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Assessing genetic divergence in Persian walnut seedling genotypes

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ABSTRACT

To assess the nature and magnitude of genetic divergence of 216 bearing trees of Persian 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 coefficients of variability were recorded for nut width and kernel weight, respectively. All 216 walnut gentotypes grouped into 10 clusters. The clustering pattern of walnut genotypes belonging to the same ecogeographical 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: Genetic divergence, walnut, nut, kernel, non hierarchial, euclideen cluster analysis, inter and intra cluster distance, variability.

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 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 (Jain, 2). More variability is a prerequisite for better selection. Although walnut cultivation has been reported from 1200 to 2200 m above sea level, in the present study seedling trees of walnut were found growing in the cold arid region of India i.e. Ladakh area is more than 3000 m above sea level where winter temperature drop more than -20^{o}C. There are many methods such as Mahalanobis D^2 statistics described by Rao (10) to represent variation. Although D^2 statistics is a quantitative measure of genetic divergence, yet the clustering pattern of the genotypes is arbitrary (Singh and Gupta, 15). 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 state of India. 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 are presented in Table 1. 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

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Table 1. Survey locations and altitude of various walnut growing areas in Ladakh region.

S.No.	Eco geographical region	Altitude (feet above mean sea level)	Latitude	Longitude
1.	Bazgo	10668	34°13'01.00"N	77°17'01.88"E
2.	Saspol	10250	34°14'67.09"N	77°10'16.08"E
3.	Gaira	10429	34°13'49.61"N	77°07'48.34"E
4.	Himpti	10203	34°16'15.66"N	77°01'56.84"E
5.	Nurla	10016	34°17'96.17"N	76°59'47.06"E
6.	Khaltsi	10103	34°19'22.05"N	76°52'97.01"E
7.	Takmachik	10050	34°22'45.80"N	76°45'50.21"E
8.	Domkhar	10150	34°24'18.09"N	76°45'99.51"E
9.	Skurbuchan	9502	34°26'00.39"N	76°42'47.09"E
10.	Lehdo	9757	34°20'48.41"N	76°48'49.96"E
11.	Beema	9176	34°36'00.86"N	76°30'52.03"E
12.	Dha	8998	34°37'21.36"N	76°28'51.20"E
13.	Garkhon	8749	34°38'04.81"N	76°25'49.07"E
14.	Darchik	8985	34°37'57.46"N	76°22'58.67"E
15.	Batalik	8906	34°39'50.49"N	76°18'42.49"E

nut and kernel character were recorded as per the description of UPOV, (18) and IPGRI, (1) and analyzed as per methods given by Panse and Sukhatme, (9). To study genetic divergence, data were analyzed using non-hierarchical Euclidean cluster analysis (Spark, 16).

RESULTS AND DISCUSSION

The range, mean, standard deviation, and coefficient of variability for various nut and kernel characters are presented in Table 2. Maximum coefficient of variability (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 percent contribution for diversity (39.38%), while the minimum eigen root value (0.007) and percent contribution towards diversity (0.05%) was observed for the last component. The percent variation explained by the first nine components was 96.36 percent (Table 3).

The distribution of 216 walnut genotypes revealed that there were ten clusters and the distribution of genotypes from different eco geographical areas was apparently random. Cluster one contained the maximum (37) number of walnut genotypes, while the lowest number of genotypes (6) fell in cluster nine. Similarly cluster 2, 3, 4, 5, 6, 7, 8 and 10 contained 11, 33, 19, 20, 20, 24, 29 and 17 genotypes from different locations (Table 4). 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 (27.71mm) was observed in cluster two. Maximum mean index of roundness (0.93), pad thickness (5.61mm), 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 5).

Similarly maximum coefficient 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 & 7, 10, 8, 2 and 7 respectively. Maximum coefficient 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 nine.

Intra- and inter-cluster distances are given in Table 6, which revealed that intra cluster distance varied between 2.006 and 2.583 in the genotypes of cluster 6 and 2, respectively. 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 contains 11 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

Table 2. Range, mean, standard deviation and coefficient of variability for various nut and kernel characters.

Characters	Range	Mean	Standard deviation	Coefficient of variability (%)
Nut weight (g)	5.61-18.47	11.76	2.57	21.85
Nut width (mm)	21.97-35.92	30.13	2.62	8.70
Nut height (mm)	26.38-48.18	36.42	4.30	11.81
Nut thickness (mm)	20.39-37.79	30.33	2.92	9.63
Index of roundness	0.63-1.04	0.84	0.09	10.71
Pad thickness (mm)	2.69-6.89	4.42	0.81	18.33
Pad width (mm)	1.56-7.63	4.59	1.22	26.58
Shell thickness (mm)	0.87-2.70	1.71	0.38	22.22
Kernel weight (g)	1.62-10.13	4.74	1.36	30.91
Kernel width (mm)	16.45-27.66	20.39	2.35	7.73
Kernel height (mm)	17.34-36.57	26.58	3.45	12.98
Kernel thickness (mm)	15.10-33.44	23.58	2.93	12.43
Kernel percentage	13.51-63.36	40.30	7.65	18.98

Table 3. Eigen vectors, eigen roots and associated variance for various components in walnuts

Characters	1	2	3	4	5	6	7	8	9	10	11	12	13
Nut weight	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	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	-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	-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	0.200	0.274	0.42	0.452	-0.187	-0.168	-0.16	0.543
Pad thickness	-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	0.181	-0.195	-0.068	0.019	0.012	-0.400	-0.629	0.545	0.076	0.021	-0.051	0.233	-0.09
Shell thickness	-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	-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	-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	-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	-0.543	-0.027	0.03	-0.018	0.039	-0.009	0.019	0.01	0.71	-0.005	0.009	0.020	-0.443
Kernel percentage	0.039	-0.251	0.684	-0.295	0.615	0.000	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 nine components=96.36%

different walnut cultivars and seedling trees in different walnut growing areas. (Pandey *et al.* 8, Mehta *et al.* 5, Sharma and Kumar 13, Joolka and Sharma, 3, Sharma and Sharma, 11, Sharma and Sharma, 14, Pandey and Sinha, 6). 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 ten clusters. Similarly, in earlier studies different clusters were found by Thakur *et al.* (17) in almond, Kaushal and Sharma, (4) in pecan and Pandey and Tripathi, (7), Sharma and Sharma, (12), (11) in walnut. The highest eigen roots and percent contribution towards diversity was contributed by fruit weight. Similarly contribution of first components was observed by Thakur *et al.* (17) in almond germplasm. The intra-cluster distance varied between 2.006 to 2.583 between clusters 6 and 2 respectively. These relatively low values of intra-cluster distance 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

Table 4. Distribution of 216 seedling trees of walnut on the basis of nut and kernel characters

Cluster	No. of seedling trees	Seedling Tree No.
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, Gaira5, 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, Lehdo14, 15, 16, 18, 19, 29, Himpti 5, Gaira8, Skurbuchan12, Takmachik2, 5, 9, 10, 12, 20, 25, 26, Bazgo 1, Batalik 1
4	19	Domkhar31, Saspol 8, 9, Khaltsi 12, 14, Lehdo 2, 20, 26, Gaira1, 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 5. Mean, standard deviation and coeffeicient of variability for various characters in different clusters.

Characters	Parameters	1	2	3	4	5	6	7	8	9	10
Nut weight	Mean	11.59	16.43	10.84	15.41	9.18	10.96	10.08	13.44	6.70	12.08
	SD	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	Mean	29.76	33.67	28.81	32.62	27.59	30.66	29.43	32.91	23.18	29.54
	SD	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	Mean	37.96	44.12	36.80	41.87	32.40	35.61	31.53	35.66	29.34	37.65
	SD	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	Mean	28.99	34.88	28.60	32.90	27.87	31.15	29.35	33.43	23.67	31.19
	SD	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	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	Mean	4.26	5.20	4.43	4.56	4.00	4.09	4.21	4.45	3.32	5.61
	SD	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	Mean	4.94	3.61	4.77	5.53	4.42	2.75	5.14	5.43	3.88	3.49
	SD	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	Mean	1.68	1.34	1.97	2.16	1.39	1.29	1.89	1.80	1.45	1.60
	SD	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	Mean	4.88	7.73	3.36	5.99	4.25	5.44	3.63	5.22	2.19	5.20
	SD	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	Mean	19.73	23.86	18.28	22.09	18.80	21.91	19.56	22.48	16.91	20.74
	SD	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	Mean	28.10	32.26	26.15	29.83	24.49	26.47	22.56	26.37	19.74	21.79
	SD	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	Mean	22.96	27.71	22.04	24.66	22.10	24.07	22.05	25.89	17.71	25.53
	SD	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	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

Table 6. Average intra and inter cluster distances among various clusters.

Clusters	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**

distance of 10.764 was recorded between cluster 2 and 9. The genotypes falling in clusters were 11 and 6 from various ecogeographical regions. Different intra and inter cluster distances were recorded previously (Pandey and Tripathil, 7, Thakur *et al.* 17 , Kaushal and Sharma, 4, Sharma and Sharma, 12, 11) 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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