

Assessing genetic divergence in seedling trees of Persian walnut (*Juglans regia*)

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ABSTRACT

A study was conducted during 2006 and 2007 at KVK Leh (Ladakh), J&K to assess the nature and magnitude of genetic divergence of 216 bearing trees of Persian (*Juglans regia* L.) walnut of seedling origin growing at various eco-geographical regions in the cold arid region of India were subjected to non-hierarchical Euclidean cluster analysis based on 13 nut and kernel characters. Minimum and maximum values of co-efficients of variability were recorded for nut width and kernel weight, respectively. All 216 walnut genotypes were grouped into 10 clusters. The clustering pattern of walnut genotypes belonging to the same eco-geographical region revealed their distribution in more than one cluster showing non-parallelism between geographic and genetic diversity. The first component presented maximum eigen root value and per cent variation. Maximum mean nut weight, kernel weight and kernel percentage was recorded in clusters 2, 2 and 6, respectively; while the average minimum shell thickness was found in cluster 6. The genotypes of cluster 2 and 9 were found highly diverse from each other and will give better segregants after hybridization which can then be used as cultivar in the future.

Key words: Euclidean cluster analysis, Genetic divergence, Inter- and intra-cluster distance, Kernel, Non-hierarchical, Variability, Walnut

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 were made in this direction in the last decade, walnut trees are mainly raised from seeds and exhibit tremendous variability for various nut and kernel characters as a result of wind pollination and walnut's monoecious nature. Walnut improvement throughout the world has taken place mostly through selection. Any reduction in genetic variability makes the crop increasingly vulnerable to diseases and adverse climatic changes (Sharma and Sharma 2005). More variability is a prerequisite for better selection. Although walnut cultivation has been reported from 1 200 to 2 200 m above mean sea level, in the present study seedling trees of walnut were found growing in the cold arid region of India, Ladakh. It is situated at more than 3 000 m above sea level where winter temperature drops down to more than -20°C . There are many methods such as Mahalanobis D^2 statistics described earlier. D^2 statistics is a quantitative

measure of genetic divergence, yet the clustering pattern of the genotypes is arbitrary. To overcome the limitations of D^2 statistics, the walnuts growing at very high altitude were subjected to non-hierarchical cluster analysis. The information on the nature and degree of genetic divergence present in cold hardy walnut populations could help to select elite trees for direct use as clones or for further improvement through hybridization.

MATERIALS AND METHODS

Investigations were undertaken during 2006 and 2007 in various walnut-growing areas of Leh and Kargil districts in Jammu and Kashmir. These two districts are jointly called the Ladakh region which is a cold and arid region. The various places surveyed were Batalik, Darchik and Gharkhon of district Kargil and Dha, Beema, Lehdo, Skurbuchan, Domkhar, Khaltsi, Takmachik, Nurla, Himpti, Gaira, Saspol and Basgo in district Leh. The elevation, latitude, longitude, and number of seedling trees from which samples were collected. Based on preliminary information from concerned farmers regarding regular bearing, data on various nut characters were recorded on 216 seedling trees, out of a total approximate population of 800 seedling trees. Data for 20 sun-dried nut and kernel character were recorded as per the description of UPOV (1988) and IPGRI (1994). To study

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genetic divergence, data were analyzed using non-hierarchical Euclidean cluster analysis (Spark 1973).

RESULTS AND DISCUSSION

The range, mean, standard deviation, and co-efficient of variability for various nut and kernel characters were recorded. Maximum co-efficient of variation (30.91%) was recorded for kernel weight, while the minimum (7.73%) was for kernel width. In the Principal component analysis, first

component showed the highest eigen roots (5.120) and maximum contribution for diversity (39.38%), while the minimum eigen root value (0.007) and per cent contribution towards diversity (0.05%) was observed for the last component. The per cent variation explained by the first 9 components was 96.36% (Table 1).

The distribution of 216 walnut genotypes revealed that there were 10 clusters and the distribution of genotypes from different eco-geographical areas was apparently random.

Table 1 Eigen vectors, eigen roots and associated variance for various components in walnuts

Character	1	2	3	4	5	6	7	8	9	10	11	12	13
Nut weight (g)	0.381	0.354	0.306	0.366	-0.027	0.168	-0.004	-0.006	0.381	0.341	0.3	0.319	0.139
Nut width (mm)	0.193	0.182	-0.062	0.148	0.206	0.097	0.492	0.54	-0.187	0.003	-0.15	0.026	-0.511
Nut height (mm)	-0.069	0.147	-0.47	0.177	0.662	-0.007	-0.081	-0.171	0.087	0.219	-0.357	0.123	0.222
Nut thickness (mm)	-0.024	-0.138	-0.033	0.137	0.053	0.841	-0.429	0.069	-0.088	-0.186	-0.018	-0.006	-0.138
Index of roundness	0.163	-0.199	-0.106	-0.2	-0.058	0.2	0.274	0.42	0.452	-0.187	-0.168	-0.16	0.543
Pad thickness (mm)	-0.072	-0.361	-0.205	-0.145	-0.007	0.076	0.264	-0.069	-0.08	-0.114	0.225	0.807	0.023
Pad width (mm)	0.181	-0.195	-0.068	0.019	0.012	-0.4	-0.629	0.545	0.076	0.021	-0.051	0.233	-0.09
Shell thickness (mm)	-0.31	-0.002	-0.07	-0.353	-0.079	0.191	-0.013	0.283	-0.174	0.763	0.161	-0.058	0.098
Kernel weight (g)	-0.257	0.205	-0.145	0.118	0.322	-0.081	-0.018	0.244	-0.088	-0.309	0.72	-0.168	0.192
Kernel width (mm)	-0.531	0.245	0.355	0.295	-0.151	-0.018	0.012	0.238	-0.205	-0.138	-0.357	0.272	0.32
Kernel height (mm)	-0.085	-0.654	0.039	0.648	0.005	-0.076	0.16	0.009	-0.042	0.237	0.081	-0.213	0.052
Kernel thickness (mm)	-0.543	-0.027	0.03	-0.018	0.039	-0.009	0.019	0.01	0.71	-0.005	0.009	0.02	-0.443
Kernel percentage (mm)	0.039	-0.251	0.684	-0.295	0.615	0	0.007	-0.008	-0.038	-0.001	-0.009	0.012	0.021
Eigen roots	5.120	2.153	1.961	1.011	0.594	0.564	0.482	0.352	0.290	0.264	0.190	0.012	0.007
Per cent variation	39.38	16.56	15.08	7.78	4.57	4.34	3.71	2.71	2.23	2.03	1.46	0.09	0.05

Per cent variation explained by first 9 components, 96.36%

Table 2 Distribution of 216 seedling trees of walnut on the basis of nut and kernel characters

Cluster	No. of seedling trees	Seedling tree numbers
1	37	Domkhar 11,16,18,30,40; Nurla 4,8,12; Saspol 5,6; Khaltsi 6,20,22,25,26; Lehdo 1,5,7,21,31; Himpti 3; Dha 4; Skurbuchan 4,7,8; Takmachik 1,4,11,17,23; Garkhon 2,6,8; Darchik 6,7,8,9
2	11	Nurla 1,2; Saspol 4; Khaltsi 4,19; Gaira 5; Dha 7; Beema 1,2; Takmachik 14,16
3	33	Domkhar 13,22,26,32,38,41; Nurla 3,9,11; Saspol 3; Khaltsi 2,5,7,17; Lehdo 14,15,16,18,19,29; Himpti 5; Gaira 8; Skurbuchan 12; Takmachik 2,5,9,10,12,20,25,26; Bazgo 1; Batalik 1
4	19	Domkhar 31; Saspol 8,9; Khaltsi 12,14; Lehdo 2,20,26; Gaira 1,3,7; Takmachik 3,15,19,22; Garkhon 4,7; Darchik 5
5	20	Domkhar 7,8,39,42; Saspol 8,9; Khaltsi 12,14; Lehdo 2,20,26; Gaira 2; Dha 8; Skurbuchan 3,9,15,16; Takmachik 21,27; Garkhon 1
6	20	Domkhar; Nurla; Saspol; Khaltsi 6,20,22,25,26; Lehdo 1,5,7,21,31; Himpti 3; Dha 4; Skurbuchan 4,7,8; Takmachik 1,4,11,17,23; Garkhon 2,6,8; Darchik 6,7,8,9
7	24	Domkhar 3, 9, 10, 15, 20, 23, 24, 25,27, 33, 35; Lehdo 11, 17, 23, 25, 32; Himpti 4; Gaira 4; Skurbuchan 1, 6, 10; Takmachik 8; Batalik 2; Darchik 10
8	29	Domkhar 1, 2, 4, 14, 17, 21, 29, 34, 38; Nurla 6; Khaltsi 9, 18; Lehdo 3, 6, 12, 13, 27, 33; Himpti 1; Gaira 6; Dha 3; Beema 5; Skurbuchan 5, 11; Takmachik 6, 7, 24; Garkhon 5; Darchik 2.
9	6	Domkhar 19; Nurla 5, 7, 10; Lehdo 30; Himpti 2
10	17	Domkhar 12, 28, 37; Nurla 13; Khaltsi 1, 3, 10, 11, 13, 15, 16, 21, 23; Dha 6, 9; Garkhon 3; Darchik 1

Table 3 Mean, standard deviation and co-efficient of variation for various characters in different clusters

Character	Parameters	1	2	3	4	5	6	7	8	9	10
Nut weight (g)	Mean	11.59	16.43	10.84	15.41	9.18	10.96	10.08	13.44	6.70	12.08
	SD \pm	1.28	1.30	1.88	1.38	1.18	1.00	1.81	1.55	0.78	1.55
	CV (%)	11.04	7.91	17.34	8.96	12.85	9.12	17.96	11.53	11.64	12.83
Nut width (mm)	Mean	29.76	33.67	28.81	32.62	27.59	30.66	29.43	32.91	23.18	29.54
	SD \pm	1.60	1.91	1.33	1.41	1.26	1.66	1.67	1.69	1.14	1.53
	CV (%)	5.38	5.67	4.62	4.32	4.57	5.41	5.67	5.14	4.92	5.18
Nut height (mm)	Mean	37.96	44.12	36.80	41.87	32.40	35.61	31.53	35.66	29.34	37.65
	SD \pm	2.88	2.53	2.54	3.49	2.34	2.06	2.08	2.02	2.14	3.60
	CV (%)	7.59	5.73	6.90	8.34	7.22	5.78	6.60	5.66	7.29	9.56
Nut thickness (mm)	Mean	28.99	34.88	28.60	32.90	27.87	31.15	29.35	33.43	23.67	31.19
	SD \pm	1.37	1.69	2.09	1.60	1.55	1.58	1.69	1.97	2.17	1.29
	CV (%)	4.73	4.85	7.31	4.86	5.56	5.07	5.76	5.89	9.17	4.14
Index of roundness	Mean	0.78	0.78	0.78	0.79	0.86	0.87	0.93	0.93	0.81	0.81
	SD \pm	0.06	0.07	0.06	0.07	0.07	0.07	0.07	0.06	0.09	0.08
	CV (%)	7.69	8.97	7.69	8.86	8.14	8.05	4.30	6.45	11.11	9.88
Pad thickness (mm)	Mean	4.26	5.20	4.43	4.56	4.00	4.09	4.21	4.45	3.32	5.61
	SD \pm	0.54	0.76	0.68	0.76	0.71	0.53	0.73	0.86	0.43	0.61
	CV (%)	12.68	14.62	15.35	16.67	17.75	12.96	17.34	19.33	12.95	10.87
Pad width (mm)	Mean	4.94	3.61	4.77	5.53	4.42	2.75	5.14	5.43	3.88	3.49
	SD \pm	0.78	1.43	0.92	1.02	0.93	0.48	0.74	0.77	0.59	0.99
	CV (%)	15.79	39.61	19.29	18.44	21.04	30.55	14.40	14.18	15.21	28.37
Shell thickness (mm)	Mean	1.68	1.34	1.97	2.16	1.39	1.29	1.89	1.80	1.45	1.60
	SD \pm	0.28	0.25	0.37	0.25	0.26	0.14	0.31	0.28	0.29	0.22
	CV (%)	16.67	18.66	18.78	11.57	18.71	10.85	16.40	15.56	20.00	13.75
Kernel weight (g)	Mean	4.88	7.73	3.36	5.99	4.25	5.44	3.63	5.22	2.19	5.20
	SD \pm	0.68	1.13	0.69	0.61	0.61	0.52	0.79	0.99	0.50	0.55
	CV (%)	13.93	14.62	20.54	10.18	14.31	9.56	21.76	18.97	22.83	10.58
Kernel width (mm)	Mean	19.73	23.86	18.28	22.09	18.80	21.91	19.56	22.48	16.91	20.74
	SD \pm	1.57	1.65	1.22	1.63	1.45	1.67	1.81	1.83	1.72	1.36
	CV (%)	7.96	6.92	6.67	7.38	7.71	7.62	9.25	8.14	10.17	6.56
Kernel height (mm)	Mean	28.10	32.26	26.15	29.83	24.49	26.47	22.56	26.37	19.74	21.79
	SD \pm	2.22	2.85	2.28	2.91	2.14	2.25	2.46	2.02	1.82	2.38
	CV (%)	7.90	8.83	8.72	9.76	8.74	8.50	10.90	7.66	9.22	8.56
Kernel thickness (mm)	Mean	22.96	27.71	22.04	24.66	22.10	24.07	22.05	25.89	17.71	25.53
	SD \pm	2.03	2.29	2.21	2.77	1.72	1.50	1.83	2.88	2.66	2.19
	CV (%)	8.84	8.26	10.03	11.23	7.78	6.23	8.30	11.12	15.02	8.58
Kernel percentage	Mean	42.18	47.00	31.21	39.05	46.50	49.86	35.99	38.91	31.10	43.41
	SD \pm	4.60	4.94	4.85	4.16	5.73	5.07	4.85	6.43	9.26	4.50
	CV (%)	10.91	10.51	15.54	10.65	12.32	10.17	13.48	16.53	27.98	10.37

Cluster 1 contained the maximum (37) number of walnut genotypes, while the lowest number of genotypes (6) fell in cluster 9. Similarly clusters 2, 3, 4, 5, 6, 7, 8 and 10 contained 11, 33, 19, 20, 20, 24, 29 and 17 genotypes from different locations (Table 2). The maximum mean value for nut weight (16.43 g), nut width (33.67 mm), nut height (44.12 mm), nut thickness (34.88 mm), kernel weight (7.73 g), kernel width (23.86 mm) kernel height (32.26 mm) and kernel thickness (27.71 mm) was observed in cluster 2. Maximum mean index of roundness (0.93), pad thickness (5.61 mm), pad width (5.53 mm), kernel percentage (49.86%), and minimum shell thickness (1.29 mm) were recorded in clusters 7 and 8, 10, 4, 6 and 6 respectively (Table 3).

Similarly, maximum co-efficient of variability for nut

weight (17.96%), nut width (5.67%), nut height (9.56%), pad thickness (19.33%) pad width (39.61%) kernel height (10.90%) were recorded in cluster 7, 2 and 7, 10, 8, 2 and 7 respectively. Maximum co-efficient of variability for nut thickness (9.17%), index of roundness (11.11%), shell thickness (20.00%), kernel weight (22.83%), kernel width (10.17%), kernel thickness (15.02%) and kernel percentage (27.98%) were in cluster 9.

Intra- and inter-cluster distances (Table 4) have revealed that intra cluster distance in genotypes varied between 2.006 of cluster 6 and 2.583 of cluster 2. The minimum inter-cluster distance of 2.257 was recorded between cluster 1 and 3. Similarly maximum inter-cluster distance of 10.764 was recorded between cluster 2 and 9. These clusters contain 11

Table 4 Average intra- and inter-cluster distances among various clusters

Cluster	1	2	3	4	5	6	7	8	9	10
1	2.116									
2	5.205	2.583								
3	2.257	6.881	2.348							
4	3.225	3.681	4.335	2.431						
5	2.642	7.066	3.147	5.586	2.170					
6	2.976	4.766	4.437	4.566	3.022	2.006				
7	3.22	7.436	2.579	5.114	2.513	4.046	2.224			
8	3.304	4.615	4.181	2.812	4.457	3.486	3.54	2.510		
9	5.94	10.764	4.873	8.822	4.181	6.717	4.725	7.929	2.258	
10	2.446	4.196	3.545	3.537	3.652	2.64	4.073	3.254	7.093	2.242

and 6 walnut genotypes from various locations, such as Nurla, Saspol, Gaira, Dha, Beema, Takmachik, Domkhar, Lehdo and Himpti.

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, 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 10 clusters. However, in earlier studies different clusters were found by Thakur *et al.* (2005) in almond, Kaushal and Sharma (2005) in pecan and Pandey and Tripathi (2007) and Sharma and Sharma (2005) in walnut. The highest eigen roots and per cent contribution towards diversity was contributed by fruit weight. Similarly, contribution of first components was observed by Thakur *et al.* (2005) in almond 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 (2.257) 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 10.764 was recorded between cluster 2 and 9. The genotypes falling in clusters were 11 and 6 from various eco-geographical regions. Different intra- and inter-cluster distances were recorded previously (Sharma and Sharma 2005, Pandey and Tripathi 2007) for various fruit crops like walnut, almond, and pecan cultivars and various genotypes were suggested for

hybridization. The 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 2 and 9 can be utilized for hybridization programme as well as for introgressing their useful traits in the commercial walnut cultivars.

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