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Genetic divergence studies regarding different growth and foliage characters of walnut (*Juglans regia* L.) germplasm

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Abstract: Genetic divergence of 29 exotic walnut cultivars introduced from abroad and 25 local selections from seedling population was studied. The analysis of variance revealed significant differences among accessions for each character under study. Based on Mahalanobis D^2 values the accessions were grouped into eight clusters. Cluster I had a maximum of 12 accessions followed by cluster II with 11 accessions. The mean intra and inter cluster distance (D) revealed that cluster VII had the highest intra cluster distance (2.171), while the inter cluster distance was the greatest between cluster II and IV (10.528). Characters like plant height, trunk girth, plant spread, plant volume, leaf length, leaf width and number of leaflets contributed the most to total divergence.

1. Introduction

Persian walnut (*Juglans regia* L.) is one of the most important nut crops grown in temperate regions and it produces edible nuts of high nutritional value. In India there are no systematic orchards of walnut containing standard cultivars. Although some efforts have been made in this direction in the last two decades and the superior selections from the seedling populations were selected through field surveys in different walnut growing districts of Himachal Pradesh (Thakur, 1993; Gupta, 1999; Sharma, 1999, 2002; Thakur *et al*., 2005). However, a large number of exotic accessions have also been introduced from abroad and a germplasm collection block has been maintained after propagation of these selections and exotic accessions on the seedling rootstock. Therefore characterization and evaluation of this germplasm is important for future use and a wider divergence is a prerequisite for breeding purposes. Information about the extent of genetic divergence is critical for the improvement of any crop in order to have heterotic responses and desirable segregants. Furthermore, information on the nature and degree of genetic divergence present in the collected germplasm could help for further improvement through hybridization.

2. Materials and Methods

The present investigation was undertaken on three- to four-year-old plants for a total of 54 indigenous selections collected through field survey at different locations in Himachal Pradesh and exotic cultivars of Persian walnut introduced directly from abroad (Table 1). These were propagated on seedling rootstocks and are presently growing at Oachghat Block of Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni-Solan (Himachal Pradesh) located around 1275 m above mean sea level and between 31°N latitude and 77°E longitude. Data for various growth [plant height (m), trunk girth (cm^2), plant spread (cm), TCSA (cm^2) and plant volume (cm^3)] and foliage characters [leaf length (cm), leaf width (cm), leaf area (cm^2), leaflet length (cm), leaflet width (cm) and number of leaflets] were recorded. Ten compound leaves were taken from each individual tree for foliage characters and average means were calculated. Standard procedures were followed to calculate the mean, standard deviation and coefficient of variation for various characters as described by Panse and Sukhatme (1995). The genetic divergence among the accessions was estimated by Mahalanobis D^2 statistic as suggested by Rao (1952).

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Table 1 - Different exotic and indigenous walnut accessions and their place of origin

S. No.	Accession	Place of origin
Exotic cultivars		
1.	Adams 10	USA
2.	Aksu≠24	China
3.	Aksu≠71	China
4.	Aksu≠81	China
5.	Aksu≠210	China
6.	Aksu≠417	China
7.	Aksu Hun ≠85	China
8.	Bulgaria 3	Bulgaria
9.	Chandler	USA
10.	Chico	USA
11.	Cisco	USA
12.	Conway Mayette	USA
13.	Corne	France
14.	Graves Franquette	USA
15.	Hotien 8	China
16.	Howard	USA
17.	Howe	USA
18.	Mayette	USA
19.	Meylannaise	France
20.	Nn 88 Godyn	Poland
21.	Parisienne	France
22.	Placentia	USA
23.	Ronde de Montignac	France
24.	Scharsch Franquette	USA
25.	Serr	USA
26.	Shinrei	China
27.	Xin Zad Fen	China
28.	Xin Zheng Zhu	China
29.	Zhong Lin≠3	China
Indigenous selections		
30.	Brij Lal Selection	H.P. (India)
31.	Chamba Selection-20	H.P. (India)
32.	Chamba Selection-32	H.P. (India)
33.	Chamba Selection-60	H.P. (India)
34.	Chamba Selection-101	H.P. (India)
35.	Chamba Selection-123	H.P. (India)
36.	Daulat Ram Selection	H.P. (India)
37.	Jaunaji Selection -2	H.P. (India)
38.	Jaunaji Selection –4	H.P. (India)
39.	Jaunaji Selection –6	H.P. (India)
40.	Jaunaji Selection-7	H.P. (India)
41.	Jaunaji Selection-10	H.P. (India)
42.	Jaunaji Selection-12	H.P. (India)
43.	Jogindernagar Selection-23	H.P.(India)
44.	Jogindernagar Selection-39	H.P. (India)
45.	Jogindernagar Selection-61	H.P. (India)
46.	Jogindernagar Selection-122	H.P. (India)
47.	Nauradhar Selection-10	H.P. (India)
48.	Nauradhar Selection-36	H.P. (India)
49.	Nauradhar Selection-53	H.P. (India)
50.	Rajgarh Selection-1	H.P. (India)
51.	Rajgarh Selection-8	H.P. (India)
52.	Rajgarh Selection-11	H.P. (India)
53.	Selection No. 8	H.P. (India)
54.	Selection No. 51	H.P. (India)

All the accessions were grouped into clusters according to the Tocher's method described in Rao (1952).

3. Results and Discussion

The analysis of variance revealed significant differences among the genotypes for each character, indicating the existence of variability among the genotypes for such characters. On the basis of the relative magnitude of D^2 values, 54 accessions were grouped, using Tocher's method, into eight clusters. Cluster I was the largest with 12 accessions followed by cluster II having 11 accessions, and cluster V having 10 accessions; clusters III, IV, VI, VII, VIII had 5, 1, 9, 4, 2 accessions, respectively (Fig. 1 and Table 2). The highest intra cluster distance was registered in cluster VII (2.171) followed by cluster II (1.951), while the minimum intra cluster value was exhibited by cluster IV (0.000). The minimum inter-cluster D^2 value was recorded between cluster I and V (1.821), indicating a close relationship and similarity of most traits of the accessions. Hence, selection of parents from these clusters should be avoided. However, the highest inter-cluster D^2 value was observed between clusters II and IV (10.528), followed by clusters I and IV (10.411), confirming a wide genetic distance between these groups (Table 3). Since these clusters exhibited the greatest inter-cluster distances, selection of parents from such clusters for hybridization programmes could help to develop novel hybrids. A similar trend of clustering patterns has been reported 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 mean value, standard deviation and co-efficient of variability on various growth and foliage traits of cluster is presented in Table 4. Cluster IV had the highest mean values for characters plant height (5.50 m), trunk girth (33.00 cm) and TCSA (86.55 cm^2), while cluster VIII exhibited the highest mean values for plant spread (110.00 cm), plant volume (1.71 cm^3), leaf length (56.50 cm), leaf width (30.25 cm), leaf area (57.35 cm^2), leaflet length (16.17 cm). Maximum mean values for leaflet width (7.19 cm) and number of leaflets (9.73) was recorded in clusters VI and II, respectively. Clusters IV and VIII can be expected to give promising and desirable recombinations in segregating generations because they comprise desirable features as revealed from their cluster means. Similarly, maximum co-efficient of variability for plant height (40.00%), trunk girth (33.11%), plant spread (42.08%), TCSA (65.21%), plant volume (87.50%) and number of leaflets (23.12%) was observed in cluster II, however, cluster I had the highest co-efficient of variability for traits leaf area (32.86%), leaflet length (13.46%) and leaflet width (19.79%).

In the principal component analysis presented in Table 5, the first vector shows the highest eigen value (4.332)

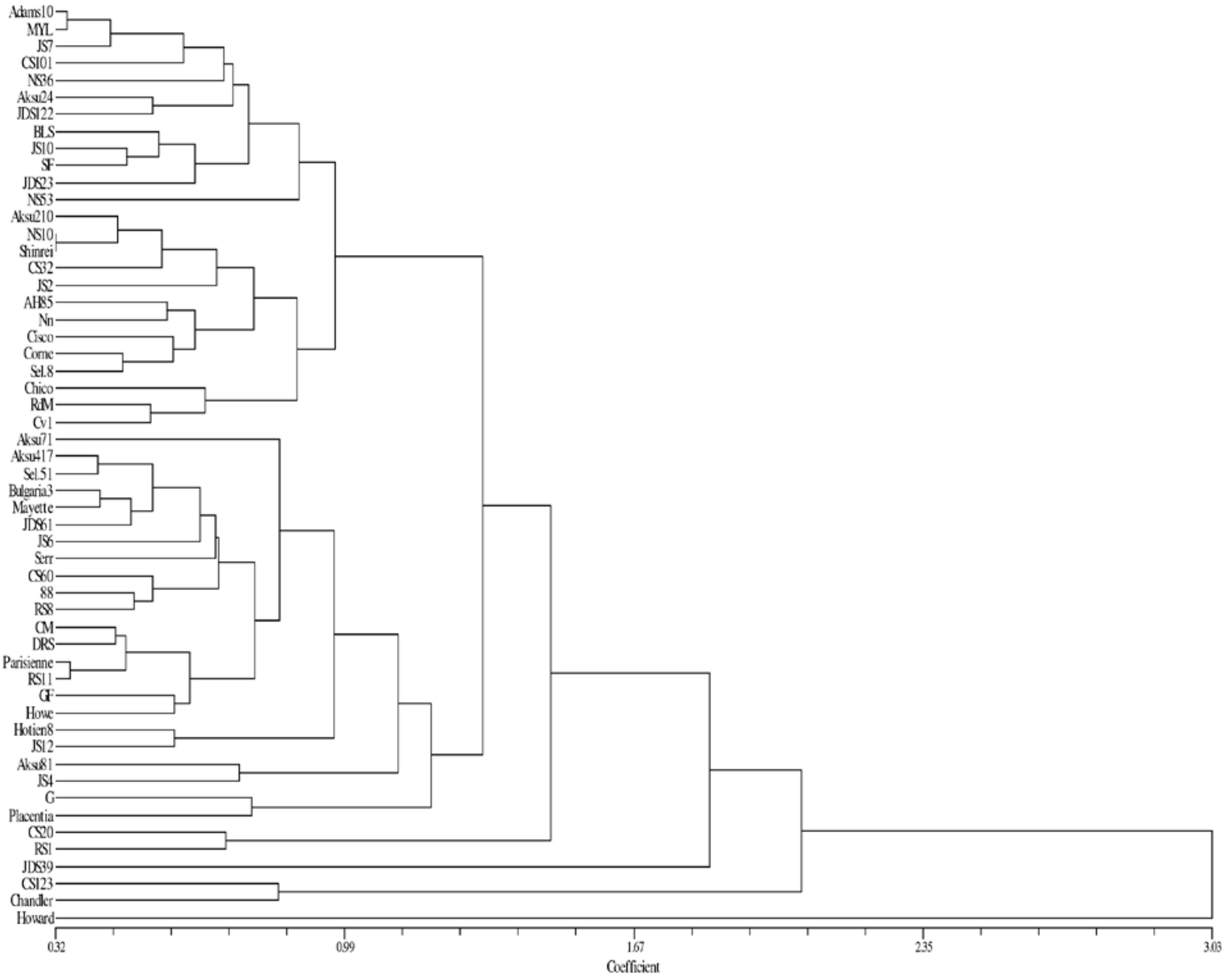

Fig. 1 - Dendrogram showing different clusters of genotypes, grouped by Tocher's method, in walnut accessions.

and accounts for 39.38% of the total variation. The first vector is the combination of leaf length, trunk girth, plant height and leaflet length. The second vector has an eigen value of 3.008 and explains 27.34% of total variation, and this factor is mainly the combination of number of leaflets and trunk girth. The third vector has an eigen value of 1.155 and total variation of 10.50%, from the combination of leaf width and plant spread. The fourth vector has an eigen value of 1.047 and total variation of 9.52%, with the maximum contribution of trunk girth. Vector fifth has an eigen value of 0.424 and total variation of 3.85%, mainly a combination of plant volume and plant spread. The sixth vector has an eigen value of 0.347 with total variation of 3.15 % and the highest contribu-

Table 2 - Distribution of 54 walnut accessions into various clusters

Clusters	Number of accessions	Accessions
I	12	Aksu≠210, Chamba Selection-32, Chamba Selection-60, Conway Mayette, Hotien 8, Jaunaji Selection-2, Jaunaji Selection-12, Jogindernagar Selection-61, Nauradhar Selection-10, Parisienne, Ronde de Montignac, Xin Zheng Zhu
II	11	Adams 10, Aksu≠24, Chamba Selection-101, Jaunaji Selection-7, Jaunaji Selection-10, Jogindernagar Selection-39, Jogindernagar Selection-122, Meylannaise, Nauradhar Selection-36, Nauradhar Selection-53, Selection No. 8.
III	5	Aksu≠81, Cisco, Daulat Ram Selection, Jaunaji Selection-4, Scharsch Franquette
IV	1	Howard
V	10	Aksu≠71, Aksu≠417, Bulgaria 3, Graves Franquette, Howe, Jaunaji Selection-6, Mayette, Rajgarh Selection-1, Selection No. 51, Xin Zad Fen
VI	9	Aksu Hun≠85, Brij Lal Selection, Chico, Corne, Jogindernagar Selection-23, Nn 88 Godyn, Serr, Shinrei, Zhong Lin ≠3
VII	4	Chamba Selection-20, Placentia, Rajgarh Selection-8, Rajgarh Selection-11
VIII	2	Chamba Selection-123, Chandler

Table 3 - Average inter- and intra-cluster distance among various clusters in walnut germplasm

Clusters	I	II	III	IV	V	VI	VII	VIII
I	**1.658**							
II	3.535	**1.951**						
III	2.295	3.975	**1.497**					
IV	10.411	10.528	8.795	**0.000**				
V	1.821	4.953	2.505	10.048	**1.396**			
VI	3.159	2.115	2.689	9.396	3.875	**1.657**		
VII	3.870	4.898	2.984	9.037	3.536	3.388	**2.171**	
VIII	7.505	5.835	6.356	8.920	7.931	5.081	5.220	**1.373**

Bold figures represent intra-cluster distances.

Table 4 - Mean, standard deviation and coefficient of variation for various clusters on the basis of growth and foliage characters of walnut germplasm

S. No.	Characters	Parameters	Clusters							
			I	II	III	IV	V	VI	VII	VIII
1	Plant height (m)	Mean	1.12	1.00	2.98	5.50	1.36	1.77	1.91	2.65
		SD	0.27	0.40	0.85	0.00	0.52	0.49	0.61	0.21
		COV	24.11	40.00	28.52	0.00	38.23	27.68	31.94	7.92
2	Trunk girth (cm)	Mean	7.15	7.55	10.40	33.00	9.45	10.75	12.12	16.00
		SD	2.05	2.50	2.25	0.00	2.60	1.70	3.12	2.83
		COV	28.67	33.11	21.63	0.00	27.51	15.81	25.74	17.69
3	Plant spread (cm)	Mean	31.71	36.36	44.00	50.00	39.50	51.94	70.00	110.00
		SD	11.48	15.30	13.87	0.00	12.95	17.36	17.80	14.14
		COV	36.20	42.08	31.52	0.00	32.78	33.42	25.43	12.85
4	TCSA (cm^2)	Mean	4.38	5.03	8.91	86.55	7.61	8.27	12.28	20.74
		SD	2.17	3.28	3.29	0.00	4.56	2.99	6.10	7.25
		COV	49.54	65.21	36.92	0.00	59.92	36.15	49.67	34.96
5	Plant volume (cm^3)	Mean	0.07	0.08	0.32	0.72	0.15	0.30	1.57	1.71
		SD	0.06	0.07	0.21	0.00	0.11	0.25	0.76	0.57
		COV	85.71	87.50	65.62	0.00	73.33	83.33	48.41	33.33
6	Leaf length (cm)	Mean	33.46	46.73	35.00	40.00	27.05	39.33	32.62	56.50
		SD	5.47	3.98	2.89	0.00	3.46	4.84	6.94	6.36
		COV	16.35	8.52	8.26	0.00	12.79	12.31	21.27	11.26
7	Leaf width(cm)	Mean	18.55	24.97	18.95	20.50	15.50	22.58	18.06	30.25
		SD	2.90	2.26	2.15	0.00	2.41	3.97	1.88	0.35
		COV	15.63	9.05	11.34	0.00	15.55	17.58	10.41	1.16
8	Leaf area (cm^2)	Mean	29.73	56.69	33.34	38.39	26.28	48.00	38.10	57.35
		SD	9.77	8.90	8.12	0.00	6.25	8.64	9.60	2.96
		COV	32.86	15.70	24.35	0.00	23.78	18.00	25.19	5.16
9	Leaflet length (cm)	Mean	10.70	14.57	10.79	12.50	8.81	13.58	10.64	16.17
		SD	1.44	1.15	1.02	0.00	1.13	1.56	1.32	0.23
		COV	13.46	7.89	9.45	0.00	12.83	11.49	12.41	1.42
10	Leaflet width (cm)	Mean	4.95	6.93	5.21	5.46	4.55	7.19	5.58	7.00
		SD	0.98	0.68	0.82	0.00	0.65	0.55	1.05	0.00
		COV	19.79	9.81	15.74	0.00	14.28	7.65	18.82	0.00
11	Number of leaflets	Mean	9.29	9.73	8.80	9.00	7.65	8.17	8.00	8.00
		SD	1.32	2.25	0.45	0.00	0.82	0.87	1.15	1.41
		COV	14.21	23.12	5.11	0.00	10.72	10.65	14.37	17.62

Table 5 - Eigen vectors, eigen roots and percent variation explained by growth and foliage characters of walnut germplasm

S. No.	Characters	Eigen roots	Per cent variation	Eigen vectors										
				1	2	3	4	5	6	7	8	9	10	11
1	Plant height (m)	4.332	39.38	0.131	0.180	0.239	0.147	0.192	0.415	0.419	0.404	0.432	0.369	0.035
2	Trunk girth (cm)	3.008	27.34	0.458	0.490	0.293	0.456	0.295	-0.145	-0.126	-0.195	-0.169	-0.196	-0.163
3	Plant spread (cm)	1.155	10.50	-0.202	-0.228	0.424	-0.383	0.496	-0.128	0.018	-0.037	-0.042	0.068	-0.557
4	TCSA (cm^2)	1.047	9.52	-0.059	-0.132	0.371	-0.170	0.326	0.211	0.099	-0.153	-0.129	-0.354	0.697
5	Plant volume (cm^3)	0.424	3.85	-0.006	-0.018	-0.379	0.051	0.579	-0.023	-0.483	0.308	-0.160	0.337	0.225
6	Leaf length (cm)	0.347	3.15	0.818	-0.293	-0.075	-0.391	-0.025	-0.076	0.057	-0.158	0.037	0.213	0.066
7	Leaf width(cm)	0.243	2.20	-0.054	0.125	0.512	-0.070	-0.326	-0.468	-0.111	0.276	-0.231	0.418	0.270
8	Leaf area (cm^2)	0.191	1.74	-0.199	0.026	-0.206	0.179	0.242	-0.426	0.469	-0.506	0.101	0.357	0.180
9	Leaflet length (cm)	0.118	1.07	0.110	-0.047	-0.209	-0.024	0.141	-0.452	0.446	0.565	-0.110	-0.432	-0.002
10	Leaflet width (cm)	0.091	0.83	-0.013	0.060	0.073	-0.090	0.049	-0.362	-0.354	0.001	0.813	-0.217	0.126
11	Number of leaflets	0.045	0.41	-0.060	0.742	-0.192	-0.627	0.024	0.058	0.074	-0.056	-0.056	-0.003	0.021

Percent variation explained by first 7 components = 95.9517

tion came from plant height, while the minimum eigen roots value 0.045 and percent contribution towards diversity (0.41%) was observed for the eleventh component, with a maximum contribution from TCSA. The percent variation explained by the first seven components was 95.95 (Table 5).

The genetic diversity among genotypes could be due to various factors, like heterogeneity, genetic architecture of populations and developmental traits, as described by (Murty and Arunachalam, 1966). Rao *et al.* (2003) reported that geographical distribution and genetic diversity are correlated and concluded that eco-geographically different cultivars also differ from each other genetically. A clustering pattern similar to our pattern was reported in walnut (Pandey and Tripathi, 2007) and hazelnut (Srivastava *et al.*, 2010). Sardana *et al.* (1997) observed that cluster means reveal the inner diversity in the material under study. De *et al.* (1988) proposed that traits contributing the most towards the D^2 values should be given priority in choosing a cluster for further selection and choice of parents for hybridization.

Based on the present findings of genetic divergence and its component analysis it can be concluded that intercrossing between genotypes of genetically diverse clusters that show a superior mean outcome may be helpful for obtaining desirable segregates. In the case of walnut germplasm the highest genetic diversity was registered between clusters II and IV. Comparison of the cluster means for 11 characters indicated that the studied traits considerably differed between the clusters. Therefore, it is suggested to cross 'Howard' with 'Chamba Selection 123' and with 'Chandler' as these accessions are among the most distant clusters, with a high mean outcome, in order to get desirable transgressive segregates.

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