population, cross breeding and back crosses as breeding methods. The aim of this paper is to examine the origin and the nut characteristics of the Hungarian bred varieties registered on the National Variety List. The grafted trees for sample collection were planted in spring of 1990 at the Experimental Fields. Strong relationships between nut height and dried nut weight, dried nut weight and kernel weight, nut width and dried nut weight, nut diameter and nut width, dried nut weight and nut diameter, kernel weight and nut diameter as described in the literature was confirmed. Another relation (nut width and kernel weight) showed a strong relation too. All of the Hungarian bred varieties have a unique genetic background. The relation is not apparent between the introduced and the local varieties in all cases. It was concluded from the genetic pattern that the French seedling varieties might have not influenced the Hungarian varieties in the extent it was supposed formerly.

1. Introduction

The Persian or English walnut (*Juglans regia* L.) production has a long term tradition in Hungary. However there is a walnut population located in Northeast Hungary, near the Hungarian and Ukrainian border, which probably used to be indigenous/native (Terpó, 1976) and different from genotypes derived from other walnut races world-wide (Ebrahimi et al., 2017; Bernard et al., 2018a), the mindful variety usage started in 1910s. The Hungarian Department of Agriculture purchased walnut seeds from France and created seedlings from them to be planted out across the country in orchards and as guard trees beside the roads. After World War II, in the 1950s the local Committee for Registration of Fruit Trees stated that seedlings derived from the French population were not enough adapted to the Hungarian climatic conditions, as the seedlings could not reach the similar harvested quantity and nut quality as in their original provenance (Szentiványi, 1998). After the failure of the domestication, selective breeding from the local population was launched. Today, Hungary produces approximately 7088 tonnes dried and in-shell fruits (FAO, 2019), the walnut production shows an increasing trend year on year. Nowadays, walnut is the third important fruit species due to its territory (9.000 ha) after apple and tart cherry (Hungarian Statistical Office (HSO, 2018).

In the first stage of the selective breeding from the local population 'Alsószentiváni 117', 'Milotai 10', and 'Tiszacsécsi 83' were registered on the National Variety List. Beside the selection work, cross breeding started in the 1970s as the second stage of the breeding programme. The best paternal parent, which gave the highest number of progenies, was the US-bred cultivar 'Pedro'. Five further cultivars were released from this stage. From the cross of 'Milotai 10' and 'Pedro' the 'Milotai bőtermő', 'Milotai kései®', and 'Milotai intenzív' were selected and approved as state registered cultivars. 'Bonifác®' and 'Alsószentiváni kései®' hybrid cultivars were derived from the cross of 'Alsószentiváni 117' and 'Pedro' (Bujdosó et al., 2014b, 2016, 2019a, 2019b). During the third stage of the breeding in the early 2000s, breeder's attention was turned to susceptibility to walnut blight (*Xantomonas arboricolae* pv. *juglandis*) (Végh et al., 2015, 2016). So far, a selected candidate called 'BD6' was enhanced during this stage of breeding (Bujdosó et al., 2020).

During the breeding process the ripening time and the fruit characteristics became important; such as early ripening time, size at least 32 mm in diameter, light shell colour, smooth shell surface, light kernel colour, at least 40 % in kernel rate and 60 % in cracking rate as breeding aims were taken into the consideration (Bujdosó et al., 2010a, 2010b,

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Table 1
Meteorological data during the data collection 2014 – 2019.

parameters	value
average yearly temperature	11.7 °C
average yearly temperature during the growing season (between April and September)	18.6 °C
average yearly luminous flux	1023 l/m^2/day
average yearly precipitation	599.4 mm
annual average of sunshine hours	2079

Bujdosó and Szentiványi, 2014a). However, in order to reach the best fruit quality, the careful selection of best fruit sites is needed beside the breeding work, as well as the realization of the best growing and post-harvest technologies (Kónya et al., 2015; Cindric et al., 2018). Moreover, origin (Sinesio and Moneta, 1997) and effect of the given year (Bujdosó et al., 2010b) influence more the final product than the varieties.

Persian walnut launched into the global production since antique times, but the genome analysis and many genetic studies have started since 1990s (Bernard et al., 2018b). Germain (1997) reported about the importance of the selection of the local population or creating hybrids using local genotype to improve the climatic adaptation, early fruiting, high productivity, disease tolerance and fruit quality. Another study was made across Italy to determine the relationship between the native populations. Although there was moderately high level of SSR polymorphisms, the effect of the fruit site was low (Pollegioni et al., 2011). Waqar Khan et al. (2010) estimated a large range of genetic distance in the Pakistan walnut population. Researchers found narrow-sense heritability (85 %) on fruit traits e.g. shell thickness, kernel and nut weight, but the kernel colour was a non-heritable trait during study of Iranian genotypes (Hansche et al., 1972). At the University of Davis the heritability for the extra light kernel colour was 49 % in average (Martínez-Garcia et al., 2017).

Using SSR markers for genetic fingerprinting has become a common, widespread tool in breeding not only for plant variety protection, but also for unfolding the origin of some historical selections (Weising et al., 2005). The identification of unique, distinct genotypes of different varieties enables us to create dendrograms based on the genetic similarities/dissimilarities, and thereby to demonstrate the connection between them. One dedicated aim of this paper is to discover more deeply the genetic background of the Hungarian bred varieties, since this is the first study dealing with not just some genetic resources derived from the Carpathian Basin, but the whole Hungarian Persian walnut assortment. Furthermore, to seek answers how the French seedlings influenced the Hungarian genome, the second aim was to check the most important nut characteristics of the aforementioned cultivars.

2. Material and methods

2.1. Plant material

The trial is located at the Experimental Fields of the National Agricultural Research and Innovation Centre Research Institute for Fruit Growing and Ornamentals (GPS coordinates:N 47°20'11,44" E 18°51'53,42"). The trial was planted in spring of 1990 on chernozem soil with high lime (pH = 8, total lime content in the top 60 cm layer 5%) and humus content (2.3–2.5 %). Considering the Arany-type cohesion index (Dobos et al., 2010) the $K_A = 40$ refers to medium compactness. Meteorological conditions of the site are presented in Table 1.

The entire Hungarian walnut assortment ('Alsószentiváni 117', 'Milotai 10', 'Tiszacsécsi 83', 'Milotai intenzív', 'Milotai bőtermő' (bőtermő means high productivity), 'Milotai kései®' (kései refers to its late leafing time), 'Alsószentiváni kései®', 'Bonifác®') as well as the novel bred 'BD6' were introduced the trial. For the examination of the genetic background 'Franquette', a historical French variety and the US-bred 'Pedro' as paternal parent of the Hungarian hybrid varieties were included in the test. The control of our trial was the US-bred 'Chandler'. The trees were grafted on a selected *Juglans regia* seedling, and planted out 10x10 m in the row and between the rows. The grafted trees were trained as a central leader canopy, replicated 5 trees of each, and the trial was not irrigated.

2.2. SSR genotyping

Total genomic DNA was extracted from fresh leaves using the ATMAB procedure, a modified cetyltrimethylammonium bromide (CTAB) method (Dumolin et al., 1995). DNA concentration was measured by spectrophotometry (Eppendorf BioPhotometer) and standardized to 10 ng/µl. Twelve SSR loci were amplified as chosen from the literature, namely WGA27, WGA72 (Woeste et al., 2002), WGA01, WGA04, WGA89, WGA118, WGA202, WGA276, WGA321, WGA331 (Dangl et al., 2005) and JR1817, JR6160 (Dang et al., 2016). Each PCR was conducted using a third, universal M13(-21) primer fluorescent-label with 6-FAM, NED, PET or VIC according to the protocol of Schuelke (2000). Reactions were carried out in a total volume of 15 µl containing 10 ng of template DNA, 1× reaction buffer (Promega GoTaq G2 Flexi, 5× reaction buffer with no magnesium), 1.5 mM of $MgCl_2$ (Promega), 20 µM of each dNTP (Promega dNTPmix, 10 mM each), 1.0 unit polymerase (Promega GoTaq G2 Flexi, 5U/µl), while 0.05 µM of Forward primer and 0.2 µM of Reverse and M13(-21) primers each (IDT Custom DNA oligos). PCRs were performed in a Veriti Personal Thermocycler (Applied Biosystems) with the following steps: an initial denaturation for 5 min at 94 °C, followed by 35 cycles of 45 s at 94 °C, 45 s at the optimum annealing temperature for each marker (T_{ANN} = 53 °C for WGA01, WGA04, WGA321, WGA331, T_{ANN} = 55 °C for WGA89, WGA118, T_{ANN} = 58 °C for WGA27, WGA72, WGA202, JR1817, JR6160 and T_{ANN} = 63 °C for WGA276), 60 s at 72 °C, furthermore eight additional cycles of 30 s at 94 °C, 45 s at 53 °C (T_{ANN} optimal for M13(-21) primer), 45 s at 72 °C, finished with a final extension step at 72 °C for 10 min. Amplification products were checked on a 2% agarose gel in 1 × TAE buffer, stained with GelRed (Biotium). Then fragments were diluted (up to 20-fold) for capillary electrophoresis and multiplexed by dye and size in formamide (Hi-Di, Applied Biosystems) using GeneScan LIZ 500 (Applied Biosystems) internal size standard. SSR genotyping was performed on an ABI 3730 DNA Analyzer (Applied Biosystems), while allele calling was carried out using GeneMapper 4.0 software (Applied Biosystems).

For SSR data analysis GenAlEx 6.5 software (Peakall and Smouse, 2006, 2012) was used. Probability of identity (P_I) was calculated for the combination of the analysed 12 loci, possible matching genotypes were checked. Genetic distance (Codom-Genotypic) option was applied for generating a pairwise, individual-by-individual genetic distance matrix. Matrix was used for subsequent PCA and for constructing UPGMA dendrogram by PAST 4.03 (Hammer et al., 2001). Mantel test was conducted by GenAlEx in order to test correlation between the genetic distance matrix and distance matrices generated from different phenothypic traits. Number of permutations was set to 9999. The R_{xy} correlation coefficient was observed with a range from –1 to +1 to test for a significant relationship between the elements of the X and Y matrices. In case of $R_{xy} = 0$ no relationship exists between the elements of the two data matrices.

2.3. Nut measurements

The nuts were harvested at the optimal ripening time, when 50 % of husks were open. Immediately after harvest the nuts were removed from the husks, washed and dried to decrease their moisture content to 10–12%. The nut characteristics (nut height, nut diameter, nut width, shell thickness, the whole dried nut weight, and kernel weight) were

Table 2
Genetic diversity indices of the analysed 12 *Juglans regia* cultivars applying 12 SSR markers (N: number of samples, N_a: number of alleles, N_e: effective number of alleles, I: Shannon's information index, H_o: observed heterozygosity, H_e: expected heterozygosity, F: fixation index).

SSR Marker	N	N_a	N_e	I	H_o	H_e	F
WGA01	12	3	2.880	1.078	0.500	0.653	0.234
WGA04	12	2	1.882	0.662	0.583	0.469	−0.244
WGA27	12	2	1.600	0.562	0.333	0.375	0.111
WGA72	12	2	1.280	0.377	0.250	0.219	−0.143
WGA89	12	4	2.215	1.013	0.583	0.549	−0.063
WGA118	12	5	2.504	1.177	0.667	0.601	−0.110
WGA202	12	6	3.000	1.328	0.667	0.667	0.000
WGA276	12	6	3.236	1.452	0.583	0.691	0.156
WGA321	12	4	3.840	1.364	0.833	0.740	−0.127
WGA331	12	3	2.165	0.837	0.667	0.538	−0.239
JR 1817	12	3	1.540	0.652	0.250	0.351	0.287
JR 6160	12	2	1.492	0.512	0.250	0.330	0.242
Min	12	2	1.280	0.377	0.250	0.219	−0.244
Max	12	6	3.840	1.452	0.833	0.740	0.287
Mean	12.000	3.500	2.303	0.918	0.514	0.515	0.009
SE	0.000	0.435	0.232	0.106	0.057	0.048	0.055

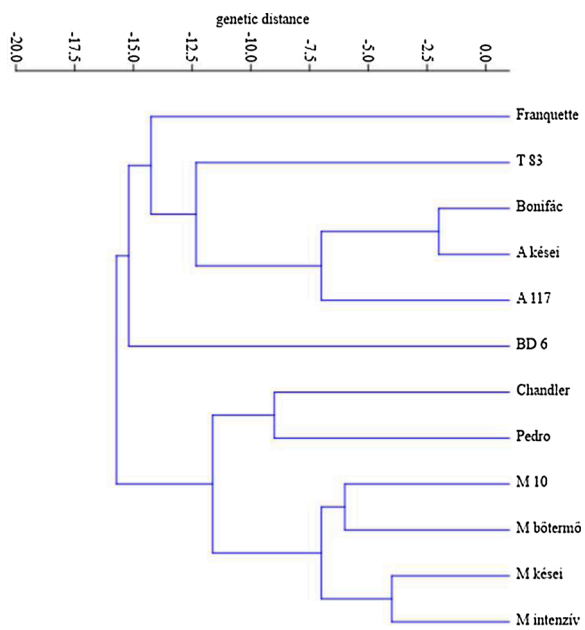

Fig. 1. Genetic distance based UPGMA dendrogram of the analysed 12 *Juglans regia* genotypes (A: Alsószentiváni, M: Milotai, T: Tiszacsécsi).

examined using UPOV walnut descriptors (Anonymous, 2017). From the measured data, the rotation index (nut diameter / nut height), and kernel rate (kernel weight / dried nut weight) were calculated. Additionally, a new index called cracking rate was introduced, which means ratio of halves to the whole kernel weight.

To separate the significance factors Multifactor Anova of Statgraphics XVII X64 software package was used. The results are presented as means ± standard error. Relationships between the observed traits in the correlation matrix were calculated by Pearson correlation coefficient.

A Principal Component Analysis (PCA) was conducted on the measured nut and kernel characteristics respectively by PAST 4.03 (Hammer et al., 2001). A pair wise, individual-by-individual distance matrix was generated in case of the nut and kernel data set using the Manhattan distance index by PAST. Distance matrices were used to construct an UPGMA dendrogram as well as to test the putative correlation between genetic and phenotypic data sets via Mantel test.

3. Results

3.1. SSR genotyping

All the nine Hungarian cultivars, as well as the French and the two US-bred varieties have a distinct, unique genotype based on the applied 12 SSR markers. The method was appropriate for genetic fingerprinting as the P_I value (in other words the probability of random matching) was very low (2.027×10^{-7}). All of the analysed 12 SSR markers proved to be polymorphic. The main genetic indices are presented in Table 2. Among the analysed 12 *Juglans regia* genotypes, the three most informative loci were WGA276, WGA321 and WGA202 regarding the number of alleles (N_a), the effective number of alleles (N_e), the Shannon's information index (I) and the heterozygosity values (H_o, H_e). The three less informative markers were WGA72, JR 6160 and WGA27 based on all the diversity indices. In case of six markers out of the applied 12 SSR loci, the values of the observed heterozygosity exceeded the expected heterozygosity resulting in a negative fixation index (F). In other words, an excess of heterozygote genotypes can be observed among the 12 analysed walnut cultivars.

Origin of the varieties can be examined by the genetic distance based UPGMA dendrogram (Fig. 1). It is clearly visible that the local selection 'Milotai 10' stands genetically closer to the US-bred cultivars and separates in a common cluster together with their hybrids. 'Milotai bőtermő' had a closer relationship to its mother cultivar ('Milotai 10') than their hybrids derived from the 'Milotai 10' and 'Pedro' cross. On the other hand, 'Tiszacsécsi 83' and 'Alsószentiváni 117' form a genetically different cluster, while the new selection 'BD6' seems to be a relatively distinct genotype, however showing a closer relationship with this later group. 'Alsószentiváni 117' separated well together with its hybrid varieties, and 'Bonifác' and' Alsószentiváni kései' are closer to each other. The position of the French cultivar, 'Franquette' is not as clear as in case of the US-bred cultivars, but it shows more similarity with the 'Tiszacsécsi 83' and 'Alsószentiváni 117' group. Based on the PCA plot of the genetic data, a clear separation of the four local selections ('Milotai 10', 'Alsószentiváni 117', 'Tiszacsécsi 83' and 'BD6') from the introduced cultivars and the hybrids can be seen. Again, the separation of the 'Milotai 10' and US lineage is unambiguous, as well as the closer positioning of 'Tiszacsécsi 83' and 'BD6'. 'Franquette' bears a closer relation to 'Tiszacsécsi 83' and 'BD6', but stands still between the US-bred varieties and the local genotypes (Fig. 2).

3.2. Nut characteristics

Nut measurement results are summarized in Table 3. The nut height varied between 36.2 and 46.7 mm, the largest nut heights were measured in the case of 'BD6', while in the case of 'Milotai intenzív' the smallest ones. The largest nut diameter was produced by 'Alsószentiváni kései' (35.4 mm), and the smallest one by' Tiszacsécsi 83' (32.2 mm). The rotation index was around 0.9 except 'BD6' reached 0.74. The nut width was between 33.6 and 38.3 mm, 'Alsószentiváni kései' had the largest value in this category, the smallest values were measured on 'Chandler' and 'Tiszacsécsi 83'. The shell thickness values ranged from 1.37 ('Milotai intenzív') to 1.89 mm ('Milotai kései').

Dried fruit and kernel data are presented in Table 4. The dried nut weight was between 11.4 and 16.6 g per nut,' BD6' reached the largest nut weight, while' Tiszacsécsi 83' the smallest one. The kernel weight was in the range of 5.5 and 7.3 g per kernel, the highest kernel weights were observed on 'Alsószentiváni 117', and the lowest on 'Chandler'. The kernel rate varied between 0.41 % and 0.53 %, the highest was calculated for 'Milotai intenzív', the lowest values for 'Alsószentiváni kései'. The cracking rate had a larger range, between 0.37 % and 0.87 %, compared to the values of the previously mentioned categories; the highest rate was calculated for 'Milotai bőtermő', the lowest for 'Alsószentiváni 117'.

The relationship of the analysed varieties regarding nut and kernel

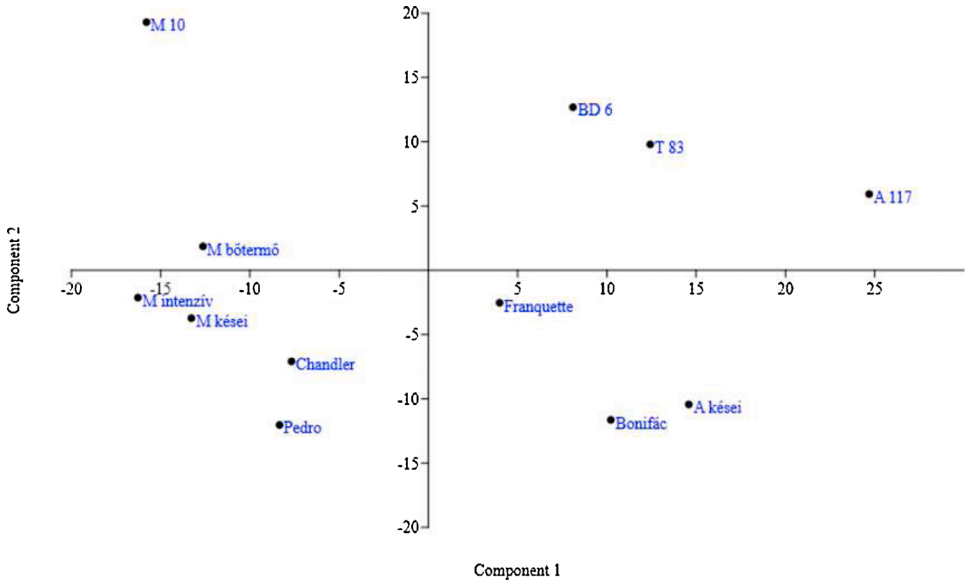

Fig. 2. PCA plot of the analysed 12 *Juglans regia* genotypes based on the applied 12 SSR markers.

Table 3
Nut characteristics of the observed varieties.

Name of the variety	Nut height (mm)[1]	Nut diameter (mm)[2]	Rotation index[3]	Nut width (mm)[4]	Shell thickness (mm)[5]
Alsószentiváni 117	38.3 ± 2.3 a	34.4 ± 1.7 a	0.90 ± 0.05 a	35.7 ± 2.4 a	1.69 ± 0.2 a
Milotai 10	36.7 ± 2.7 bc	33.2 ± 2.3 cd	0.90 ± 0.03 a	34.8 ± 2.1 ab	1.56 ± 0.2 bcd
Tiszacsécsi 83	37.5 ± 3.1 ab	32.2 ± 3.1 e	0.85 ± 0.05 c	33.6 ± 2.5 b	1.46 ± 0.2 cde
Milotai intenzív	36.2 ± 2.6 c	34.4 ± 2.5 abc	0.94 ± 0.00 b	35.3 ± 2.1 a	1.37 ± 0.2 de
Milotai bőtermő	38.4 ± 2.3 a	34.8 ± 2.0 ab	0.90 ± 0.10 a	37.3 ± 2.2 c	1.61 ± 0.2 ab
Chandler	38.2 ± 2.0 a	33.0 ± 1.8 de	0.86 ± 0.10 c	33.6 ± 1.8 b	1.55 ± 0.2 bcd
Bonifác	38.3 ± 5.7 a	34.3 ± 1.8 abc	0.90 ± 0.10 a	35.0 ± 1.9 a	1.73 ± 0.3 ab
Alsószentiváni kései	38.2 ± 2.0 abc	35.4 ± 1.1 ab	0.92 ± 0.05 ab	38.3 ± 1.7 c	1.71 ± 0.2 ab
Milotai kései	38.7 ± 2.1 a	33.9 ± 1.4 acd	0.87 ± 0.04 c	35.9 ± 2.0 ab	1.89 ± 0.2 f
BD6	46.7 ± 2.4 d	34.6 ± 1.5 a	0.74 ± 0.2 d	37.1 ± 2.5 c	1.63 ± 0.3 ab

[1] : SD ($p \geq 0.05$) = 1.3.
[2] SD ($p \geq 0.05$) = 0.9.
[3] SD ($p \geq 0.05$) = 0.02.
[4] SD ($p \geq 0.05$) = 1.2.
[5] SD ($p \geq 0.05$) = 0.13.

characteristics can be explained by a PCA plot (Figs. 3 and 4) and also by an UPGMA dendrogram (Fig. 5a–b). The clear separation of 'BD6' is evident based on the nut data, and also visible based on the kernel data. The other Hungarian varieties are closer to each other and to 'Chandler'. The most similar Hungarian varieties to 'Chandler' are' Tiszacsécsi 83' and 'Milotai 10'. 'Alsószentiváni 117' and most of the hybrids represent a moderately distinct group compared to the control 'Chandler', however not as distinct as 'BD6' based on nut data.

3.3. Correlation between traits

Table 5 contains the correlation coefficients of all observed categories described in this paper. The highest correlation was observed between rotation index and nut height (0.768), between dried nut weight and kernel weight (0.674), between nut height and dried nut weight (0.673), nut width and dried nut weight (0.665), and between nut diameter and nut width (0.630). While the dried nut weight had a high correlation with nut height and nut width, this character showed only a moderate correlation with the nut diameter (0.534) in our study.

Correlation of shell thickness was low in our database collected from the Hungarian assortment. The highest value was only 0.300 measured between shell thickness and dried nut weight. The kernel weight correlated well with the dried nut weight (0.674), but some correlation could be detected also with nut diameter (0.551), and nut height (0.470). The kernel ratio reached the highest correlation with kernel weight (0.466) and dried nut weight (0.462) in this study.

Mantel tests were conducted (detailed data not shown here) in order to test putative correlation between the genetic matrix and distant matrices of the measured nut and kernel characteristics. No genetic correlation was found with any of the traits.

4. Discussion

There was a keen interest in the genotypes having a large nut size in the Hungarian walnut breeding program. The largest nut height was observed in case of 'BD6', which was a significant difference from the other cultivars involved in the trial. The nut diameter is important for the growers, because 32 mm in diameter is the minimum border of the first grade category, which means more income for the growers compared to the lower grades requiring smaller nut sizes. During the data collection period all examined varieties reached the 32 mm in diameter, the novel bred lateral bearing cultivars ('Milotai intenzív', 'Milotai bőtermő', 'Bonifác', Alsószentiváni kései', 'BD6') reached the premium + grade, because their average nut diameter was at least 34 mm in diameter. Typical for the Hungarian bred walnut cultivars is that they have a round nut shape; the novel bred 'BD6' is the only variety

Table 4
The whole dried fruit weight, kernel weight, kernel rate and cracking index of the varieties involved in the trial.

Name of the variety	Dried fruit weight (g)[6]	Kernel weight (g)[7]	Kernel rate (%)[8]	Cracking rate (%)[9]
Alsószentiváni 117	14.7 ± 2.2 a	7.3 ± 1.0 a	0.50 ± 0.10 a	0.37 ± 0.30 a
Milotai 10	12.6 ± 2.4 e	5.8 ± 1.4 ef	0.46 ± 0.10 ab	0.72 ± 0.10 cd
Tiszacsécsi 83	11.4 ± 1.7 f	5.6 ± 0.9 f	0.49 ± 0.06 ab	0.69 ± 0.30bc
Milotai intenzív	11.6 ± 2.8 f	6.0 ± 1.6 def	0.53 ± 0.10 a	0.79 ± 0.10 def
Milotai bőtermő	13.7 ± 2.3 d	6.6 ± 1.5 bc	0.48 ± 0.10 ab	0.87 ± 0.20 f
Chandler	11.8 ± 1.3 e	5.5 ± 1.0 f	0.46 ± 0.10 abc	0.86 ± 0.10 ef
Bonifác	13.8 ± 1.3 a	6.3 ± 0.9 cde	0.46 ± 0.10 abc	0.62 ± 0.10 b
Alsószentiváni kései	15.5 ± 2.5 abc	6.4 ± 1.7 cde	0.41 ± 0.07 bc	0.62 ± 0.20 bc
Milotai kései	15.2 ± 1.7 ab	6.5 ± 1.0 cd	0.42 ± 0.04 bc	0.78 ± 0.07 de
BD6	16.6 ± 2.2 bc	7.1 ± 1.7 ab	0.42 ± 0.10 c	0.68 ± 0.10 bc

[6] SD ($p \geq 0.05$) = 1.0.
[7] SD ($p \geq 0.05$) = 0.7.
[8] SD ($p \geq 0.05$) = 0.04.
[9] SD ($p \geq 0.05$) = 0.1.

with an elongated shape (larger nut height than diameter).

Walnut cultivars / genotypes with large fruit size were selected just some breeding programs; in Ukraine (the nut diameter varied between 36.7 and 40.1 mm, Kondratenko et al., 2005), and in Vietnam (ranged from 40.4–49.7 mm, Son et al., 2019). Cultivars / genotypes from the other breeding programs have similar or a smaller nut size compared to cultivars appearing on the Hungarian National Variety List (Solar and Stampar, 2005; Botu et al., 2010; Gandev, 2019; Akça et al., 2020, Skender2020). Interesting to see, that the walnut cultivars derived from the Bulgarian (Gandev, 2019) and Iranian breeding program (Hassani et al., 2020) had a bit thicker shell thickness value (around 1 mm) than measured on the cultivars from the Carpathian race.

Our results about the nut characteristics are similar to the results reported in different breeding programs (Zeneli et al., 2005; Pop et al., 2013; Hassani et al., 2014; Solar and Stampar, 2005; Solar et al., 2014). However, there are some extreme values can be read in the literature: kernel ratio of the genotypes derived from the Kazakh population can be varied between 33.5 % and 70.9 % (Akça et al., 2020), or in Iran this character was between 35.5 % and 71.0 % (Hassani et al., 2020), up to 63.8 % for some Albanese genotypes (Zeneli et al., 2005), 62.1 % and 66.2 % for some other Iranian genotypes (Ebrahimi et al., 2015; Rezaei et al., 2018), some new Serbian cultivars can reach 51.2 % and 57.8 % kernel ratio (Cerović et al., 2010). Some genotypes derived from the Northern provinces of Vietnam can be ranged from 38.1–70.8 g fruit weight (Son et al., 2019), Cosmulescu et al. (2017) found genotypes having fruit weight up to 20.9 g near Bechet, Romania. In previous studies high correlations were found between nut height and dried nut weight, dried nut weight and kernel weight, nut width and dried nut weight, nut diameter and nut width, dried nut weight and nut diameter, kernel weight and nut diameter (Sharma and Sharma, 2001; Eskandari et al., 2005; Amiri et al., 2010; Cosmulescu and Botu, 2012; Mahmoodi et al., 2016, 2019; Poggetti et al., 2017). Except dried nut weight and nut diameter, as well as kernel weight and nut diameter relations, all other mentioned ones were confirmed also in this study. Furthermore, nut width and kernel weight showed a good correlation, too.

Comparing our results with a recent, detailed study regarding the Iranian core collection (Mahmoodi et al., 2019), we can conclude, that the correlation between some of the measured nut characteristics are congruent with the Iranian data, while others are quite different. The nut diameter and the nut height correlation were similar to what Mahmoodi et al. (2019) reported, with a value of 0.58 and 0.537 in the Iranian and in the Hungarian assortment respectively. The correlation of nut width and nut height was slightly higher in the Hungarian assortment (0.545), than in the Iranian population (0.53). It is opposite for the correlation of nut width and nut diameter; this value was 0.630 in the Hungarian assortment and 0.66 in the Iranian core population.

Correlation of shell thickness was low in our database compared to the Iranian data (Mahmoodi et al., 2019); the highest value was measured between shell thickness and dried nut weight (0.3), and this value was 0.53 in the Iranian population.

The kernel weight correlated well with the dried nut weight (0.67), nut diameter (0.55), and nut height (0.47) in our dataset derived from the Hungarian assortment. In the Iranian core collection, the kernel weight also correlated well to the categories mentioned before, but all the correlation coefficients were higher; 0.84 nut fruit weight, 0.68 nut diameter and 0.54 nut height (Mahmoodi et al., 2019).

The kernel ratio reached the highest correlation with kernel weight (0.466) and dried nut weight (0.462) in our study; regarding the similar

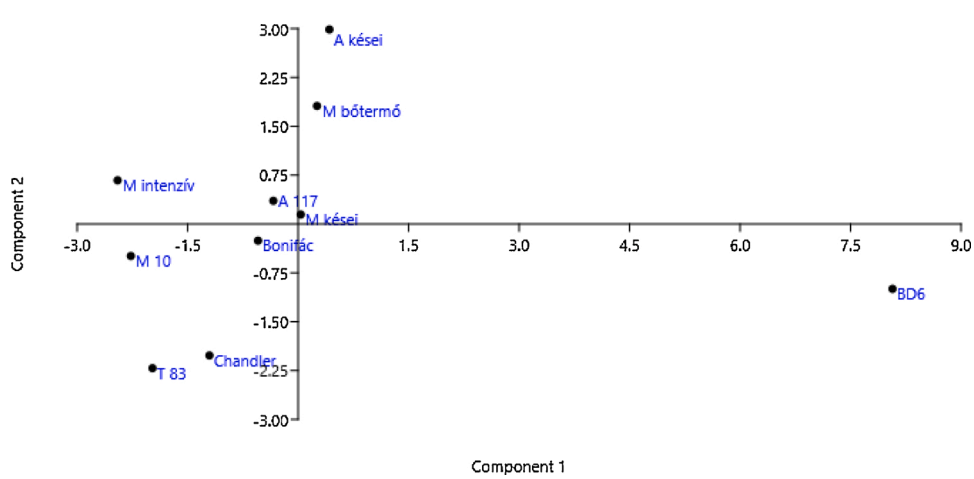

Fig. 3. PCA plot of the analysed varieties based on nut characteristics.

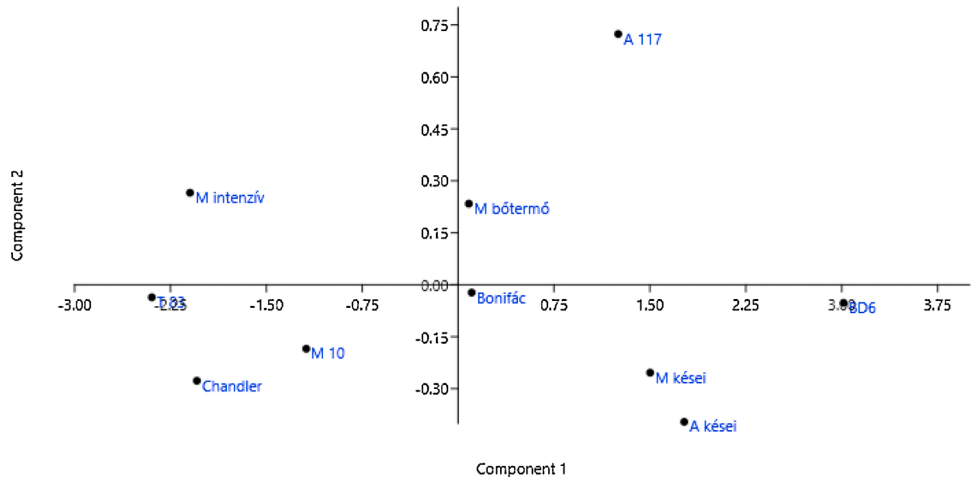

Fig. 4. PCA plot of the analysed varieties based on dried fruit and kernel characteristics.

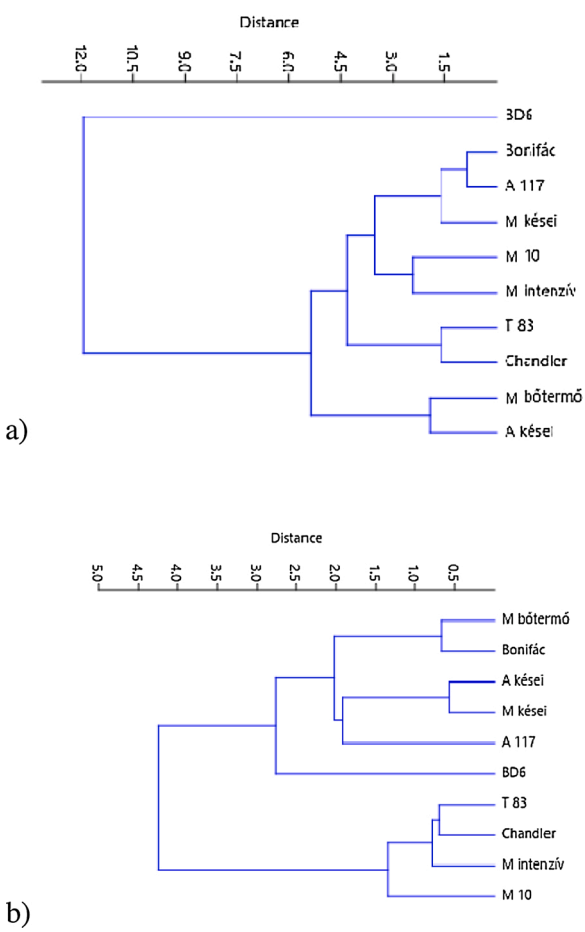

Fig. 5. UPGMA dendrograms constructed based on Manhattan distance matrices of nut (a) and kernel (b) characteristics.

relations in the Iranian core collection kernel ratio and kernel weight showing almost the same correlation (0.43), while the correlation between kernel ratio and dried nut weight was much lower (0.11) (Mahmoodi et al., 2019). Finally, the cracking index showed low correlations with all observed characters.

Analysing the putative correlation between the genetic distances and the measured nut traits by Mantel test, there was no evidence that an obvious linkage could be found between the nut characteristics and any of the selected genotypes. In other words, all the Hungarian bred cultivars imply the potential of the superior quality regarding nut and kernel features. Moreover, the absence of genetic correlation is not surprising, since the Hungarian variety assortment is the result of a long - lasting selection work for specified nut traits, consisting of genotypes that were derived from the local field selection or from F1 offspings selection of directional crosses.

The genetic investigation shed light also on the origin of the Hungarian varieties selected in the Carpathian Basin. The results seem to contradict the former theory supposing French origin, and support rather an authentic local origin from indigenous population in case of most of the selected varieties. The only exception is 'Milotai 10' in which case a potential influence from the US-bred cultivars can be observed.

5. Conclusions

Some previous studies examined individual Hungarian bred varieties or genotypes derived from the Carpathian Basin, while this is the first time the origin of the full Hungarian Persian walnut assortment was investigated. The results confirmed that the varieties, registered on the Hungarian National Variety List, are unique genotypes based on the applied 12 SSR markers. Strong relationships are confirmed between nut height and dried nut weight, dried nut weight and kernel weight, nut width and dried nut weight, nut diameter and nut width, dried nut weight and nut diameter, kernel weight and nut diameter. One more relation (nut width and kernel weight) was found, which showed a strong relation. Beside their unique genetic background the Hungarian walnut cultivars reached the premium fruit grade categories. There was no evidence that an obvious linkage could be found between the nut characteristics and any of the selected genotypes derived from the Hungarian variety assortment, which is the result of a long - lasting selection work for specified nut traits.

CRediT authorship contribution statement

Geza Bujdoso: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing - original draft, Writing - review & editing. **Klara Cseke:** Conceptualization, Data curation, Formal analysis,

Table 5
Correlation matrix of investigated characteristics.

	ND	NW	DNW	RI	ST	KW	KR	CI
NH	0.537	0.545	**0.673**	**0.768**	0.118	0.470	0.176	0.310
ND		**0.630**	0.534	0.234	0.109	0.551	0.031	0.044
NW			**0.665**	0.141	0.100	0.565	0.089	0.077
DNW				0.367	0.300	**0.674**	0.462	0.109
RI					0.100	0.100	0.200	0.104
ST						0.137	0.151	0.070
KW							0.466	0.100
KR								0.054

NH: nut height, ND: nut diameter, NW: nut width, DNW: dried nut weight, RI: rotation index, ST: shell thickness, KW: kernel weight, KR: kernel ratio, CI: creaking index.

Methodology, Validation, Visualization, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors report no declarations of interest.

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