

GENETIC DIVERSITY BASED ON MORPHOLOGICAL TRAITS AND GERmplasm CONSERVATION OF WALNUT IN KASHMIR, PAKISTAN

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ABSTRACT

Persian walnut is an economically important nut crop of temperate regions of the world. In the present study 225 local walnut accessions from 37 sites of Azad Jammu and Kashmir were analysed for morphological traits to investigate variability and identify superior one. Nut length, nut weight, shell thickness and nut diameter varied from 20.87–47.34 mm, 6.44–17.7 g, 1.61–2.54 mm and 27.25–44.31 mm respectively. Kernel weight and percentage varied from 2.81–8.65 g and 32.53–77.34%. Data were analysed statistically using multivariate analysis such as Principal Components Analysis and Cluster Analysis. Pearson correlation coefficient among traits revealed a positively significant correlation between nut weight and nut thickness ($r = 0.45987$), kernel weight and nut weight ($r = 0.94777$), kernel% and nut thickness ($r = 0.48346$), shell thickness and kernel% ($r = 0.21961$), a negative correlation with significant differences was found between kernel weight and nut thickness ($r = -0.40968$), kernel% and nut length ($r = -0.10844$) and between kernel% and nut weight ($r = -0.95209$). The highest and most significant positive correlation (0.96024) was observed between kernel weight and kernel% and ($r = 0.94777$) between kernel weight and nut weight. A wide range of variability was observed among walnut accessions and BA2, BN2, CN3 and BN4 accessions are reported as superior and conserved for future breeding programs due to their distinct characteristics.

Key words: walnut, morphological diversity, germplasm conservation, cluster analysis

INTRODUCTION

Persian walnut (*Juglans regia* L.) a member of *Juglans* genus belongs to the *Juglandaceae* family. The genus *Juglans* includes 21 species which are commonly grown in temperate zone [McGranahan and Leslie 2012]. The genus is native to the western Himalayan and Iran, China and Balkans are considered as the centre of origin for this nut species [Arzani et al. 2008]. China, Turkey and Iran are the major walnut producers in Asia. Overall 65% productivity was recorded in the last decades in Asian countries followed by America 19.1% [FAO 2019]. Pakistan neighbouring to Iran is also a country having good productivity of walnut in temperate region especially in Azad Kashmir, Khyber-Pakhtunkhwa and Gilgit Baltistan.

In the years (2010–2015) the production of walnuts recorded was 11.5 thousand tons to 15.4 thousand tons [Naylor 2016].

The huge genetic variation in walnut populations in Turkey presents good prospects for walnut breeders to choose new cultivars directly or use them in cross breeding practices [Asma 2012]. High variation found in nut traits such as nut sizes, shape, shell thickness, colour of kernels, kernel percent, taste of kernels and other morphological traits has been described in walnut trees of different regions of the world which might be due to propagation of walnut through seed [Cosmulescu 2013, Akca et al. 2015, Khadivi-khub and Ebrahimi 2015, Cosmulescu et al. 2018].

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Western Himalayan region especially the state of Jammu and Kashmir is a rich area of a variety of fruit cultivars [Hussain et al. 2016]. Genetic variability in walnut populations has been observed very high and exists in many parts of the world. A good variety considered is that having jumbo size nut, soft shell and light coloured kernel [Chen et al. 2014, Sharma et al. 2014,]. Morphological characters are considered to be an option for selection and classification of superior germplasm [Ebrahimi et al. 2015]. The study area is situated at a very important geographical place and is well known for rich biodiversity. The climatic conditions of the study area are suitable for walnut production [Khan et al. 2011]. The mechanism of grafting and budding is very rare, so walnut grows by seed dispersal in Kashmir. The scientific knowledge regarding walnut, information of local walnut cultivars and its documentation from Azad Jammu and Kashmir is lacking so far. Hence, the main aim of current study

was to evaluate the morphological variations among walnut populations and to identify promising accessions from Azad Jammu and Kashmir. In this context this is the very first attempt in walnut documentation from AJK, so that these varieties may be used as breeding material in future cultivar development programs.

MATERIALS AND METHODS

The present research was conducted in Neelum valley, Jhelum valley and Haveli Districts of the state of Azad Jammu and Kashmir (AJ&K), Pakistan.

Sample collection. Local accessions were collected from 37 different sites of study areas in Azad Jammu and Kashmir during harvest-ing period (Fig. 1). A total of 225 walnut accessions were selected for the collection of samples in order to study morphological traits after several thorough surveys and observations. The accessions with similar traits were eliminated and

Table 1. Morphological traits with their codes used for walnut accessions

Traits	2	3	5	7	9
Tree vigor	very strong	strong	medium	weak	very weak
Branch density	very dense	dense	sparse	very sparse	..
Growth habit	upright	semi upright	spreading
Leafing time	18–31 March	01–14 April	15–25 April	26 April–05 May	06–20 May
Leafing group	very early	early	medium	late	very late
Leaf shape	elliptic	narrow elliptic	broad elliptic	lanceolate	..
Dichogamy	protandrous	protogynous	homogamous
Catkin abundance	many	medium	few
Bearing habit	terminal	lateral	mixed
Time of maturity	18–25 August	26 August–10 September	11–22 September	23 Sep–03 October	04–15 October
Hardness	extra soft	soft	medium	hard	very hard
Ease of removal	very easy	easy	medium	difficult	very difficult
Nut size	very large	large	medium	small	very small
Shell colour	very light	light	medium	brown	dark brown
Shell texture	very smooth	smooth	slightly grooved	embossed	very embossed
Tip shape	obtuse	emarginate	mucronate	cuspidate	truncate
Base shape	obtuse	emarginate	mucronate	cuspidate	truncate
Nut shape	round	broad ovate	triangular	elongated	very elongated
Plumpness	very strong	strong	medium	weak	very weak
Kernel colour	very light	light	medium	brown	dark brown

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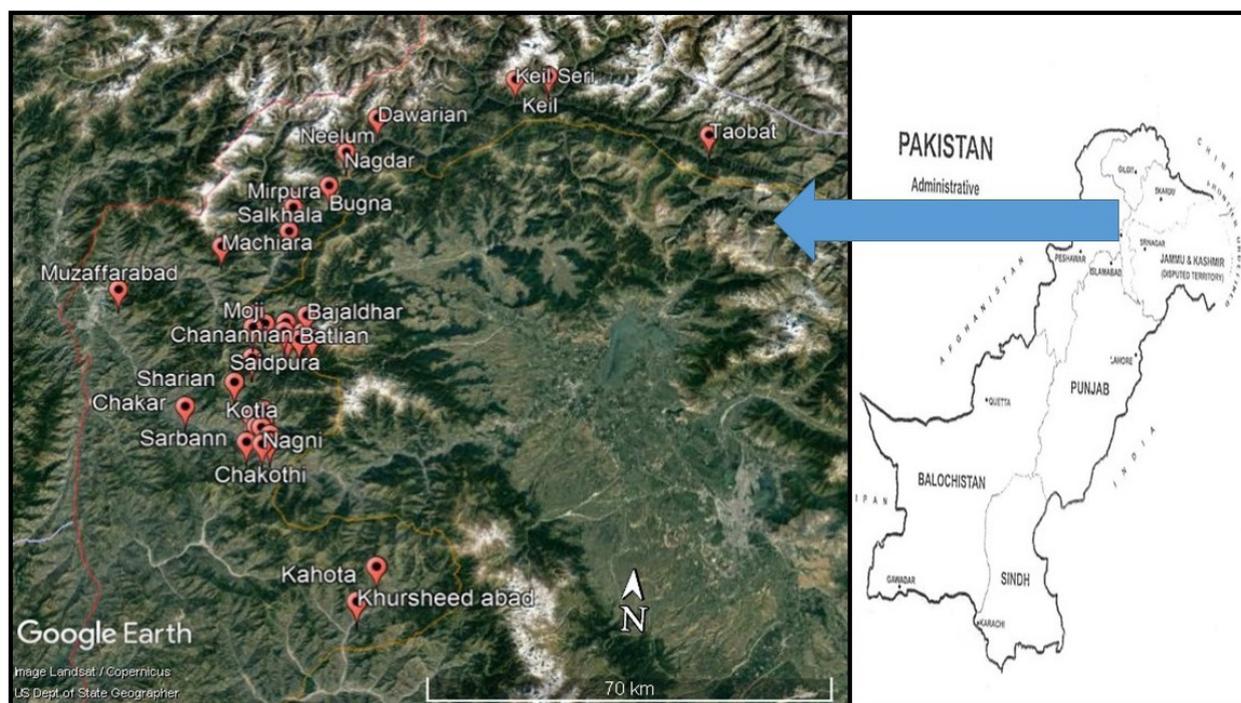


Fig. 1. Map of the study area in Azad Jammu and Kashmir, Pakistan [Google Maps 2020]

37 accessions were selected based on several horticultural characteristics. The selected walnut trees from different sites with codes are given in Table 2.

Morphological characterization. Morphological characteristics were measured according to standards proposed by walnut descriptor [IPGRI 1994] with minor modifications (Tab. 1). Thirty-three traits were measured to assess variations among the walnut populations during two successive years (2016–2017). Measurements of quantitative and qualitative morphological traits were based on 5 replicates (each with 10 nuts chosen randomly) and the mean values were used. Some variables were measured by laboratory equipment. Quantitative traits (nut length, nut width, kernel length, shell thickness and suture height) were measured by using vernier calliper and weight for nut and kernel was measured using electronic balance with 0.001 g precision. Qualitative traits such as nut shape (Fig. 4), kernel traits (plumpness and colour), shell traits (colour, texture and hardness), kernel removal from nuts were determined based on rating and coding (Tab.1) according to walnut descriptor [IPGRI 1994].

Kernel ratio was calculated using the formula (ratio of kernel weight to nut weight) as obtained by Acka et al. [2015]. Phenological and flowering traits included leafing (bud-break) date, leafing group, dichogamy type and flowering habit [Arzani et al. 2008].

Statistical analyses. The data were computed for descriptive statistics, such as mean, standard deviation and coefficient of correlation (Tab. 3) by using statistical software package i.e. MS Excel program 2007 [Mousivand et al. 2013]. PAST software version 3.06 was used for multivariate analysis including principal component analysis and cluster analysis [Braak and Smilauer 2002] to find out the relationship between studied accessions.

RESULT AND DISCUSSION

Morphological characterization of *Juglans regia*

The morphological traits of 37 accessions were presented in Table 2 i 7. Study revealed that nut diameter has value between 27.25 mm (QK7) to 44.31 mm (BN4), nut thickness varied from 30.29 mm (DN1) to

Table 2. Pomological characteristics of selected walnut accessions

Accessions	Nut diameter (mm)	Nut thickness (mm)	Nut length (mm)	Nut weight (g)	Kernel weight (g)	Shell thick (mm)	Kernel%
TT1	32.62	35.86	35.77	11.522	5.64	1.86	48.95
DN1	27.95	30.29	28.71	7.312	2.812	2.38	38.46
KL5	30.19	35.05	32.77	9.826	3.668	1.75	37.33
BA1	29.77	36.33	29.6	9.522	4.288	1.61	45.03
KL1	31.13	38.73	31.39	7.578	4.978	1.82	65.69
MP5	31.25	35.76	32.63	10.39	3.688	2.18	35.5
BA2	31.55	39.35	31.72	6.444	4.984	1.98	77.34
KS2	28.43	31.01	30.05	9.918	3.226	2.06	32.53
CN1	29.97	34.28	29.91	10.188	4.84	1.84	47.51
NT1	31.25	34.67	35.13	11.658	4.638	1.68	39.78
SP2	34.71	39.02	34.25	15.156	5.018	1.75	33.11
BD1	28.55	38.69	44.73	10.216	4.308	1.97	42.17
TL1	30.84	37.34	33.35	13.294	4.71	2.17	35.43
BN1	32.41	37.76	37.85	15.85	6.55	2.14	41.33
BN2	43.7	45.02	45.15	9.466	7.321	2.36	77.34
BN3	28.85	31.34	31.43	10.106	4.784	1.94	47.34
SP1	32.15	35.75	32.75	10.578	7.453	1.86	70.46
BN4	44.31	46.17	43.55	7.43	5.321	2.04	71.62
QK1	29.75	34.28	31.55	7.958	4.002	1.92	50.29
NT2	32.38	35.69	32.75	11.884	4.768	2.34	40.12
CN2	32.16	39.68	31.13	10.356	6.897	1.65	66.6
QK2	28.43	31.61	36.05	9.016	5.23	2.46	58.01
SP3	29.97	35.69	29.95	13.064	8.654	1.98	66.24
GA1	39.98	46.34	46.15	11.044	4.236	1.74	38.36
GA2	37.13	40.01	46.27	7.964	3.552	1.72	44.6
GA3	37.85	39.35	45.95	9.78	3.896	2.06	39.84
NT3	43.73	46.34	35.39	13.234	4.406	2.08	33.29
NT4	41.55	46.25	47.34	17.7	5.752	2.43	39.88
QK3	28.55	38.57	29.23	8.514	4.138	1.76	48.6
QK4	28.85	31.34	32.35	11.818	4.738	1.88	40.09
QK5	27.65	31.61	20.87	10.328	3.838	1.86	37.16
QK6	29	33.11	30.05	9.578	4.118	1.87	42.99
QK7	27.25	30.71	31.25	8.888	3.722	1.72	41.88
SB1	30.86	33.47	33.05	8.06	3.136	2.54	38.91
SB2	27.28	31.67	27.93	15.646	7.04	2.12	45
SA1	32.82	35.69	36.75	13.898	4.6	1.96	33.1
CN3	30.23	34.79	36.26	6.984	5.123	1.88	73.35

Accessions: BA – Bugna, NM – Neelum, SA – Salkhala, DN – Dawarian, NR – Nagdar, CN – Chanannian, KS – Kundal Shahi, KL – Keil, NT – Nowkot, QK – Qaiserkot, RN – Reshian, SP – Saidpura, TS – Tredasharif, MI – Moji, GA – Gaepura, BD – Bajaldahar, TT – Taobutt, MP – Mirpura, KS – Keilseri, TL – Tilawari, SB – Serbann. The numbers 1–7 within abbreviations show the sequence of accessions from each other.

46.74 mm (NT3), nut length found between 20.87 mm (QK5) to 47.34 mm (NT4), weight of nut varied from 6.444 g (BA2) to 17.7 g (NT4), weight of kernel varied from 2.812 g (DN1) to 8.65 g (SP3), shell thickness varied from 1.61 mm (BA1) to 2.54 mm (SB1) and kernel percentage found between 32.53% (KS2) to 77.34% (BA2). Highest kernel percentage shown by BA2 revealed that soft accessions are best in terms of kernel ratio. Quantitative morphological nut, kernel and shell traits revealed that range of nut length was from 20.87 to 47.34 mm, whereas the diameter of nut ranged from 27.25 to 44.31 mm. Cosmulescu and Botu [2012] investigated morphological traits of walnut trees in Oltenia, Romania and found that nut length ranged from 28.20 to 49.70 mm and diameter from 25.70 to 40.60 mm. Weight of nut is one of the most important trait which influences the quality and market value of walnut. Weight is one of the most common important parameter influencing the quality of nuts. The highest weight of nut (17.7 g) observed among the selected accessions was less than the weight (23.81 g) for walnuts shown by Sen and Tekintas [1990] in Adilcevaz, Turkey, nut weight (20 g) reported by Atefi [2001] in Kamal-Abad, Iran, 18.60 g observed by Sharma and Sharma [2001] for walnut accessions in Himachal Pradesh, India and 18.40 g for Romanian walnuts reported by Cosmulescu and Botu [2012] but higher than the weight (17.04 g) of walnut nuts for Anatolia region, Turkey observed by Yarilgac et al. [1999] and nut weight (16.01 g) investigated for walnut accessions in North Anatolia region, Turkey [Aslantaş 2006].

Kernel weight is important characteristic on the basis of which kernel percentage or ratio is calculated.

Kernel weight and colour determine the quality of walnuts. We found the kernel weight between 2.81 to 8.65 g. Desirable colour for kernel is light brown and weight of kernel should be between 6–10 g or weight of kernel should be at least 50% of weight of the nut [Arzani et al. 2008]. The kernel percentage is a trait of enormous importance in the selection of superior varieties and walnut improvement programs. We found kernel percentage in our selections between 32.53% and 77.34%. The high percentage of kernel increases the quality and market value of nuts. In our study the highest percentage of kernel (77.34%) was higher than 63.80% found by Zeneli et al. [2005], 67.14% by Aslantaş [2006] and 71.70% by Cosmulescu and Botu [2012] but less than 79.60% reported by Arzani et al. [2008]. Ten of all the investigated walnuts accessions had percentage or ratio of kernel higher than 50 can be the future trees for walnut breeding programs. Accession number BA2 and BN2 had the highest percentage of kernel (77.34%), followed by CN3 (73.35%), BN4 (71.61%), SP1 (70.46%), SP3 (66.24%) and KL1 (65.69%). Twenty-seven accessions were recorded to have lower kernel ratio or percentage of kernel (kernel%) ranging from 32.53% (KS2) to 48.95% (TT1). We also recorded the age of selected genotypes that varies from 20 to 110 years and inferred that kernel and nut traits are not influenced by the over age of walnut trees. Similar results were reported by Sharma and Sharma [2001].

The results with descriptive statistics for 13 quantitative morphological traits of local walnut accessions are summarized in Table 4. The highest coefficient of variability (38.62%) was observed in suture height with standard deviation of 1.05. Correlation of coefficient

Table 3. Correlation coefficient between pair of quantitative characters

	NT (mm)	NL (mm)	NW (g)	KW (g)	ST (mm)	Kernel%
NT (mm)		0.68541	0.45987	-0.40968	-0.31106	0.48346
NL (mm)	0.68541		-0.073919	0.072409	0.35345	-0.10844
NW (g)	0.45987	-0.073919		0.94777	0.23182	-0.95209
KW (g)	-0.40968	0.072409	0.94777		-0.21202	0.96024
ST (mm)	-0.31106	0.35345	0.23182	-0.21202		0.21961
Kernel%	0.48346	-0.10844	-0.95209	0.96024	0.21961	

Key: NT – nut thickness, NL – nut length, NW – nut weight, KW – kernel weight, ST – shell thick, kernel% – percentage of kernel.

Table 4. Statistical analysis of quantitative traits of 37 walnut accessions

Traits	Min.	Max.	Mean (\pm SD)	Variance	CV%
Nut diameter	27.25	44.31	32.30 \pm 4.81	43.74	14.91
Nut thickness	30.29	46.34	36.72 \pm 4.67	48.11	12.70
Nut length	20.87	47.34	34.62 \pm 6.16	62.69	17.81
Nut weight	6.44	17.7	10.60 \pm 2.67	9.68	25.21
Kernel weight	2.81	8.65	4.87 \pm 1.31	2.32	26.88
Shell thickness	1.61	2.54	1.98 \pm 0.24	0.14	12.29
Kernel%	32.53	77.34	47.71 \pm 13.67	224.66	28.88
Kernel length	18.51	43.43	30.52 \pm 6.10	55.78	19.97
Suture height	0.47	4.85	2.73 \pm 1.05	1.31	38.62
Tree DBH (cm)	45	180	80.05 \pm 27.10	1011.51	33.85
Age of tree	20	110	64.38 \pm 21.04	545.51	32.68
No. leaflets	7	11	8.78 \pm 1.40	3.29	15.91
No. of veins	11	19	14.02 \pm 2.44	9.80	16.70

CV% – percentage of coefficient of variance.

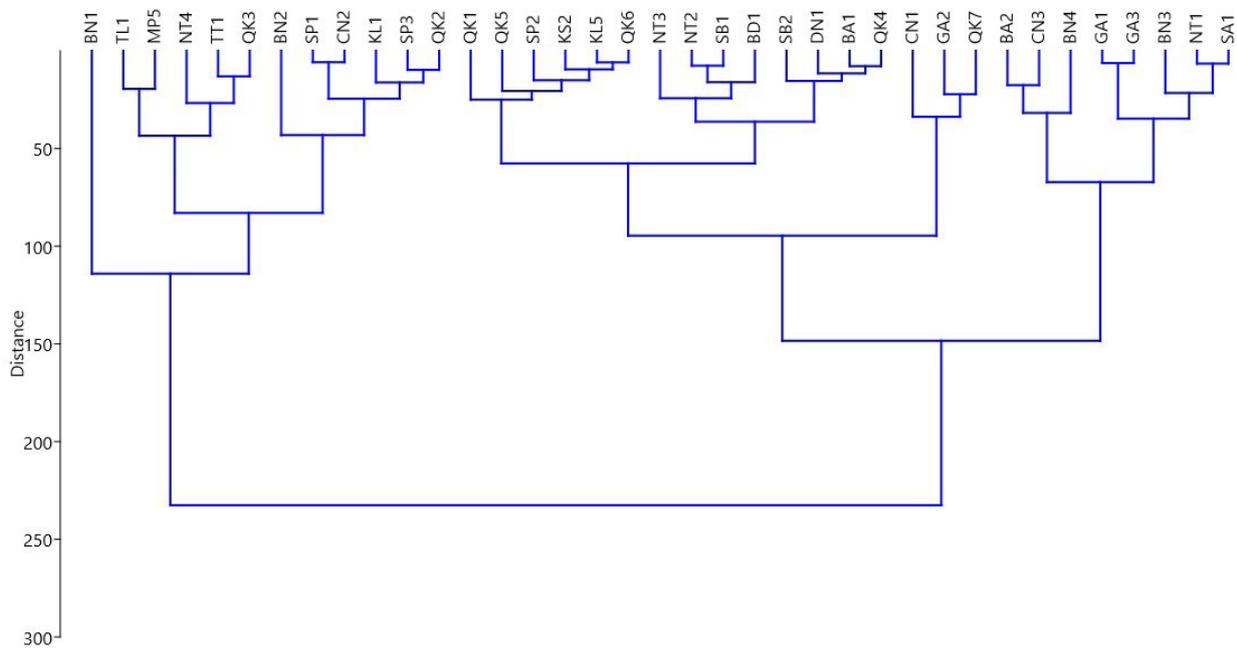


Fig. 2. Classification of 37 walnut accessions using cluster analysis by Wards method

Accessions: BA – Bugna, NM – Neelum, SA – Salkhala, DN – Dawarian, NR – Nagdar, CN – Chanannian, KS – Kundal Shahi, KL – Keil, NT – Nowkot, QK – Qaiserkot, RN – Reshian, SP – Saidpura, TS – Tredasharif, MI – Moji, GA – Gaepura, BD – Bajaldahar, TT – Taobutt, MP – Mirpura, KS – Keilseri, TL – Tilawari, SB – Serbann. The numbers 1–7 within abbreviations show the sequence of accessions from each other.

Table 5. Statistical analysis of qualitative morphological traits for walnut accessions

Traits	Min.	Max.	Mean (\pm SD)	Variance	CV%
Tree vigor	2	5	3.57 \pm 1.19	1.42	33.39
Branch density	2	7	4.22 \pm 1.75	3.06	41.51
Growth habit	2	5	2.95 \pm 1.18	1.39	39.96
Leafing time	2	9	4.35 \pm 2.61	6.79	59.88
Leafing group	2	9	4.35 \pm 2.61	6.79	59.88
Leaf shape	2	7	4.27 \pm 1.63	2.65	38.1
Dichogamy type	2	5	3.03 \pm 1.28	1.64	42.28
Catkin abundance	2	5	2.92 \pm 1.19	1.41	40.68
Bearing habit	2	5	3.27 \pm 1.35	1.81	41.18
Time of maturity	2	9	4.73 \pm 2.71	7.37	57.4
Hardness	2	9	4.30 \pm 1.94	3.77	45.18
Ease of removal	2	9	4.35 \pm 2.45	6.01	56.35
Nut size	2	9	5.62 \pm 2.09	4.35	37.11
Shell colour	2	7	4.05 \pm 1.39	1.94	34.37
Shell texture	2	9	4.54 \pm 1.74	3.03	38.36
Tip shape	3	9	5.38 \pm 1.62	2.63	30.16
Base shape	2	9	3.73 \pm 2.76	7.59	73.87
Nut shape	2	5	2.73 \pm 1.24	1.54	45.4
Plumpness	2	7	3.35 \pm 1.18	1.4	35.32
Kernel colour	2	9	4.24 \pm 1.71	2.91	40.21

CV% – percentage of coefficient of variance.

cient between different traits of walnut accessions included in this study revealed significantly positive correlation among different variables such as nut weight and nut thickness ($r = 0.45987$), kernel weight and nut weight ($r = 0.94777$), kernel% and nut thickness ($r = 0.48346$), shell thickness and kernel% ($r = 0.21961$), a negative correlation with significant differences was also observed between kernel weight and nut thickness ($r = -0.40968$), between kernel% and nut length ($r = -0.10844$), between kernel% and nut weight ($r = -0.95209$). The highest and most significant positive correlation (0.96024) was observed between kernel weight and kernel% and between kernel weight and nut weight ($r = 0.94777$)– Table 3.

The cluster analysis based on Euclidean distance allowed assessment of similarity or dissimilarity and clarified some of the relationships among the studied walnut accessions. The cluster analysis, applied on quantitative morphological (nut, kernel, shell and tree) traits,

has classified the accessions into four groups on the basis of kernel ratio, tree age, tree circumference and number of veins. First cluster consisted of two sub-clusters. Sub-cluster-1 consisted of five accessions i.e. TL1, MP5, NT4, TT1 and QK3 while sub-cluster-2 consisted of six accessions i.e. BN2, SP1, CN2, KL1, SP3 and QK2. Second cluster also consisted of two sub-clusters. Sub-cluster-1 consisted of six accessions i.e. QK1, QK5, SP2, KS2, KL5 and QK6 while sub-cluster-2 consisted of eight accessions i.e. NT3, NT2, SB1, BD1, SB2, DN1, BA1 and QK4. Third cluster consisted of only three accessions i.e. CN1, GA2 and QK7. Fourth cluster also consisted of two sub-clusters. Sub-cluster-1 consisted of three accessions i.e. BA2, CN3 and BN4 while sub-cluster-2 consisted of five accessions i.e. GA1, GA3, BN3, NT1 and SA1. An accession i.e. BN1 was separately classified as outlier accession (Fig. 2). The results of cluster analysis confirmed the results of PCA for the studied accessions. The high diversity

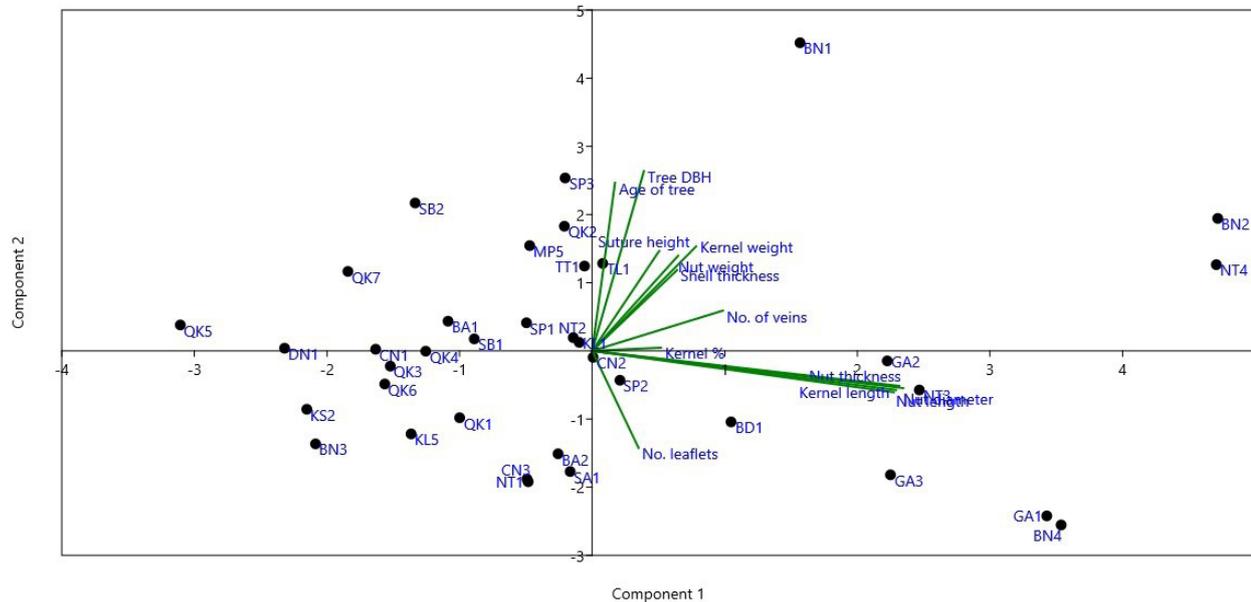


Fig. 3. Principal component analysis for the classification of walnut accessions

Accessions: BA – Bugna, NM – Neelum, SA – Salkhala, DN – Dawarian, NR – Nagdar, CN – Chanannian, KS – Kundal Shahi, KL – Keil, NT – Nowkot, QK – Qaiserkot, RN – Reshian, SP – Saidpura, TS – Tredasharif, MI – Moji, GA – Gaepura, BD – Bajaldahar, TT – Taobutt, MP – Mirpura, KS – Keilseri, TL – Tilawari. The numbers 1–7 without abbreviations show the sequence of accessions from each other.

Table 6. Principal component analysis (PCA) and cluster analysis of walnut accessions based on principal component

Principal component axis	Eigenvalue	Percentage of variance
PC1	3.68247	28.327
PC2	2.30833	17.756
PC3	1.76327	13.564
PC4	1.21282	9.3294
PC5	1.07641	8.2801
PC6	0.895281	6.8868
PC7	0.724756	5.575
PC8	0.694801	5.3446
PC9	0.311491	2.3961
PC10	0.254171	1.9552
PC11	0.0467556	0.35966
PC12	0.0210784	0.16214
PC13	0.00836396	0.064338

Table 7. The morphological and pomological characteristics of selected walnut accessions

S #	Acc	TV	BD	GH	LT	LG	LS	DT	CA	FH	TM	HR	ER	NSI	SC	ST	TS	BS	NS	KP	KC
1	TT1	3	2	2	2	2	3	2	3	5	3	3	3	5	5	7	9	3	3	5	7
2	DN1	5	2	2	2	2	5	2	3	5	3	5	5	5	5	5	5	9	5	3	5
3	KL5	5	5	3	3	3	2	5	5	3	3	7	7	3	5	7	7	2	5	3	3
4	BA1	5	5	3	5	5	2	5	2	3	5	5	5	5	7	5	7	9	5	5	5
5	KL1	5	7	5	9	9	5	2	2	2	9	5	5	5	3	3	5	2	2	2	3
6	MP5	3	5	3	9	9	5	2	2	2	9	3	2	5	3	3	7	9	5	2	3
7	BA2	3	2	2	9	9	3	3	3	5	9	3	2	5	3	2	5	3	2	3	3
8	KS2	5	3	2	9	9	5	2	5	5	9	3	2	3	3	5	5	9	5	5	9
9	CN1	3	3	2	2	2	5	2	2	2	3	7	9	2	3	3	5	2	2	3	3
10	NT1	3	5	2	2	2	5	3	2	2	2	7	7	3	5	5	5	2	2	3	3
11	SP2	5	7	5	2	2	3	3	5	2	2	7	9	3	3	3	7	2	2	5	5
12	BD1	5	7	5	2	2	3	5	5	2	2	7	9	2	3	3	7	2	2	5	5
13	TL1	5	2	2	3	3	2	5	2	5	3	5	5	7	3	3	5	2	2	5	7
14	BN1	5	2	2	3	3	2	5	2	3	3	3	2	2	3	3	3	2	3	3	3
15	BN2	2	3	3	7	7	5	2	3	5	7	3	2	3	2	3	3	7	2	2	2
16	BN3	3	3	3	2	2	5	2	2	5	2	3	2	5	3	3	7	9	5	3	7
17	SP1	3	3	2	2	2	2	2	2	3	2	3	3	7	3	5	7	3	2	3	5
18	BN4	3	2	2	9	9	3	2	3	5	9	3	2	3	3	3	2	2	3	3	3
19	QK1	5	5	5	3	3	7	3	5	5	9	3	3	7	7	5	3	2	2	3	3
20	NT2	2	5	2	9	9	5	5	3	2	9	2	2	5	3	5	3	2	2	2	3
21	CN2	3	7	5	5	5	5	2	2	5	5	3	2	5	5	5	7	3	5	3	3
22	QK2	2	5	2	7	7	5	5	2	2	7	3	3	7	5	7	5	2	2	3	5
23	SP3	2	5	2	3	3	7	3	5	2	3	7	9	7	5	7	7	2	2	3	5
24	GA1	3	2	3	3	3	3	3	3	5	3	3	3	5	2	3	5	2	2	3	5
25	GA2	5	3	3	7	7	3	2	3	5	7	3	3	5	2	3	5	2	2	7	7
26	GA3	3	5	2	3	3	5	2	2	3	3	3	3	5	7	7	5	7	2	3	5
27	NT3	3	5	2	3	3	5	3	2	3	3	5	3	7	5	7	7	3	5	3	5
28	NT4	5	7	5	5	5	7	2	3	2	7	5	5	9	5	7	7	3	2	3	3
29	QK3	5	3	3	7	7	3	5	3	5	5	3	3	7	5	5	7	2	2	3	3
30	QK4	3	2	5	3	3	5	3	5	2	7	9	9	9	5	9	5	3	3	2	2
31	QK5	2	2	2	2	2	5	2	2	2	3	2	2	7	2	2	5	5	2	5	3
32	QK6	2	3	2	2	2	5	2	2	3	2	3	5	9	5	5	5	2	2	2	3
33	QK7	3	5	3	3	3	3	2	3	5	3	3	3	7	3	3	3	2	2	3	3
34	SB1	2	7	3	5	5	2	5	2	2	5	3	3	7	3	3	3	9	2	3	3
35	SB2	2	5	5	7	7	7	5	3	2	9	3	3	5	5	5	7	9	2	2	3
36	SA1	3	5	2	2	2	5	2	2	2	2	5	5	9	3	3	3	2	2	3	7
37	CN3	2	3	3	7	7	5	2	2	5	7	3	2	3	3	2	3	5	2	2	3

Acc – accessions, TV – tree vigour, BD – branch density, GH – growth habit, LT – leafing time, LG – leafing group, LS – leaflet shape, DT – dichogamy type, CA – catkin abundance, FH – flowering habit, TM – time of maturity, HR – hardness, ER – ease of removal, NSI – nut size, SC – shell colour, ST – shell texture, TS – tip shape of nut, BS – base shape of nut, NS – nut shape, KP – kernel plumpness, KC – kernel colour.

Accessions: BA – Bugna, NM – Neelum, SA – Salkhala, DN – Dawarian, NR – Nagdar, CN – Chanannian, KS – Kundal Shahi, KL – Keil, NT – Nowkot, QK – Qaiserkot, RN – Reshian, SP – Saidpura, TS – Tredasharif, MI – Moji, GA – Gaepura, BD – Bajaldahar, TT – Taobutt, MP – Mirpura, KS – Keilseri, TL – Tilawari, SB – Serbann. The numbers 1–7 without abbreviations show the sequence of accessions from each other.

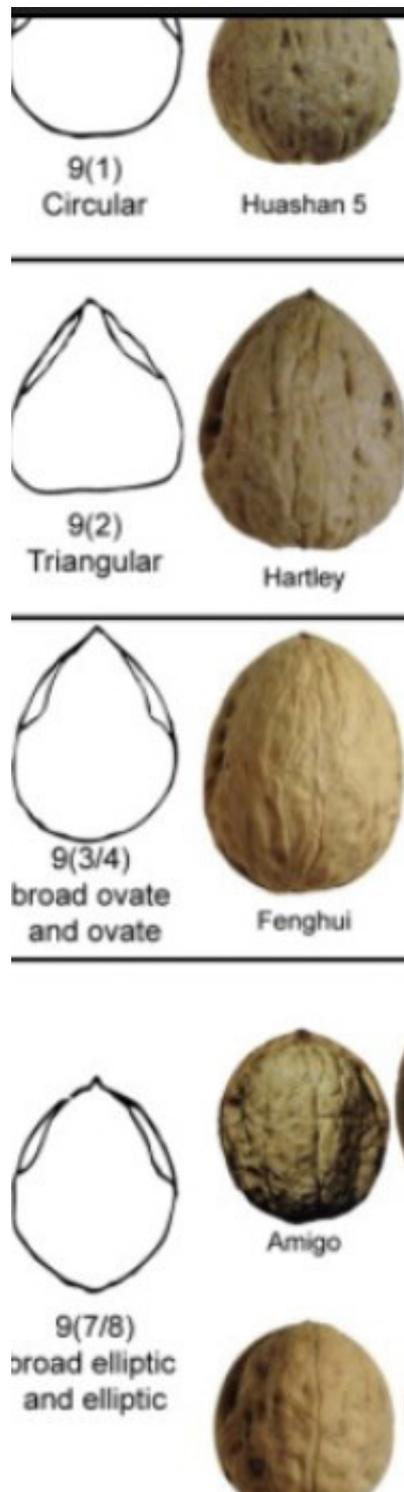


Fig. 4. Diversity in nut shapes among studied walnut accessions

among accessions may be related to sexual propagation, which leads to intra-population diversity. Also, artificial selection and natural hybridization may have contributed to the variations.

Principal components analysis (PCA) was used to identify the most significant traits in the dataset. It determines the main factors and parameters to discriminate among accessions. Accessions were also classified by using principal components analysis (PCA) – Figure 3. The PCA of quantitative traits in local walnut accessions revealed maximum variations. The PC-1 showed the highest Eigenvalue (3.6824) with 28.327% variance while the lowest Eigenvalue (0.00836396) with 0.064338% variance was shown by the PC-13. The PCA for walnut accessions revealed maximum variation in the first four PC's contributing 68.9764% variance (Tab. 6). The PCA of quantitative traits revealed that kernel% is the most important parameter in discriminating the local walnut accessions. The PCA revealed that the accessions BN1, BN2 and NT4 were different from others. These accessions were actually landraces from Leepa Valley (Fig. 3). Our results are in harmony with [Jonah et al. 2014].

CONCLUSION

This study was conducted in one of the most important walnut growing regions of Azad Jammu and Kashmir. The results of current study revealed a greater amount of variability in walnut indicating better chances for improvement of germplasm based upon morphological characteristics. These results indicate that valuable walnut accessions, particularly those with desirable nut and kernel characteristics, exist in Azad Jammu and Kashmir. Principal component analysis and cluster analysis permitted discrimination of significant traits that are useful for breeding. The high variability among walnut accessions could be the result of sexual propagation. This study also confirmed the importance of conserving the promising walnut genetic resource. These accessions are the potential source for future breeding programs of nut crops.

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REFERENCES

- Akca, Y., Bilgen, Y., Ercisli, S. (2015). Selection of superior persian walnut (*Juglans regia* L.) from seedling origin in Turkey. *Acta Sci. Pol., Hortorum Cultus*, 14(3), 103–114.
- Arzani, K., Mansouri-Ardakan, H., Vezvaei, A., Roozban, M.R. (2008). Morphological variation among Persian walnut (*Juglans regia*) varieties from central Iran. *New Zealand J. Crop Hort. Sci.*, 36(3), 159–168. <https://doi.org/10.1080/01140670809510232>
- Aslantaş, R. (2006). Identification of superior walnut (*Juglans regia*) varieties in north-eastern Anatolia, Turkey. *New Zealand J. Crop Hort. Sci.*, 34(3), 231–237. <https://doi.org/10.1080/01140671.2006.9514412>
- Asma, B.M. (2012). Pomological and phenological characterization of promising walnut (*Juglans regia* L.) varieties from Malatya, Turkey. *Acta Sci. Pol., Hortorum Cultus*, 11(4), 169–178.
- Atefi, J. (2001). Comparison of some promising Iranian walnut clones and foreign varieties. *Acta Hort.*, 544, 51–59. <https://doi.org/10.17660/ActaHortic.2001.544.5>
- Braak, C.J. ter, Smilauer, P. (2002). CANOCO reference manual and CanoDraw for Windows user's guide: software for conical community ordination (version 4.5): www.canoco.com.
- Chen, L., Ma, Q., Chen, Y., Wang, B., Pei, D. (2014). Identification of major walnut cultivars grown in China based on nut phenotypes and SSR markers. *Sci. Hortic.*, 168, 240–248. <http://dx.doi.org/10.1016/j.scienta.2014.02.004>
- Cosmulescu, S., Botu, M. (2012). Walnut biodiversity in south-western Romania resource for perspective cultivars. *Pak. J. Bot.*, 44(1), 307–311.
- Cosmulescu, S. (2013). Phenotypic diversity of walnut (*Juglans regia* L.) in Romania – opportunity for genetic improvement. *South-Western J. Hortic. Biol. Env.*, 4(2), 117–126.
- Cosmulescu, S., Stefanescu, D., Ionescu, M.B. (2018). Genetic diversity among *Juglans regia* genotypes based on morphological characters of nut. *Erwerbs-Obstbau*, 60(2), 137–143. <http://dx.doi.org/10.1007/s10341-017-0347-5>
- Ebrahimi, A., Khadivi-Khub, A., Nosrati, Z., Karimi, R. (2015). Identification of superior walnut (*Juglans regia*) genotypes with late leafing and high kernel quality in Iran. *Sci. Hortic.*, 193, 195–201.
- FAO (2019). FAOSTAT Production Crops. Available: <http://faostat.fao.org/site/567/default.aspx#ancor> [date of access: 16.01.2014].
- Hussain, I., Sulatan, A., Shinwari, Z.K., Raza, G., Ahmed, K. (2016). Genetic diversity based on morphological traits in walnut (*Juglans regia* L.) landraces from Karakoram region-I. *Pak. J. Bot.*, 48(2), 653–659.
- IPGRI (1994). Descriptors for walnut (*Juglans spp.*). International Plant Genetic Resources Institute, Rome.
- Jonah, O.F., Paula, E.R. de-, Kherani, E.A., Dutra, S.L.G., Paes, R.R. (2014). Atmospheric and ionospheric response to stratospheric sudden warming of January 2013. *J. Geophys. Res. Space Phys.*, 119(6), 4973–4980. <https://doi.org/10.1002/2013JA019491>
- Khadivi-Khub, A., Ebrahimi, A. (2015). The variability in walnut (*Juglans regia* L.) germplasm from different regions in Iran. *Acta Physiol. Plant.*, 37(3), 57. <http://dx.doi.org/10.1007/s11738-015-1806-y>
- Khan, B., Abdukadir, A., Qureshi, R., Mustafa, G. (2011). Medicinal uses of plants by the inhabitants of Khunjerab National Park, Gilgit, Pakistan. *Pak. J. Bot.*, 43(5), 2301–2310.
- McGranahan, G., Leslie, C. (2012). Walnut. In: *Fruit Breeding*, Badenes, M.L., Byrne, D.H. Springer, Boston, 827–846. https://doi.org/10.1007/978-1-4419-0763-9_22
- Mousivand, M., Hassani, D., Payamnoor, V., Jafar, A.M. (2013). Comparison of tree, nut, and kernel characteristics in several walnut species and inter-specific hybrids. *Crop Breed. J.*, 3(1), 25–30. <https://dx.doi.org/10.22092/cbj.2013.100447>
- Naylor, R.L. (2016). Oil crops, aquaculture and the rising role of demand: A fresh perspective on food security. *Global Food Security*, 11, 17–25. <https://doi.org/10.1016/j.gfs.2016.05.001>
- Sen, S., Tekintas, F. (1990). A study on the selection of Adilcevaz walnuts. *Fruit Breed. Genetics*, 317, 171–174.
- Sharma, O., Sharma, S. (2001). Genetic divergence in seedling trees of Persian walnut (*Juglans regia* L.) for various metric nut and kernel characters in Himachal Pradesh. *Sci. Hortic.*, 88(2), 163–171.
- Sharma, R.M., Kour, K., Singh, B., Yadav, S., Kotwal, N., Rana, J.C., Anand, R. (2014). Selection and characterization of elite walnut (*Juglans regia* L.) clone from seedling origin trees in North Western Himalayan region of India. *Australian J. Crop Sci.*, 8(2), 257.
- Yarilgac, T., Koyuncu, F., Koyuncu, M., Kazankaya, A., Sen, S. (1999). Some promising walnut selections (*Juglans regia* L.). *Acta Hort.*, 544, 93–96. <http://dx.doi.org/10.17660/ActaHortic.2001.544.10>
- Zeneli, G., Kola, H., Dida, M. (2005). Phenotypic variation in native walnut populations of Northern Albania. *Sci. Hortic.*, 105(1), 91–100. <http://dx.doi.org/10.1016%2Fj.scienta.2004.11.003>

