Genetic divergence studies in different nut and kernel characters of diverse walnut (*Juglans regia* L.) germplasm

Shivali Sharma, K. Kumar and Amit Kumar*

*Department of Fruit Science, UHF, Nauni-Solan (Himachal Pradesh), *Division of Fruit Science, SKUAST-Jammu, India. *E-mail: khokherak@rediffmail.com*

Abstract

Exotic cultivars introduced from abroad and local selections from seedling population of walnut were subjected to Non-hierarchical Euclidean cluster analysis based on 12 nut and kernel characters. Studied accessions were grouped into four clusters, where cluster 1 and 3 had majority of the accessions. The clustering pattern of walnut genotypes belonging to the same eco-geographical region revealed their distribution in more than one cluster showing between geographic and genetic diversity. The first component presented maximum eigen root value and per cent variation. The mean intra and inter cluster distance (D) revealed that cluster 4 had highest intra cluster distance (2.206), while the inter cluster distance was maximum between cluster 3 and 4 (5.806). Maximum mean nut yield, nut length, nut weight and kernel weight was recorded in cluster 4, whereas, maximum kernel percentage and fat percentage was recorded in cluster 1 and 2, respectively. Minimum shell thickness was observed in cluster 2. The accessions of cluster 3 and 4 were found highly diverse from each other and will give better segregants after hybridization which can be used as a parent in further breeding programme.

Key words: Growth, divergence, exotic cultivars, local selections, walnut germplasm

Introduction

Horticulturists have exploited the genetic diversity from native walnut population by selecting plus trees for economically important characters for developing improved genotypes. Otherwise, in India most of the walnut plantations are of age-old trees of seedling origin which come into bearing late and produce nuts of inferior quality. As such, characterization and evaluation becomes necessary for determining adaptability under new environmental conditions (Lanseri *et al.*, 2001; Botu *et al*., 2007). Determination of nature and magnitude of genetic diversity and variability of the character under improvement is pre-requisite for any breeding programme. The choice of genetically diverse parent for hybridization under transgressive breeding programme is dependent upon categorization of breeding material on the basis of appropriate criteria (Sharma, 1996). Therefore, the information about the extent of genetic divergence is critical for improvement of any crop in order to have heterotic response and desirable segregants. To achieve this from the last twenty years, field surveys were carried out in different walnut growing districts of Himachal Pradesh to select the superior selections from the seedling populations (Thakur, 1993; Gupta, 1999; Sharma, 1999, 2002).

A large number of selected genotypes along with exotic accessions introduced from abroad are being maintained as germplasm collection after grafting the same on seedling rootstock. The study was conducted with an objective to generate information on nature and degree of genetic divergence present in the germplasm for further improvement through hybridization.

Materials and methods

Experimental site and studied material: The present investigation was undertaken on 3-4 years old plants of indigenous selection collected through field survey of different locations in Himachal Pradesh and exotic cultivars of persian walnut introduced directly from abroad (Table 1).

Table 1. Indigenous and exotic accessions with their place of origin

Accession	Place of origin	Accession	Place of origin
Aksu≠71	China	Mayette	USA
Aksu≠417	China	Placentia	USA
Chico	USA	Serr	USA
Cisco	USA	Xin Zad Fen	China
Graves Franquette	USA	Zhong Lin≠3	China
Howard	USA	Daulat Ram Selection	H.P. (India)

These selections and exotic cultivars were propagated on seedling rootstocks and are presently growing at experimental farm of Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni-Solan (H.P.) located around 1275 m above mean sea level and between 31° N latitude and 77° E longitude.

Observation recorded: Nut yield was calculated by weighing total number of nuts produced by each accession. Yield efficiency was calculated as suggested by Westwood (1993).

$$\text{Yield efficiency (kg/cm}^2) = \frac{\text{Yield (kg) / tree}}{\text{Trunk cross-sectional area}}$$

Trunk cross sectional area (TCSA) was calculated as per method given by Westwood (1993) as TCSA is equal to πr^2, where, r is equal to radius of trunk.

A random sample of 10 nuts from each plant was taken and

observations on twelve nut and kernel characters were recorded as per descriptors. Nut and kernel weight (g) was weighed on digital balance whereas kernel percentage was calculated as under:

$$\text{Kernel (\%)} = \frac{\text{Kernel weight}}{\text{Nut weight}} \times 100$$

Nut diameter (mm) through suture and perpendicular to suture, nut length (mm) and shell thickness (at near centre of half shell in mm) were measured with the help of digital vernier calliper (Mitutoyo, Japan - CD - 6" CS). For estimation of kernel protein (%), nitrogen content was estimated by conventional Kjeldahl method in Kjeltech auto analyser from Foss Tecator, Sweden. Protein content (%) was worked out by multiplying N content with a factor of 5.3 suggested for tree nuts (Khanizadeh *et al.* 1995), however, kernel fat (%) was determined by the method of Folch *et al.* (1957).

Data analysis: Data recorded on the nut and kernel characters were statistically analyzed using standard procedures (Panse and Sukhatme, 1995). Genetic divergence was computed using non-hierarchical Euclidean cluster analysis as suggested by Spark (1973) within the germplasm and classification of accessions into homogenous groups. This method characterizes genetic divergence on the basis of similarity and dissimilarity denoted by aggregate effects of agronomic traits under study.

Results

The mean, standard deviation and co-efficient of variation for nut and kernel characters were recorded and the analysis of variance revealed significant differences among the genotypes for each character, indicating the existence of considerable variability among the genotypes for the character studied. In the principal component analysis, first component showed the highest eigen roots (4.958) and maximum contribution for diversity (41.32 %) with the main contribution of kernel weight, nut yield, nut diameter through suture and fat, while the minimum eigen roots value (0.001) and per cent contribution towards diversity (0.01 %) was observed for the eleventh component, having maximum contribution of nut weight. Second component *i.e.* nut yield showed the eigen value of 3.615 and explains 30.12 per cent of total variation and is mainly the contribution of protein and nut yield. The third vector had an eigen value of 1.273 having the

total variation of 10.61 per cent and it is the combination of nut diameter perpendicular to suture and nut diameter through suture, whereas the fourth component had an eigen value of 1.112 with a total variation of 9.27 per cent and the maximum contribution was of kernel weight. Fifth vector *i.e.* nut diameter perpendicular to suture had an eigen value of 0.470 which explains total variations for this vector was 3.91 per cent with it is mainly combination of nut length and nut weight. However, last component of the study recorded eigen root value (0.000) with 0.00 per cent contribution towards diversity. The per cent variation explained by the first 5 components was 95.23 per cent (Table 2).

The distribution of 12 walnut accessions revealed that there were four clusters and the distribution of accessions from different eco-geographical areas was apparently random. Cluster 1 and 3 contained the maximum (4) number of walnut accessions while cluster 2 and 4 contained minimum (2) number of walnut accessions (Table 3). Maximum mean value for number of nuts/plant (17.00), nut yield (180.49 g/plant), nut diameter perpendicular to suture (32.40 mm), nut length (34.00 mm), shell thickness (3.06 mm), nut weight (10.47 g) and kernel weight (4.31 g) was observed in cluster 4. Maximum mean value for nut diameter through suture (31.00 mm), protein (9.52 %) and fat (55.50 %) was recorded in cluster 2, however, maximum kernel percentage (56.72 %) was observed in cluster 1 (Table 4). Similarly, maximum co-efficient of variability for number of nuts/plant (97.71 %), nut yield (80.17 %), yield efficiency (48.15 %), nut length (9.22 %), shell thickness (27.94 %), nut weight (9.43 %), kernel weight (19.80 %), protein percentage (44.72 %) were recorded in cluster 1, however, nut diameter through suture (20.04 %), kernel percentage (19.97 %) and fat percentage (15.37 %) scored maximum coefficient of variability in cluster 4.

Intra- and inter-cluster distances revealed that intra cluster

Table 3. Distribution of 12 walnut accessions into various clusters

Cluster	Number of accessions	Accessions
1	4	Cisco, Mayette, Placentia, Serr
2	2	Daulat Ram Selection, Graves Franquette
3	4	Aksu≠71, Aksu≠417, Chico, Xin Zad Fen
4	2	Howard, Zhong Lin≠3

Table 2. Eigen vectors, eigen roots and per cent variation explained by yield, nut and kernel characters of walnut germplasm

S. No.	Character	Eigen roots	Variation (%)	Eigen vectors				
				1	2	3	4	5
1.	Number of nuts/plant	4.958	41.32	-0.133	0.126	-0.303	0.314	0.326
2.	Nut yield (g/plant)	3.615	30.12	0.422	0.454	0.165	-0.296	0.198
3.	Yield efficiency (kg/cm²)	1.273	10.61	-0.170	-0.250	-0.104	0.294	0.345
4.	Nut diameter through suture (mm)	1.112	9.27	0.364	0.177	0.488	0.178	0.305
5.	Nut diameter perpendicular to suture (mm)	0.470	3.91	-0.361	-0.336	0.531	0.139	0.111
6	Nut length (mm)	0.242	2.02	-0.085	0.160	-0.300	-0.029	0.476
7.	Shell thickness (mm)	0.189	1.58	-0.112	0.009	0.059	-0.356	-0.065
8.	Nut weight (g)	0.062	0.52	-0.041	0.039	0.353	0.118	0.391
9.	Kernel weight (g)	0.049	0.41	0.597	-0.218	-0.160	0.545	-0.189
10.	Kernel (%)	0.029	0.24	-0.017	-0.018	0.319	0.134	-0.359
11.	Protein (%)	0.001	0.01	-0.353	0.676	0.034	0.370	-0.280
12.	Fat (%)	0.000	0.00	0.094	-0.199	-0.056	-0.288	0.053

Per cent variation explained by first 5 components = 95.23%

Table 4. Mean, standard deviation and coefficient of variation for various clusters on the basis of yield, nut and kernel characters of walnut germplasm

S No.	Characters	Parameters	Clusters			
			1	2	3	4
1	Number of nuts/ plant	Mean	8.75	1.50	16.75	17.00
		SD	6.55	0.71	5.30	7.07
		COV	97.71	47.33	31.64	41.59
2	Nut yield (g/plant)	Mean	47.82	13.25	70.78	180.49
		SD	38.34	5.30	15.22	86.05
		COV	80.17	40.00	21.50	47.67
3	Yield efficiency (kg/cm²)	Mean	0.005	0.006	0.014	0.013
		SD	0.002	0.0002	0.005	0.0008
		COV	48.15	22.58	37.21	41.86
4	Nut diameter through suture (mm)	Mean	26.95	31.00	23.04	26.34
		SD	1.74	2.47	0.37	5.28
		COV	6.46	7.97	1.61	20.04
5	Nut diameter perpendicular to suture (mm)	Mean	28.50	26.93	24.86	32.40
		SD	2.21	2.40	0.81	2.71
		COV	7.75	8.91	3.26	8.36
6	Nut length (mm)	Mean	29.50	33.33	26.45	34.00
		SD	2.72	0.08	2.26	2.14
		COV	9.22	0.24	8.54	6.29
7	Shell thickness (mm)	Mean	2.04	1.99	1.61	3.06
		SD	0.57	0.32	0.43	0.11
		COV	27.94	16.08	26.71	3.59
8	Nut weight (g)	Mean	5.30	9.00	4.21	10.47
		SD	0.50	0.71	0.17	0.71
		COV	9.43	7.89	4.04	6.78
9	Kernel weight (g)	Mean	3.03	4.25	2.24	4.31
		SD	0.60	0.35	0.16	0.57
		COV	19.80	8.23	7.14	13.23
10	Kernel (%)	Mean	56.72	47.22	53.23	41.40
		SD	7.27	0.22	5.04	8.27
		COV	12.82	0.46	9.47	19.97
11	Protein (%)	Mean	5.30	9.52	5.20	4.12
		SD	2.37	2.52	1.50	0.17
		COV	44.72	26.47	28.85	4.12
12	Fat (%)	Mean	44.97	55.50	47.75	46.00

Table 5. Average inter and intra-cluster distance among various clusters in walnut germplasm

Clusters	1	2	3	4
1	**2.162**			
2	3.990	**0.837**		
3	2.903	5.703	**1.300**	
4	4.591	5.260	5.806	**2.206**

Bold figures represent intra-cluster distances

distance in accessions varied between 0.837 of cluster 2 and 2.206 of cluster 4 (Table 5). The minimum inter cluster distance of 2.903 was recorded between cluster 1 and 3. Similarly, maximum inter-cluster distance of 5.806 was recorded between cluster 3 and 4. These clusters contain 4 and 2 walnut accessions *viz*. Aksu#7, Aksu#417, Chico, Xin Zad Fen, Howard and Zhong Lin#3.

Discussion

In the present investigation a wide range of diversity was observed for various nut and kernel characters from various eco-geographical regions. Similar variation for these characters was obtained by various workers with different walnut cultivars and seedling trees in different walnut growing areas (Mehta *et al*., 2005, Sharma and Kumar, 2005 and Pandey *et al*., 2006). The tendency of different walnut genotypes to occur in clusters cutting across eco-geographical regions, demonstrates that geographical isolation is not the only factor causing genetic diversity. All present genotypes were clustered into 4 clusters. However, in earlier studies different clusters were found by Barua and Sharma (2003) in apple, Thakur *et al*. (2005) in almond, Kaushal and Sharma (2005) in pecan and Sharma and Sharma (2005), Pandey and Tripathi (2007) and Sharma *et al*. (2010) in walnut. The highest eigen roots and per cent contribution towards diversity was contributed by number of nuts/plant. Similarly, contribution of first components was observed by Srivastava *et al*. (2010) in hazelnut germplasm. The relatively low values of intra-cluster distance (Table 4) depicted the presence of a narrow range of variability within a cluster. The inter cluster distance was least (2.903) between cluster 1 and 3 which indicates that the genotypes of these cluster are very close to each other and cannot be used for hybridization programme. The maximum inter-cluster distance of 5.806 was recorded between cluster 3 and 4. The genotypes falling in clusters were 4 and 2 from various eco-geographical regions. Different intra- and inter-cluster distances were recorded previously (Sharma and Sharma, 2005; Pandey and Tripathi, 2007 and Sharma *et al*., 2010) for walnut seedling trees and various genotypes were suggested for hybridization.

In conclusion, parents for hybridization could be selected on the basis of their large inter cluster distance for isolating useful recombinants in the segregating generation. To improve various nut and kernel characters the genotypes falling in cluster 3 and 4 can be utilized for hybridization programme as well as for introgressing their useful traits in the commercial walnut cultivars.

References

Barua, U. and R.K. Sharma, 2003. Estimation of genetic divergence in apple (*Malus x domestica* Borkh.). *Indian J. Pl. Gen. Res.*, 16: 4-6

Botu, M., I. Botu, G. Achin and M. Tudor, 2007. Walnut cultivars in Romanian conditions. *Acta Hort.*, 760: 555-561

Folch, J., M. Lees and S. Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, 226: 497-506.

Gupta, S.K. 1999. Characterization and evaluation of walnut (*J. regia* L.) germplasm accessions. M.Sc. Thesis, Dr. Y.S. Parmar University of Horticulture and Forestry, Solan, India.

Kaushal, R.K. and S.D. Sharma, 2005. Non-hierarchical Euclidean cluster analysis studies in seedling trees of pecan (*Carya illinoensis* (Wang) K. Koch.) growing in Himachal Pradesh. *Acta Hort.*, 696: 97-101

Khanizadeh, S., D. Buszardand C.G. Zarkadas, 1995. Misuse of the Kjeldahl method for estimating protein content in plant tissues. *HortScience*, 30(7): 1341-1342.

Lansari, A., A.E. Hassani, D. Nabil and E. Germain, 2001. Preliminary results on walnut germplasm evaluation in Morocco. *Acta Hort.*, 544: 27-35

Mehta, K., O.C. Sharma, A.S. Kashyap and B.S. Thakur, 2005. Variability for various nut and kernel characters in some promising seedling walnuts (*Juglans regia* L.). *Acta Hort.*, 696: 91-95

Pandey, G. and A.N. Tripathi, 2007. Estimation of genetic divergence in walnut. *Indian J. Hort.*, 64: 399-401

Pandey, G., A.N. Tripathi and S. Mandal, 2006. Promising genotypes of Persian walnut (*Juglans regia* L.) from Uttaranchal. *Indian J. Hort.*, 63: 256-259

Panse, V.G. and P.V. Sukhatme, 1995. *Statistical Methods for Agricultural Workers*. ICAR Pub., New Delhi, p. 296.

Sharma, J.R. 1996. *Statistical and Biometrical Techniques in Plant Breeding*. New Age International, New Delhi.

Sharma, O.C. and S.D. Sharma, 2005. Intra specific divergence in seedling trees of Persian walnut (*Juglans regia*) for metric traits, foliage and floral characters in Himachal Pradesh, India. *Plant Gen. Res. Newslett.,* 144: 45-53

Sharma, R. and K. Kumar, 2005. Selecting walnuts from seedling tree populations in some villages of district Sirmour (HP), India. *Acta Hort.,* 696: 85-89

Sharma, O.C. 1999. Studies on variability and selection of superior persian walnut (*Juglans regia* L.) seedling trees in Himachal Pradesh. Ph.D. Thesis, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan, India.

Sharma, R. 2002. Selecting walnuts from seedling tree populations in some villages of district Sirmour (H.P.). M.Sc. Thesis, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni-Solan, India.

Spark, D.N. 1973. Euclidean cluster analysis algorithm AS. 58. *Appl. Stat.*, 22: 126-130.

Srivastava, K.K., K.A. Zargar and S.R. Singh, 2010. Genetic divergence among *Corylus colurna* genotypes based on morphological characters of hazelnut. *Biodi. Res. Conser.,* 17: 13-17

Thakur, D. 1993. Genetic variability in bearing seedling walnuts (*J. regia* L.) in Kullu valley. M.Sc. Thesis, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni-Solan, India.

Thakur, B.S., O.C. Sharma, K. Mehta, D.P. Sharma and A.S. Rehalia, 2005. Genetic divergence in almond (*Prunus amygdalus* Batsch) growing in Kinnaur, Himachal Pradesh. *Acta Hort.,* 696: 69-74.

Westwood, M.N. 1993. *Temperate Zone Pomology*. Seventh Edition. H. Freeman and Company, San Francisco. p. 523.