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In-situ assessment of the traditional Lebanese walnut germplasm using morphological characteristics and ISSR markers

A. Chehade [1 (*)], **F. Ibrahim** [1], **F. Jibbawi** [1], **A. Elbitar** [2], **H. Hammoud** [3 (*)]

[1] *Lebanese Agricultural Research Institute, Tal Amara Station, Department of Plant Biotechnology, Plant Genetic Resources Unit, P.O. Box 287, Zahle, Lebanon.*

[2] *Lebanese Agricultural Research Institute, Tal Amara Station, Department of Plant Biotechnology, Plant Tissue Culture Unit, P.O. Box 287, Zahle, Lebanon.*

[3] *Lebanese University, Faculty of Science, Conservation and Management of Natural Resources (Master program), Hadath, Lebanon.*

Key words: Diversity, ISSR markers, *Juglans regia L.,* Lebanon, morphological descriptors.

Abstract: The characterization of walnut cultivars is an important step towards conservation and exploitation of the Lebanese walnut diversity. In this study, we aimed to evaluate the genetic and phenotypic diversity within traditional Lebanese walnut cultivars grown in different regions of Lebanon. A total of 35 accessions were submitted to an ampelographic assessment; 26 of them were genetically studied using Inter Simple Sequence Repeat (ISSR) markers. A set of 27 morphological characters, including 13 qualitative traits and 14 quantitative traits related to the tree, leaf, nut, and kernel, was examined. Seven specific characters of the walnut were revealed to be the most discriminating. These characters included: leaflet shape, shell texture, shell color, nut shape, nut weight, kernel weight, and kernel color. Based on these traits, the clustering analysis identified three distinct groups that are clearly differentiated by the leaflet shape and margin. Molecular assessment was conducted using nine ISSR primers already used for walnut trees. These primers generated 72 bands, of which 67 were polymorphic. The average of the observed alleles was equal to eight in each locus. ISSR clustering based on Jaccard distance did not reveal similarity between accessions with the molecular studies. The highest genetic distance was observed between genotypes T34 and T2, differing by 45 bands (62.5%), while the lowest was between genotypes T2 and T6, differing by only four bands (5.5%). Accordingly, the results revealed a high diversity among Lebanese walnut accessions that should be further investigated to determine the major walnut genotypes and to evaluate the effect of agroclimatic conditions on the morphotypes.

(*) **Corresponding author:**
alichehade@hotmail.com.
haidar.hamoud.17@gmail.com.

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ORCID:
CA: 0009-0003-1361-2807
EA: 0009-0002-5184-2294

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1. Introduction

Walnuts belong to the genus *Juglans* of the family *Juglandaceae* of which the most commonly grown species is the Persian walnut (*Juglans regia*). Walnuts are native to Central Asia, Eastern Europe and North America. Walnut trees usually require higher altitudes for growing well (1200 -2000 m above sea levels). They are adapted to sunny climate in summers and moderate winters. However, the trees grow well in cool and dry conditions, with optimum rainfall exceeding 800 mm. Walnuts, due to their high nutritional value, are prioritized by the Food and Agriculture Organization (FAO) (Hassan *et al.,* 2013). They are consumed in numerous ways and are also pressed for oil (Gao *et al*., 2024). Nuts are highly valued in the pharmaceutical and food industries for their high content of proteins, carbohydrates, lipids, and micronutrients. Additionally, walnuts contain bioactive compounds such as sterols, dietary fiber, and polyphenols (Sharma *et al*., 2022), which possess antimicrobial, antifungal, anti-inflammatory, antiviral, and anticancer properties. Walnuts provide value for the ecosystem in terms of offering a habitat and nourishment for many animals. The wood of some species of walnut is highly prized for its color, hardness, and grain, being used for furniture and other purposes.

The geographical nature of Lebanon is the ideal habitat for the growth of walnut trees. It is considered one of the traditional and economically important crops, and its cultivation is widespread in most Lebanese regions, especially in the north of Lebanon and the Bekaa region, near the rivers (Janta, Labweh, Ajar), in irrigated areas, and in home gardens. According to the agricultural census (MOA, 2012), the area of nut trees was approximately 1282.5 ha, with a majority of walnut trees, covering an approximate area of 1205 ha.

In Lebanon, walnut genotypes are highly diverse, often derived from nuts or cuttings, resulting in numerous landraces that are adapted to different conditions. There are many local traditional accessions but no named cultivars. Walnut accessions are distinguished and denominated according to the shape of the fruit as Dmagh shape (Brain shape), Kalb shape (Heart shape) or the hardness of the nuts (Ferk, Kasi). These accessions may harbor important traits for future breeding programs and for enhancing the genetic database of international cultivars. Over the last few decades, the traditional walnut germplasm has been threatened by various anthropogenic pressures, particularly the progressive replacement of local cultivars by more advantageous, improved varieties imported from abroad. Moreover, walnut germplasm is threatened by urbanization, climate change, outbreaks of new diseases and pests, and de-vegetation due to timber harvesting. Little information is available about the genetic diversity of Lebanese walnuts. Therefore, the preservation of traditional cultivars, which are at risk of disappearance, is a critical necessity in order to maintain the local genetic diversity of walnuts. Accordingly, characterizing the autochthonous cultivars is an important step towards conserving and exploiting the diversity of the Lebanese walnut.

Several techniques have been developed that can be used to estimate the genetic diversity of walnuts, including morphological characteristics (Atefi, 1997; Einollahi and Khadivi, 2024) and various molecular markers, such as ISSR (Potter *et al.*, 2002; Abbasi Holasou *et al*., 2023), RFLP (Fjellstrom and Parfitt, 1995; Bernard *et al*., 2018), SSR (Wang *et al.,* 2008; Wani *et al.*, 2024) and RAPD (Fatahi *et al.*, 2010). The objectives of this study were to determine the level and distribution of genetic diversity of the Lebanese traditional walnut accessions using morphological traits and ISSR markers.

2. Materials and Methods

Field survey

Field survey was performed during vegetation (Early Spring 2024) and production periods (September 2024) with the aim of collecting traditional cultivars growing in family gardens and commercial plantations. Samples of 35 walnut accessions were collected from 12 cultivated stands that are located in major walnut producing villages, which spread over four main agricultural areas (the North plain, Bekaa plain, Mount Lebanon and the South). These sites are subjected to varying climatic conditions (precipitation and temperature) and different agricultural practices. They are situated at an altitude between 262 and 1566 m, a latitude ranging from N33.27762° to N34.46924° and a longitude between E35.34504° and E36.2242° (Table 1, Fig. 1). Trees were selected based on their age (minimum of 10 years) and their sanitary state. Samples consisted of 20 mature fruits and leaves were collected from one tree per accession. The

Table 1 - Geographic and climatic information of 12 walnut sites surveyed in Lebanon

Sites	Zone	Latitude (°/N)*	Longitude (°/E) (z)	Altitude (m) (z)	Precipitation (mm)	Average annual mean surface air temperature (°C)	Number of accessions
Nabi sheeth	Beqaa	33.52478	36.06654	1187	570-750	13.6	5
Janta	Beqaa	33.51527	36.0416	1106	580-750	13.2	4
Nahle	Beqaa	34.03535	36.17998	1434	500-700	13	5
Labweh	Beqaa	34.12528	36.20107	872	350-450	15.5	2
Anjar	Beqaa	33.44001	35.56798	882	550-680	15.28	5
kfardabch	Beqaa	33.94350	36.03667	1180	540-640	13.10	2
Tal Al-amara	Beqaa	33.86388	35.98658	910	540-650	15	1
sohmor	Beqaa	33.53194	35.69833	850	600-800	15	2
Maaroub	south	33.27762	35.34504	262	650-750	20	1
Fnaydeq	North	34.46924	36.2242	1566	750-1000	14	5
Alay	Mount Lebanon	33.80485	35.60151	911	1000-1200	15.69	2
Bisour	Mount Lebanon	33.75450	35.57685	350	1000-1200	15.69	1

(z) Data listed by the Global Positioning System (GPS).

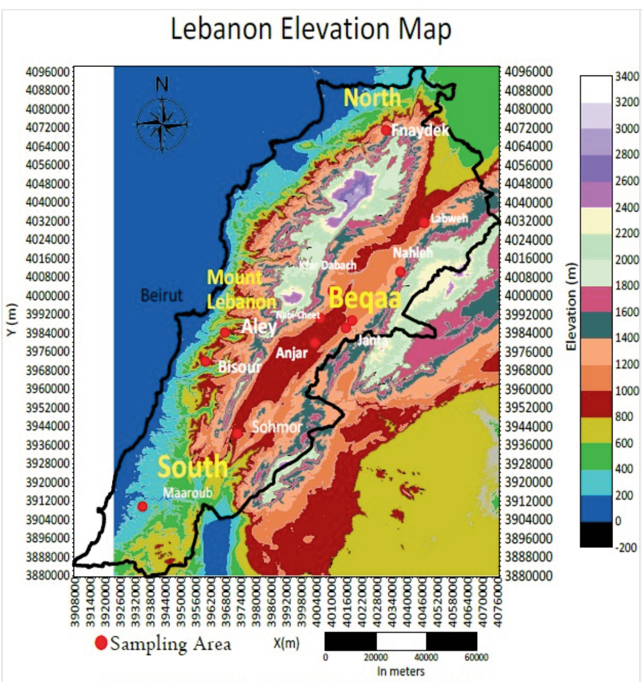

Fig. 1 - Geographic distribution of the studied walnut accessions as visualized with DIVA-GIS program.

sampled young leaves were stored at -20°C pending DNA extraction.

Morphological descriptors

Twenty-seven morphological traits related to the tree, leaf, nut and kernel extracted from the list of walnut descriptors previously developed by International Plant Genetic Resources Institute (IPGRI, 1994) were used in this study, of which thirteen qualitative (Growth habit, leaflet shape, leaflet margin, leaflet color, rachis color, nut shape, shell texture, shell color, shell strength, nut length of tip, nut shape of base, nut shape of apex, kernel color) and fourteen quantitative (Leaf length, leaf petiole, leaf width, number of leaflets, leaflet length, leaflet width, leaflet length/leaflet width, leaflet petiole, nut width, nut length, nut length/ nut width, nut weight, kernel weight, kernel percentage).

Molecular characterization

DNA for each walnut accession was extracted from 300 mg of young leaves using cetyl-trimethyl ammonium bromide (CTAB) procedure described by Doyle and Doyle (1987). DNA quantity and quality were determined spectrophotometrically at 260 nm and 280 nm (using a NanoDrop) and by electrophoresis on a 1% agarose gel stained with ethidium bromide and visualised under UV light.

Nine ISSR primers (UBC807, UBC810, UBC811, UBC814, UBC818, UBC819, UBC821, UBC826, UBC865) previously developed for the assessment of the walnut germplasm (Christopoulos *et al.*, 2010; Aiqing *et al.*, 2014; Shamasbi *et al.*, 2018; Çilesiz, 2025) were used in this study based on their good results for amplification and high power of discrimination on walnut.

Microsatellite amplifications were performed in a total volume of 20 µl with 2 µl of PCR buffer (10mM Tris-HCl, 50 mM KCl, 0.1% Triton x 100 and 0.02% of gelatin), 2.5 mM MgCl2, 0.2 mM of each dNTP, 2.5 µM primer, 1,5 U of Taq DNA polymerase and 50 ng of walnut genomic DNA. PCR was carried out using a Bio-rad thermocycler. PCR reactions were run with a temperature pattern of initial DNA denaturation at 95°C for 4 minutes, followed by 35 cycles of DNA denaturation at 94°C for 45 seconds, primer annealing at 45-47-51.3°C according to primer for 45 seconds, the extension stage with 72°C for 2 minutes and a final extension step of 7 min at 72°C. Five µl of the PCR products were separated on a 2% agarose gel electrophoresis prepared in TBE 1X stained with ethidium bromide to check the PCR amplification and determine the size of the amplified fragments.

Data analysis

Qualitative characteristics have been described and scored according to IPGRI standards (IPGRI, 1994). For quantitative traits, the mean ± standard deviation was calculated. Means were compared using analysis of variance (Anova) followed by the Least Significant Difference (LSD) tests, performed with SAS software (SAS, 1995).

Trait evaluation was performed by using the principal component analysis (PCA). The relationships between walnut leaves and fruits based on their quantitative and qualitative traits were analyzed using Hierarchical Cluster Analysis with Euclidean distance in the PAST software (Hammer *et al*., 2001).

To assess the information given by ISSR markers, amplified bands were scored as 1 for presence or 0 for absence. The following parameters were calculated as follow: number of total alleles per locus, the percentage of polymorphic band (PPB), Polymorphism Information Content for the dominant markers (PIC = 2 x f x (1-f), where f is the frequency of present allele) and power of discrimination ($PD = 1 - \Sigma gi^2$, where gi is the frequency of the i_{th} genotype). Genetic distances were calculated according to Jaccard. Trees clustering the data with the unweighted pair-group method (UPGMA) with SAHN-clustering and tree programs of PAST software (Kriege *et al.,* 2014).

3. Results and Discussion

Morphological characterization analysis

In this study, a set of 27 descriptors was examined for 35 walnut accessions collected from various agroclimatic areas in Lebanon, encompassing 13 qualitative and 14 quantitative traits related to the tree, leaf, nut, and kernel. Residual normality was assessed for 14 quantitative traits using the Shapiro-Wilk test. Six traits (Leaf length, leaf width, leaf petiole, leaflet length, leaflet width and kernel weight) were normally distributed (P>0.05) and were analyzed with Anova test followed by LSD. The walnut samples of these traits showed significant differences among walnut trees (p<0.05) and pairwise comparisons using LSD indicated which walnut trees differed significantly.

The remaining eight traits violated the normality assumption (p<0.05) were showed statistically significant differences between walnut tress by using nonparametric procedures (Mann-Whitney U test, data not shown).

Morphological characteristics of the Trees and leaves

The growth habit of the tree showed diversity among the studied accessions, varying between erect, semi-erect, and spreading. Differences were clearly observed in the leaflet shape (Fig. 2), which ranged from narrow elliptic (Two accessions) to elliptic (23 accessions) and broad elliptic (10 accessions), predominantly exhibiting entire margins. The leaflet and rachis color of nearly 71% and 66% of the accessions were green and green to yellow, respectively. The two accessions, T27 (Tal Al-Amara) and T44 (Bisour), exhibit unique traits, such as narrow-elliptic leaflets and serrate margins, which

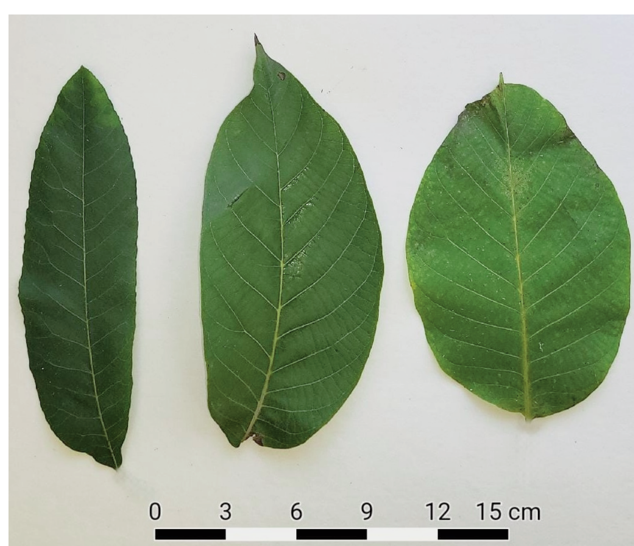

Fig. 2 - Leaflet shapes, from left to right: Narrow elliptic, Elliptic, and Broad elliptic.

emphasise morphological diversity (Table 2).

The accessions showed significant differences in the leaf length ranging from 21.81 ± 3.88 cm (T44, Bisour) to 44.60 ± 4.29 cm (T16, Nahle) and leaf width between 18.69 ± 3.66 cm (T44, Bisour) and 40.88 ± 3.75 cm (T43, Alay) (Table 3). Petiole length average of the leaf was between 4.20 ± 0.71 cm (T44, Bisour) and 10.75 ± 1.55 cm (T43, Alay). The leaflet number of the leaf was between 3 (T11, Janta) to 15 (T27, Tal Amara). The average leaflet length ranged between 9.32 ± 1.38 cm (T44, Bisour) and 20.59 ± 1.47 cm (T43, Alay) and width between 2.70 ± 0.31 cm (T44, Bisour) and 8.99 ± 0.66 cm (T43, Alay). The petiole length of the leaflet of nearly 77% of the accessions was 0.1 cm. The ratio leaflet length to leaflet width in the majority of the samples exceeded a ratio of two, reflecting the narrow elliptic shape of the leaflets. Accordingly, Bisour accession (T44) had the lowest leaf length, leaf width, petiole length, leaflet length, leaflet width, while Alay accession (T43) had the highest leaf width, petiole length, leaflet length, and leaflet width.

Table 2 - Qualitative descriptors of the Leaf and tree recorded for the 35 walnut accessions

Sample	Site	Growth habit	Leaflet shape	Leaflet margin	Leaflet color	Rachis color
T1	Nabi sheeth	Spreading	Broad elliptic	Entire	Green	Yellow
T2		Semi-erect	Broad elliptic	Entire	Green	Green to Yellow
T6		Spreading	Elliptic	Entire	Dark Green	Green to Yellow
T7		Spreading	Broad elliptic	Entire	Green	Green to Yellow
T8		Spreading	Broad elliptic	Entire	Dark Green	Green to Yellow
T9	Janta	Spreading	Broad elliptic	Entire	Green	Green to Yellow
T10		Spreading	Broad elliptic	Entire	Green	Green to Yellow
T11		Semi-erect	Broad elliptic	Entire	Dark Green	Green to Yellow
T12		Spreading	Elliptic	Entire	Green	Green to Yellow
T13	Nahle	Spreading	Elliptic	Entire	Green	Green to Yellow
T14		Semi-erect	Broad Elliptic	Entire	Green	Green
T15		Semi-erect	Elliptic	Entire	Green	Green
T16		Erect	Broad elliptic	Entire	Dark Green	Green
T17		Spreading	Elliptic	Entire	Green	Green to Yellow
T18	Labweh	Spreading	Elliptic	Entire	Green	Green
T19		Spreading	Elliptic	Entire	Green	Green
T20	Anjar	Spreading	Elliptic	Entire	Green	Green to Yellow
T21		Spreading	Elliptic	Entire	Green	Green to Yellow
T22		Erect	Elliptic	Entire	Green	Green to Yellow
T23		Spreading	Elliptic	Entire	Green	Green to Yellow
T24		Spreading	Elliptic	Entire	Green	Green to Yellow
T25	kfardabch	Spreading	Elliptic	Entire	Green	Green to Yellow
T26		Spreading	Elliptic	Entire	Green	Green to Yellow
T27	Tal Al-amara	Semi-erect	Narrow elliptic	Serrate	Dark Green	Green
T28	Maaroub	Semi-erect	Elliptic	Entire	Green	Green to Yellow
T31	sohmor	Spreading	Elliptic	Entire	Green	Green to Yellow
T32		Spreading	Elliptic	Entire	Green	Green
T34	Fnaydeq	Spreading	Elliptic	Entire	Green	Green to Yellow
T35		Semi-erect	Elliptic	Entire	Green	Green to Yellow
T36		Spreading	Broad Elliptic	Entire	Dark Green	Green
T37		Spreading	Elliptic	Entire	Dark Green	Green
T39		Semi-erect	Elliptic	Entire	Green	Green
T41	Alay	Spreading	Elliptic	Entire	Dark Green	Green to Yellow
T43		Spreading	Elliptic	Entire	Dark Green	Green to Yellow
T44	Bisour	Erect	Narrow elliptic	Serrate	Dark Green	Green

Table 3 - Quantitative characteristics of walnut leaves recorded for the 35 walnut accessions

Sample	Site	Leaf length (cm)	Leaf width (cm)	Leaf petiole (cm)	Leaflet length (cm)	Leaflet width (cm)	Leaflet length/Leaflet width	Leaflet petiole (cm)	Number of leaflet
T1	Nabi sheeth	30.90 ± 4.13 cdefg [z]	28.10 ± 4.19 efghijkl	8.50 ± 1.66 cdefgh	14.15 ± 2.10 efghij	8.13 ± 1.11 bcd	1.11 ± 0.13	0.1	3
T2		32.00 ± 4.00 cdefg	29.50 ± 0.50 bcdefghijk	7.75 ± 0.87 fghij	14.88 ± 1.81 defg	7.00 ± 0.46 fghi	2.12 ± 0.15	0.1	7
T6		34.75 ± 7.09 cd	30.00 ± 3.56 bcdefghij	9.50 ± 1.47abcde	15.55 ± 1.64 cdef	6.29 ± 0.49 hijk	2.47 ± 0.15	0.1	7
T7		29.95 ± 6.06 cdefgh	28.30 ± 3.00 defghijkl	6.59 ± 1.17 ij	13.90 ± 2.29 fghijk	6.87 ± 0.87 fghi	2.04 ± 0.33	0.1	7
T8		26.70 ± 6.02 fghijk	30.10 ± 5.03 bcdefghij	7.90 ± 0.22 efghi	14.15 ± 3.03 efghij	7.02 ± 1.42 fghi	2.01 ± 0.11	0.1	5
T9	Janta	27.50 ± 3.83 efghijk	25.50 ± 3.35 ghijkl	6.08 ± 0.80 j	10.90 ± 2.04 op	5.70 ± 1.18 mn	1.93 ± 0.21	0.1	5
T10		34.17 ± 7.08 cd	31.67 ± 5.39 bcdefgh	8.50 ± 0.87 cdefgh	14.88 ± 2.91 def	7.56 ± 1.43 def	1.98 ± 0.23	0.1	7
T11		23.33 ± 1.89 ijk	23.33 ± 2.52 jklm	7.67 ± 2.52 fghij	10.63 ± 2.40 op	5.75 ± 1.25 klm	1.87 ± 0.30	0.1	3
T12		26.46 ± 4.69 fghijk	24.48 ± 4.29 ijklm	7.90 ± 0.82 efghi	11.72 ± 2.48 lmno	4.78 ± 0.72 n	2.44 ± 0.25	0.1	5
T13	Nahle	29.90 ± 4.16 cdefgh	32.10 ± 5.41 bcdefg	7.70 ± 0.97 fghij	16.10 ± 2.51 cbd	7.65 ± 0.41 cdef	2.10 ± 0.28	0.1	5
T14		41.00 ± 3.67 ab	35.40 ± 3.83 abc	8.90 ± 1.43 bcdefg	17.60 ± 1.97 b	8.43 ± 0.93 abc	2.09 ± 0.11	0.1	8
T15		35.80 ± 3.49 bc	34.70 ± 2.17 abcde	9.60 ± 1.08 abcd	15.50 ± 1.80 def	7.26 ± 0.71 efg	2.16 ± 0.38	0.1	7
T16		44.60 ± 4.29 a	35.00 ± 2.03 abcd	9.50 ± 2.06 abcde	17.25 ± 1.18 bc	8.80 ± 0.65 ab	1.96 ± 0.13	0.1	10
T17		32.40 ± 3.27 cdef	35.90 ± 3.09 ab	9.90 ± 1.14 abc	17.60 ± 1.47 b	8.02 ± 1.24 bcde	2.22 ± 0.25	0.1	5
T18	Labweh	26.20 ± 2.08 ghijk	25.60 ± 1.82 fghijkl	7.60 ± 0.96 fghij	13.05 ± 1.14 hijklm	5.53 ± 0.29 klmn	2.36 ± 0.17	0.1	7
T19		28.00 ± 3.67 efghij	26.10 ± 1.78 fghijkl	9.68 ± 1.69 abcd	13.19 ± 0.99 ghijkl	6.27 ± 0.59 ijk	2.11 ± 0.18	0.1	6
T20	Anjar	35.40 ± 1.95 bcd	28.30 ± 3.82 defghijkl	8.96 ± 2.77 bcdefg	15.75 ± 1.92 cde	6.66 ± 0.62 ghij	3.28 ± 0.03	0.3	7
T21		35.17 ± 4.67 bcd	30.33 ± 3.44 bcdefghij	9.17 ± 0.68 abcdef	15.36 ± 1.07 def	6.88 ± 0.87 fghi	2.25 ± 0.17	0.3	7
T22		30.33 ± 3.82 cdefg	32.50 ± 1.91 bcdef	9.50 ± 0.58 abcde	14.81 ± 1.30 defg	6.06 ± 0.88 jkl	2.47 ± 0.23	0.3	7
T23		34.17 ± 4.07 cd	29.67 ± 3.06 bcdefghij	7.33 ± 0.58 ghij	14.60 ± 1.45 defghi	6.70 ± 0.67 ghij	2.19 ± 0.24	0.3	8
T24		30.38 ± 2.72 cdefg	27.50 ± 3.87 fghijkl	7.00 ± 2.71 hij	13.25 ± 2.15 ghijkl	6.03 ± 0.57 jklm	2.19 ± 0.29	0.3	7
T25	kfardabch	32.83 ± 5.48 cde	29.50 ± 6.50 bcdefghijk	8.00 ± 1.00 defghi	12.91 ± 3.13 ijklm	5.40 ± 0.93 lmn	2.37 ± 0.20	0.3	7
T26		31.13 ± 4.98 cdefg	31.33 ± 4.04 bcdefghi	8.33 ± 0.58 cdefgh	15.69 ± 1.81 cde	7.11 ± 0.87 fg	2.23 ± 0.32	0.3	6
T27	Tal Al-amara	34.52 ± 4.84 cd	26.16 ± 4.39 fghijkl	7.10 ± 1.69 hij	10.76 ± 1.10 op	3.65 ± 0.50 o	2.97 ± 0.25	0.1	15
T28	Maaroub	31.59 ± 6.87 cdefg	27.22 ± 7.19 fghijkl	8.91 ± 1.42 bcdefg	12.63 ± 1.45 jklmn	5.74 ± 0.41 klm	2.20 ± 0.23	0.1	7
T31	sohmor	23.80 ± 3.59 ijk	21.90 ± 5.59 lm	8.30 ± 0.45 cdefgh	10.98 ± 2.55 op	5.25 ± 0.80 mn	2.07 ± 0.19	0.1	5
T32		26.80 ± 1.52 efghijk	24.24 ± 2.13 jklm	8.26 ± 1.30 defghi	12.20 ± 0.92 klmno	5.33 ± 0.27 lmn	2.29 ± 0.13	0.1	6
T34	Fnaydeq	22.13 ± 4.84 jk	22.00 ± 4.30 lm	8.17 ± 1.26 defghi	10.73 ± 2.10 op	5.51 ± 1.25 klmn	1.97 ± 0.18	0.1	6
T35		22.90 ± 4.71 jk	24.40 ± 3.23 ijklm	7.08 ± 0.73 hij	11.90 ± 1.61 lmno	5.93 ± 0.78 jklm	2.02 ± 0.20	0.1	6
T36		29.36 ± 5.52 defghi	25.10 ± 6.02 hijklm	10.50 ± 0.79 ab	12.64 ± 2.14 jklmn	7.06 ± 1.29 fgh	1.80 ± 0.21	0.1	6
T37		26.33 ± 7.68 ghijk	22.68 ± 8.66 klm	7.80 ± 1.15 fghij	11.98 ± 1.68 lmno	5.94 ± 0.53 jklm	2.02 ± 0.20	0.1	6
T39		24.00 ± 1.26 hijk	22.67 ± 2.64 klm	8.25 ± 1.17 defghi	11.46 ± 1.20 mno	5.42 ± 0.90 lmn	2.13 ± 0.07	0.1	7
T41	Alay	27.67 ± 2.79 efghijk	28.75 ± 4.58 cdefghijkl	8.85 ± 0.90 bcdefg	14.65 ± 2.35 defgh	5.97 ± 0.49 jklm	3.16 ± 0.11	0.1	5
T43		42.23 ± 5.47 a	40.88 ± 3.75 a	10.75 ± 1.55 a	20.59 ± 1.47 a	8.99 ± 0.66 a	3.02 ± 0.11	0.1	5
T44	Bisour	21.81 ± 3.88 k	18.69 ± 3.66 m	4.20 ± 0.71 k	9.32 ± 1.38 p	2.70 ± 0.31 p	1.92 ± 0.04	0.3	11
LSD		60.565	4.578.488	16.962	17.163	0.78			

[z] For each trait, means followed by different letters are significantly different at P≤0.05 (Least Significant Difference (LSD) test).

Morphological characteristics of the nuts and kernels

For the 35 accessions, the nut and kernel traits showed significant diversity. The shell texture varies from smooth (Nine accessions) to rough (Four accessions), with medium texture being the most common (22 accessions) (Fig. 3). The shell color ranges from very light to dark shades. Regarding the tip of the nut, 91% of the accessions had a medium tip, and the rest varied between a long and absent tip, as observed in specific accessions, such as T17 and T27 (Table 4). Significant differences were observed in the nut shape, walnuts exhibit a wide range of forms, including round (Eight accessions), ovate (Three accessions), elliptic (Two accessions), Broad Elliptic (Seven accessions), elongate (One accession), short trapezoid (Seven accessions), long

Fig. 3 - Shell texture, from Left to right: Rough, Medium, and Smooth.

trapezoid (Seven accessions) (Fig. 4). The majority of the walnut accessions had a round apex and base. For instance, the Tal-Amara sample features an ovate nut with a round apex and base. Shell strength is generally intermediate, with some accessions exhibiting strong or weak shells. Kernel coloration ranges from light to amber hues.

Nuts had generally presented an average length ranging from 2.50±0.00 cm (T27, Tal Amara) to 5.22±0.26 cm (T1, Nabi sheeth) and width from 1.18±0.10 cm (T39, Fnaydeq) to 4.15±0.15 cm (T1, Nabi sheeth),

Table 4 - Qualitative traits of nut and kernel recorded for the 35 walnut accessions

Sample	Site	Shell texture	Shell color	Nut tip	Nut shape	Apex shape	Base shape	Shell strength	Kernel color
T1	Nabi sheeth	Rough	Light	Medium	Long trapezoid	Emarginate	Round	Intermediate	Amber
T2		Medium	Medium	Medium	Short trapezoid	Obtuse	Round	Intermediate	Light amber
T6		Medium	Medium	Medium	Broad Elliptic	Round	Round	Strong	Light amber
T7		Medium	Light	Medium	Broad Elliptic	Round	Round	Intermediate	Light
T8		Medium	Very light	Medium	Long trapezoid	Truncate	Truncate	Strong	Light
T9	Janta	Smooth	Light	Medium	Broad Elliptic	Round	Cuneate	Strong	Light
T10		Rough	Medium	Medium	Short trapezoid	Truncate	Truncate	Intermediate	Light amber
T11		Smooth	Medium	Medium	Round	Round	Round	Intermediate	Amber
T12		Medium	Medium	Medium	Short trapezoid	Truncate	Truncate	Intermediate	Amber
T13	Nahle	Medium	Medium	Medium	Round	Obtuse	Truncate	Intermediate	Light amber
T14		Medium	Light	Medium	Broad Elliptic	Obtuse	Round	Intermediate	Light amber
T15		Medium	Medium	Medium	Long trapezoid	Truncate	Truncate	Intermediate	Light
T16		Medium	Medium	Medium	Round	Round	Truncate	Weak	Light amber
T17		Smooth	Light	Absent	Round	Round	Truncate	Intermediate	Amber
T18	Labweh	Smooth	Light	Medium	Round to ovate	Obtuse	Cuneate	Weak	Light
T19		Medium	Dark	Medium	Broad Elliptic	Round	Round	Weak	Light amber
T20	Anjar	Medium	Dark	Medium	Short trapezoid	Truncate	Round	Intermediate	Amber
T21		Medium	Light	Medium	Short trapezoid	Obtuse	Truncate	Intermediate	Amber
T22		Smooth	Medium	Medium	Round	Round	Truncate	Intermediate	Light amber
T23		Smooth	Light	Medium	Round to ovate	Obtuse	Truncate	Intermediate	Light
T24		Rough	Dark	Medium	Short trapezoid	Truncate	Round	Intermediate	Amber
T25	kfardabch	Medium	Light	Medium	Long trapezoid	Round	Round	Intermediate	Amber
T26		Medium	Dark	Medium	Round	Round	Truncate	Intermediate	Light amber
T27	Tal Al-amara	Smooth	Very light	Absent	Ovate	Round	Round	Paper	Light
T28	Maaroub	Smooth	Very light	Medium	Broad Elliptic	Truncate	Cuneate	Intermediate	Light amber
T31	sohmor	Medium	Dark	Medium	Short trapezoids	Emarginate	Round	Intermediate	Amber
T32		Medium	Dark	Medium	Round	Truncate	Truncate	Intermediate	Light
T34	Fnaydeq	Medium	Medium	Medium	Broad Elliptic	Round	Round	Intermediate	Light amber
T35		Medium	Medium	Medium	Long trapezoids	Round	Truncate	Intermediate	Light amber
T36		Medium	Medium	Medium	Long trapezoid	Truncate	Round	Intermediate	Light amber
T37		Medium	Dark	Medium	Round	Truncate	Truncate	Intermediate	Light amber
T39		Medium	Dark	Medium	Long trapezoid	Obtuse	Round	Intermediate	Amber
T41	Alay	Rough	Dark	Long	Elliptic	Obtuse	Cuneate	Weak	Light amber
T43		Medium	Medium	Medium	Elliptic	Round	Round	Weak	Amber
T44	Bisour	Smooth	Dark	Medium	Elongated	Round	Cuneate	Paper	Light

Fig. 4 - Nut shapes. From left to right, Top: Short trapezoid, Ovate, Long trapezoid; Bottom: Elliptic, Broad elliptic, Round.

resulting in length/width ratios typically around 1.2 (Table 5). The accessions showed significant differences in the nut and kernel weight ranging from 2.51 ± 0.02 g (T27, Tal Amara) to 19.81 ± 3.92 g (T7, Nabi sheeth) and from 0.70±0.02 (T27, Tal Amara) to 8.06 ± 0.33 g (T8, Nabi sheeth) respectively, contributing to kernel percentages from 27% (T36, Fnaydeq) to 60% (T8, Nabi sheeth). Accordingly, Tal Amara accession (T27) was distinguished by the lowest nut length, nut weigth, kernel weight while Nabi sheeth accession (T1) showed the highest nut length and nut width. Results from other Research showed that the fruit weight in Iran varied between 8.58 and 19.8 g, and the kernel percentage varied from 17.57 to 62.6% (Sarikhani Khorami *et al.,* 2014). Additionally, research on 58 walnut genotypes in the Himachal Region, India, revealed that fruit weight ranged from 6.4 to 20.55 g, and kernel percentage varied from 12 to 62.5% (Sharma and Sharma, 2001).

Validation of the variables

To validate the descriptors used in this study, a principal component analysis was performed on 27 morphological characters for the 35 different walnut accessions. This analysis will enable us to identify the most discriminating characters.

The principal components revealed that the first three components contributed 68% of the total variation (Table 6). The nut shape dominates the first component, which provides 48% of the total variation. The second component, which accounts for 13% of the total variation, includes leaflet shape, nut, and kernel weight. The third component, representing 7% of the total variation, comprises the shell texture, nut, and kernel color.

The biplot analysis was performed using PC1 and PC2 (Fig. 5), which together accounted for 61% of the variance (Table 6). The green arrows (Fig. 5) indicate the direction and magnitude of each trait's contribution to the first and second principal components. Among the 27 descriptors used in this study, the biplot analysis allowed to extract four traits (Q: Nut Shape, B: Leaflet shape, W: Nut weight and Y: Kernel weight) contributed strongly to the separation of the accessions. Most of the variation among the accessions was explained by nut shape (Q, component 1), resulting a broad horizontal distribution of the accessions. Highly correlated morphological components pointed in roughly the same direction. Accordingly, nut weight (W) was positively correlated with kernel weight (Y). Positive correlations were also observed among leaf traits such as Leaf length (F), Leaf width (H), leaflet length (J) and leaflet width (K).

PCA biplot segregated walnut accessions based on distinct morphological traits. For example, seven accessions (T1, T2, T7, T8, T9, T10 and T36) with broad elliptic leaflet shape and medium to high nut and kernel weights clustered together. The three accessions T11, T16 and T17 characterized by a round nut shape and intermediate to high leaflet width formed a separate group. Additionally, fourteen accessions (T6, T12, T14, T15, T19, T20, T21, T24, T25, T28, T31, T34, T35 and T39) with elliptic leaflet shape were grouped together.

The results confirmed the efficiency of the ampelographic descriptors in distinguishing among walnut varieties, consistent with previous studies on walnut trees (Karimi *et al.*, 2014), *Ensete ventricosum* (Haile *et al.*, 2023), grapevine (Chehade *et al*., 2022; Khater *et al.*, 2025).

Morphological clustering of the accessions

The dendrogram constructed based on the seven discriminating descriptors validated by PCA allowed for clustering the 35 evaluated accessions into three main groups at an Euclidean distance of -9 (Fig. 6).

The first group clustered 11 accessions: Labweh (T27, T18), Janta (T11), Sohmor (T32), kafardabch (T26), Fnaydeq (T37), Anjar (T22, T23), Nahle (T17, T16, T13). The majority of the accessions in this group share an elliptic leaflet shape, a round nut shape, medium shell color, and low to medium nut

Table 5 - Quantitative traits of nut and kernel recorded for the 35 walnut accessions

Sample	Site	Nut length (cm)	Nut width (cm)	Nut length/ Nut width	Nut weight (g)	Kernel weight (g)	kernel %
T1	Nabi sheeth	5.22 ± 0.17	4.15 ± 0.15	1.26 ± 0.08	18.78 ± 4.42	5.46 ± 1.49 bcde [z]	30.0 ± 7.0
T2		3.99 ± 0.15	3.27 ± 0.12	1.22 ± 0.02	12.78 ± 0.90	6.31 ± 0.57 ab	50.0 ± 6.0
T6		3.79 ± 0.46	3.17 ± 0.17	1.19 ± 0.11	12.54 ± 1.11	5.19 ± 0.48 bcdefg	42.0 ± 4.0
T7		4.32 ± 0.15	3.39 ± 0.29	1.28 ± 0.13	19.81 ± 3.92	6.67 ± 1.35 ab	33.0 ± 7.0
T8		4.16 ± 0.13	3.44 ± 0.24	1.22 ± 0.12	14.56 ± 5.18	8.06 ± 0.33 a	60.0 ± 16.0
T9	Janta	3.93 ± 0.10	3.38 ± 0.06	1.16 ± 0.02	12.84 ± 0.95	5.14 ± 0.64 bcdefg	40.0 ± 2.0
T10		4.06 ± 0.19	3.24 ± 0.05	1.25 ± 0.05	12.57 ± 0.79	4.11 ± 0.57 fghijk	33.0 ± 4.0
T11		3.18 ± 0.08	3.08 ± 0.08	1.03 ± 0.00	9.70 ± 0.77	4.02 ± 0.40 ghijkl	41.0 ± 1.0
T12		3.70 ± 0.26	3.58 ± 0.26	1.03 ± 0.01	13.96 ± 2.05	4.87 ± 1.52 cdefgh	34.0 ± 8.0
T13	Nahle	3.74 ± 0.11	3.39 ± 0.20	1.11 ± 0.05	13.00 ± 0.98	4.47 ± 0.44 efghij	34.0 ± 2.0
T14		3.94 ± 0.23	3.25 ± 0.17	1.21 ± 0.05	12.41 ± 2.33	4.53 ± 1.18 efghij	36.0 ± 3.0
T15		4.20 ± 0.28	3.45 ± 0.11	1.24 ± 0.14	15.63 ± 2.53	6.25 ± 0.70 abc	38.0 ± 5.0
T16		3.62 ± 0.28	3.00 ± 0.14	1.21 ± 0.09	9.49 ± 0.52	4.33 ± 0.72 efghijk	45.0 ± 5.0
T17		3.65 ± 0.07	3.50 ± 0.06	1.04 ± 0.01	12.16 ± 1.56	5.05 ± 0.33 bcdefg	39.0 ± 2.0
T18	Labweh	3.49 ± 0.11	2.84 ± 0.13	1.23 ± 0.08	9.55 ± 0.68	4.97 ± 0.45 cdefgh	52.0 ± 2.0
T19		3.64 ± 0.11	2.77 ± 0.10	1.31 ± 0.05	9.14 ± 0.27	3.45 ± 0.34 jkl	38.0 ± 4.0
T20	Anjar	3.35 ± 0.1	3.28 ± 0.03	1.02 ± 0.02	11.56 ± 2.49	5.37 ± 0.26 bcde	49.0 ± 19.0
T21		3.54 ± 0.23	3.11 ± 0.13	1.14 ± 0.10	9.14 ± 1.58	3.94 ± 0.90 hijkl	43.0 ± 3.0
T22		3.52 ± 0.23	3.29 ± 0.11	1.07 ± 0.07	12.56 ± 1.07	5.52 ± 0.65 bcde	44.0 ± 2.0
T23		3.46 ± 0.18	3.31 ± 0.12	1.05 ± 0.06	10.37 ± 1.24	4.58 ± 1.01 efghij	44.0 ± 5.0
T24		3.95 ± 0.11	3.23 ± 0.19	1.22 ± 0.04	11.39 ± 1.90	5.93 ± 0.85 bcd	52.0 ± 2.0
T25	kfardabch	4.43 ± 0.20	3.37 ± 0.20	1.32 ± 0.08	12.79 ± 1.34	5.97 ± 0.73 bc	47.0 ± 2.0
T26		3.71 ± 0.21	3.20 ± 0.14	1.16 ± 0.05	12.87 ± 1.75	5.00 ± 0.74 cdefgh	39.0 ± 1.0
T27	Tal Al-amara	2.50 ± 0.00	1.20 ± 0.00	2.08 ± 0.00	2.51 ± 0.02	0.70 ± 0.02 m	28.0 ± 0.0
T28	Maaroub	3.84 ± 0.13 f	3.00 ± 0.12	1.28 ± 0.06	12.23 ± 1.32	4.72 ± 0.93 defghi	38.0 ± 4.0
T31	sohmor	3.34 ± 0.17	3.21 ± 0.14	1.03 ± 0.08	12.19 ± 3.73	5.54 ± 0.45 bcde	40.0 ± 2.0
T32		3.38 ± 0.18	3.16 ± 0.19	1.07 ± 0.07	11.40 ± 1.11	4.62 ± 1.01 efghij	40.0 ± 6.0
T34	Fnaydeq	3.24 ± 0.09	2.82 ± 0.15	1.15 ± 0.04	8.63 ± 1.63	2.97 ± 1.15 l	33.0 ± 8.0
T35		3.92 ± 0.18	3.16 ± 0.09	1.24 ± 0.06	12.17 ± 1.92	4.40 ± 1.08 efghij	36.0 ± 7.0
T36		4.08 ± 0.25	3.23 ± 0.15	1.26 ± 0.03	11.93 ± 2.72	3.07 ± 0.30 l	27.0 ± 6.0
T37		3.28 ± 0.21	3.33 ± 0.17	0.99 ± 0.05	11.23 ± 1.67	3.95 ± 1.93 hijkl	34.0 ± 13.0
T39		4.14 ± 0.33	1.18 ± 0.10	2.14 ± 0.19	14.90 ± 2.77	5.92 ± 1.42 bc	40.0 ± 8.0
T41	Alay	4.56 ± 0.39	1.44 ± 0.12	2.44 ± 0.25	9.94 ± 1.22	3.63 ± 1.03 ijkl	36.0 ± 5.0
T43		3.96 ± 0.43	1.31 ± 0.12	2.30 ± 0.15	9.54 ± 1.50	4.10 ± 1.18 fghijkl	42.0 ± 7.0
T44	Bisour	3.74 ± 0.30	1.95 ± 0.20	2.01 ± 0.40	6.47 ± 0.38	3.17 ± 0.19 kl	49.0 ± 3.0
LSD						12.224	

[z] For each trait, means followed by different letters are significantly different at P≤ 0.05 (Least Significant Difference (LSD) test).

and kernel weight.

The second group contained eight accessions: Janta (T10, T9), Nabi-Sheeth (T1, T2, T7, T8), Fnaydeq (T36) and Nahle (T14), which share, on one hand, broad elliptic leaflet shapes. On the other hand, the majority of these accessions had medium to high nut and kernel weight.

The third and largest group consisted of 16 accessions: Alay (T41, T43), Kfardabch (T25), Bisour (T44), Maaroub (T28), Nabi-Sheeth (T6), Labweh (T19), Nahle (T15), Fnaydeq (T34, T35, T39), Janta (T12), Anjar (T21, T24, T20), Sohmor (T31). The majority of these accessions are characterised by an elliptic leaflet shape, an amber shell color, and low to medium nut and kernel weights.

Three cases of similarity were observed within the evaluated accessions: the first case was revealed between T18 (Labweh) and T23 (Anjar), the second case between T26 (Kafardabch) and T37 (Fnaydeq) and the third case was detected between T20 (Anjar)

Table 6 - Principal components analysis of the 27 morphological characters evaluated for the 35 walnut accessions

Variables	Factor 1	Factor 2	Factor 3
Growth Habit	-0.2605	0.1511	-0.4106
Leaflet Shape	-0.1451	**0.9298 ***	0.1581
Leaflet Margin	0.1059	-0.4854	0.5637
Leaflet Color	0.28	0.0429	0.2204
Rachis Color	-0.07275	0.3707	-0.1822
Leaf Length	0.1052	0.2692	-0.004267
Leaf Petiole	0.1797	-0.0005028	-0.3164
Leaf Width	0.07981	0.2107	0.01889
Number of Leaflet	0.1045	-0.4429	0.4273
Leaflet Length	0.1623	0.2281	-0.3419
Leaflet Width	0.1665	0.4709	-0.3174
leaflet L/ Leaflet W	0.006946	-0.5072	0.07997
Leaflet Petiole	0.007297	-0.3471	-0.09709
Shell Texture	-0.4299	0.2027	**-0.62417**
Shell Color	0.03561	-0.3428	**-0.6081**
Nut tip	-0.2038	0.06415	-0.2275
Nut Shape	**-0.9978**	-0.05072	0.02065
apex shape	-0.243	0.2149	-0.3307
base shape	0.2937	0.1901	-0.4649
shell strength	-0.2361	0.5061	-0.2951
Nut Length	-0.2389	0.3161	-0.1177
Nut width	-0.184	0.4765	-0.5116
Nut L/Nut W	-0.02781	-0.4988	0.1539
Nut Weight	-0.1082	**0.6299**	-0.2755
Kernel Weight	-0.1296	**0.6**	-0.1125
Kernel Percentage	-0.02273	-0.195	0.1534
Kernel Color	-0.1722	0.01275	**-0.6292**
Percentage of total variation	**48**	**13**	**7**

*= The characters in bold are discriminant.

and T31 (Sohmor).

Analysis of ISSR

The molecular analysis of walnut accessions revealed a high degree of variability. The ISSR markers exhibited distinct polymorphism among the various Juglans accessions. A total of 67 polymorphic bands were detected across 26 accessions out of 35 studied (nine accessions not amplified) through the use of 9 ISSR primers: UBC 807, UBC 810, UBC 814, UBC 811, UBC 819, UBC 865, UBC 818, UBC 821, and UBC 826 (Table 7). Similarly, 67 polymorphic bands were reported in Kashmir valley by Shah *et al.* (2019) in studying the genetic diversity 96 walnut genotypes using 19 ISSR markers, while 82 polymorphic bands were obtained in Iran by Sharifi *et al.* (2021) in studying 82 walnut accessions using 10 ISSR markers; 123 polymorphic bands were obtained by Kabiri *et al.* (2019) for 66 Moroccan walnuts using 11 ISSRs markers.

The size of the amplified products ranged from 195 to 1300 bp (Table 7). The number of polymorphic bands varied between five (UBC810) and nine (UBC818 and UBC821), with an average of 7.4 per primer. Five primers had one monomorphic band (UBC 810, UBC814, UBC819, UBC826 and UBC 865). The percentage of polymorphism varied between 83% for the ISSR primer UBC 810 and 100%

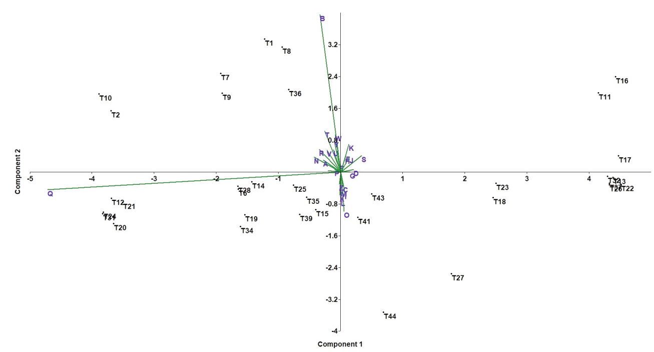

Fig. 5 - PCA biplot for 27 traits among 35 walnut accessions. The contribution of each trait to principal components 1 and 2 is shown in green arrows. A: growth habit; B: leaflet shape; C: leaflet margin; D: leaflet color; E: rachis color; F: leaf length; G: Leaf petiole; H: leaf width; I: number of leaflets; J: leaflet length; K: Leaflet width; L: leaflet length/leaflet width; M: leaflet petiole; N: Shell texture; O: Shell color; P: nut tip; Q: Nut shape; R: apex shape; S: base shape; T: shell strength; U: nut length, V: nut width; W: Nut weight; X: nut length/nut width; Y: Kernel weight; Z: kernel percentage; kernel color.

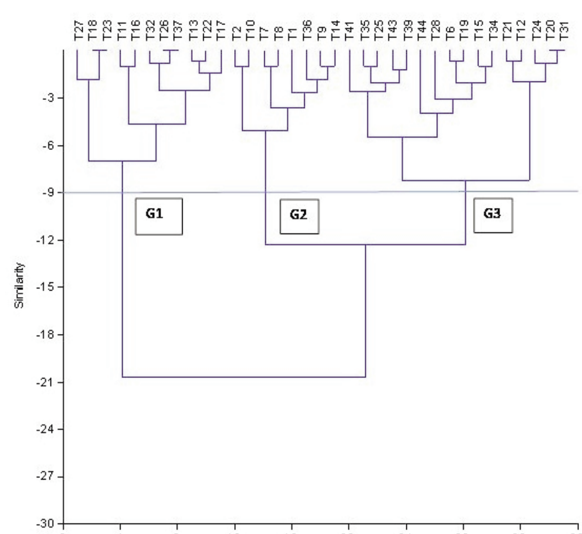

Fig. 6 - Dendogram of the 35 Lebanese walnut accessions, constructed with the most seven discriminant morphological traits, using Euclidean distance and UPGMA clustering.

Table 7 - Primer sequences, annealing temperature, band sizes, monomorphic band, polymorphic band, percentage of polymorphism, polymorphic information content and discriminating power of the nine ISSR markers used in this study

Primers	Sequence 5' to 3'	Annealing temperature (°C)	Band sizes (bp)	Total number of bands	Mono-morphic band	Poly-morphic band	% Poly-morphism	Polymorhic Information Content (PIC)	Dicrimination power
UBC 807	A(GA)7GGT	45	225-800	7	0	7	100%	0.29	0.9
UBC 810	(GA)8T	45	225-800	6	1	5	83%	0.27	0.78
UBC 811	CAC(CA)6AT	47	250-1100	8	0	8	100%	0.29	0.79
UBC 814	(CT)8A	45	275-1200	8	1	7	88%	0.35	0.87
UBC 818	(CA)8G	45	250-700	9	0	9	100%	0.31	0.914
UBC 819	(GT)8A	47	250-900	8	1	7	88%	0.26	0.86
UBC 821	(GT)8T	51.3	260-1300	9	0	9	100%	0.41	0.94
UBC 826	(AC)8C	51.3	195-650	8	1	7	88%	0.24	0.8
UBC 865	(ATG)5TCC	47	275-950	9	1	8	89%	0.37	0.9
Mean	-	-	-	8	-	7.4	92.8	0.31	0.86

for UBC 807, UBC 811, UBC 818 and UBC 821, with an average 92.8%. This finding is higher than that shown by Malvolti *et al.* (2010) for Italian walnut (73.8%) and Christopoulos *et al.* (2010) for Greek walnut (82.8%). Nevertheless, it is similar with the result reported by Aiqing *et al.* (2014) for Chinese walnut (92.31%).

The Polymorphism Information Content (PIC) of the 9 primers varied from 0.24 to 0.41 with an average of 0.31. The results indicate that UBC 821 with GT dinucleotide repeat which has the highest polymorphism. The minimum polymorphism content was obtained from the UBC826 with AC dinucleotide repeat. The average PIC obtained in our present results was higher than those previously found in ISSR studies in walnut by Potter (2002), Christopoulous (2010), Mahmoodi *et al.* (2012), Ji *et al.* (2014) and Shah *et al.* (2019).

The calculated discrimination power (PD) ranged from 0.78 for the ISSR primer UBC 810 to 0.94 for the ISSR primers UBC 821, with an average of 0.86, indicating a high diversity of the loci and confirming the efficiency of these primers in studying the polymorphism of the Lebanese walnut accessions. This value is similar to the work of Kabiri *et al.* (2019) for 66 Moroccan walnuts using ISSRs markers.

Molecular classification of the accessions

The allelic data were utilized to generate a dendrogram through the Jaccard distance and the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) clustering method, revealing the genetic relationships among walnut accessions (Fig. 7). The dendrogram did not reveal similarity between accessions as observed in the molecular studies. The highest genetic distance was observed between genotypes T34 (sohmor) and T2 (Nabi sheeth), differing by 45 bands (62.5%), while the lowest was between two genotypes from the same location (Nabi sheeth) T2 and T6, differing by only 4 bands (5.5%). In addition, this dendrogram enabled the distinction of four groups at a Jaccard similarity distance of -9.

Group 1 included seven accessions with an elliptic leaflet shape and an entire margin. These accessions shared a common amplification of 225 bp and 275 bp

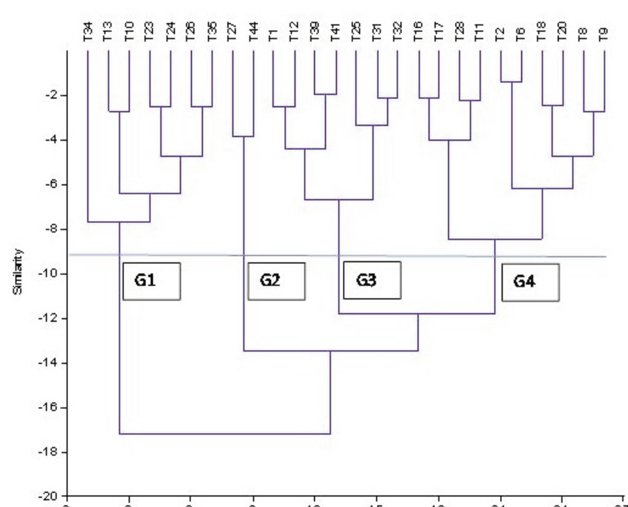

Fig. 7 - Dendogram of the 26 Lebanese walnut accessions, constructed from 9 ISSR, using Jaccard distance and UPGMA clustering.

using the UBC 807 primer, 300 bp and 400 bp using the UBC 818 primer, and 275 bp using the UBC 814 primer.

Group 2 consisted of two accessions (T27 and T44), which had a narrow, elliptic leaflet shape with a serrate margin and a dark green color. The two accessions had 19 common bands, including: five bands of UBC 821 (400 bp, 480 bp, 600 bp, 700 bp, and 900 bp), three bands of each: UBC 807 (300 bp, 400 bp, and 500 bp) and UBC 818 (275 bp, 350 bp, and 500 bp), two bands of each: UBC 819 (450 bp, and 500 bp) and UBC 826 (400 bp, and 650 bp), one band of each: UBC 810 (300 bp), UBC 814 (375 bp), UBC 811 (680 bp) and UBC 865 (750 bp). Indeed, this group exhibits a specific molecular pattern characterized by the presence of a unique band at 250 bp for UBC 811 and the absence of two bands that are present in other groups (275 bp at UBC 807 and 300 bp at UBC 818). This result confirms that these two accessions are not related to *Juglans regia* L, but they are related to *Carya illinoinensis* (Pecan) L.

Group 3 included seven accessions characterized by an elliptic leaflet shape with entire margin and medium nut tip which share 23 bands, comprising: five bands of UBC 818 (275 bp, 300 bp, 350 bp, 550 bp, and 700 bp), four bands of UBC 807 (225 bp, 275 bp, 400 bp, and 500 bp), three bands of each: UBC 810 (300 bp, 350 bp, and 480 bp), UBC 811 (425 bp, 680 bp, and 750 bp), UBC 819 (500 bp, 600 bp, and 800 bp), two bands of each: UBC 865 (700 bp, and 750 bp), UBC 826 (400 bp, and 550 bp) and one band of UBC 814 (700 bp)

Group 4 included ten accessions characterized by entire leaflet margin with twenty one common bands comprising: four bands of UBC 807 (275 bp, 400 bp, 500 bp and 800 bp), three bands of each: UBC 865 (700 bp, 750 bp and 950 bp), UBC 814 (275 bp, 375 bp and 700 bp), UBC 811 (425 bp, 680 bp and 750 bp), UBC 818 (275 bp, 300 bp and 700 bp), two bands of UBC 826 (225 bp and 550 bp), one band of each: UBC 810 (480 bp), UBC 819 (500 bp), UBC 821 (700 bp). Indeed, this group exhibits a specific molecular pattern characterized by the presence of a unique band at 800 bp for UBC 807.

Accordingly, the results of the genetic structure by using ISSR markers of the walnut trees were not correlated with the geographical regions of the sites. All the groups comprised accessions from different geographical regions.

The results of this study revealed a large morphological diversity and high genetic variation among the Lebanese walnut accessions. The dendrogram based on ISSR markers did not correspond to the one generated from morphological traits. These traits can be influenced by environmental factors, whereas molecular data are largely unaffected by such factors. Therefore, results derived from molecular data are generally considered more precise and reliable for assessing genetic relationships. Similar results were obtained in Iran by Sharifi *et al.* (2021) in studying walnut genetic diversity investigation using phonological and morphological characteristics and ISSR markers.

4. Conclusions

This study involves a morphological and molecular characterization of walnut accessions grown in various areas of Lebanon, with the objective of assessing the genetic diversity among walnut cultivars. Numerous discriminant traits were initially responsible for the diversity detected within the accessions, such as leaflet shape, shell texture, shell color, nut Shape, nut weight, kernel weight, and kernel color. These traits can be used by researchers and nursery owners to select the best single accession of walnut cultivars and meet the needs of growers for particular desired traits. Such traits include kernel weight and kernel percentages are a desirable character for commercial acceptance of a variety. In this context, T8 accession had the highest kernel weight and kernel percentages, making it suitable for propagation by nursery owners to meet farmers' demand.

No clear structure of the accessions with their geographical growing areas was observed in this study. Such results have been reported in different crops by several studies, e.g. on chest-nut (Marinoni *et al.*, 2013), almond (Chalak *et al.*, 2007) and olives (Chehade *et al.*, 2015). This variability could be attributed to the free exchange of planting material between different Lebanese villages. The combination of ampelographic descriptions and molecular markers generated effective differentiation of potential clones within the walnut cultivars studied. No significant correlation was found between the results of morphological and molecular characterization. This correlation could be further improved, first by extending the number of accessions, second by involving more descriptors allowing for the characterization of more traits and secondly, at the molecular level, by using a larger set of molecular markers such (SSR, SNP).

Finally, the walnut accessions listed in our study should be gathered into specific collections located in different environmental conditions, in order to evaluate their agronomic potential (bloom, maturity dates, yield, nutritive composition and disease resistance). Based on the morphological, molecular characterization and agronomic evaluation of walnut accessions, conservation strategies should be implemented. The selected material should constitute a potential wealth of genetic diversity, which can be used for the improvement of walnut trees in Lebanon and elsewhere.

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