Genetic Diversity Analysis in Aromatic Rice Germplasm using Agro-Morphological Traits

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During the present investigation, an attempt was made to analyze the relatedness and distances among different aromatic rices using 20 agro-morphological traits. The genetic divergence among 45 indigenous aromatic rice germplasm was estimated for 20 agro-morphological characteristics. Based on this analysis using agro-morphological characteristics, all the genotypes were grouped into 10 different clusters. Cluster I, II, III, IV, V, VI, VII, VIII, IX & X, comprised of 20, 8, 5, 2, 2, 2, 2, 2, 1, 1 genotypes, respectively. Among different traits, weight of panicle, plant height, 1000-grain weight and flag leaf length played major role in making of clusters. The maximum intra-cluster divergence was observed for cluster VI and least for clusters IX & X. As far as the inter cluster divergence is concerned cluster IX & X were highly divergent, and least inter cluster divergence was recorded between cluster VIII & II. This analysis was also followed by Dendrogram study which grouped all genotypes in 3 major clusters. The genotypes from these clusters may be used as potential donors for future hybridization programme to develop varieties with more grain as well as kernel length.

Key Words: Basmati rice, Genetic divergence, Agro-Morphological traits

Introduction

Rice (*Oryza sativa*) is the world's single most important food crop and a primary food source for more than a third of world's population. It is no longer a luxury food but has become the only cereal that constitutes a major source of calories for the urban and the rural (Sasaki and Burr, 2000).The intra-specific variation in rice is extensive and sub-specific classification has always been important for rice breeders and geneticists. India is one of the original centers of rice cultivation. The harvesting area of the rice in India is the largest in the world. India stands next only to China in terms of production and occupies second position in rice exports, next only to Thailand among the rice trading countries of world (The Hindu Survey of Agriculture, 2005).Aromatic rices forms a separate group and, is nature's gift exclusive to Indian sub continent (Glaszman 1987). The aroma associated with some domesticated rice varieties may have arisen from a gene mutation during evolution or be the outcome of a separate domestication event (Bradbury *et al.* 2005a). Among aromatic rices, *basmati* is accepted as best scented longest and slenderest rice in world. India is leading country in the export of scented rice and due to its importance, the basmati rice is considered as *Scented pearl* (Siddiq, 1996).An ever increasing global demand for aromatic rice has been noted in the recent times. Chaudhary *et al.* (2003) have discussed the economic aspects of aromatic rice detailing current trends, consumption pattern and global market demands. Presently, only traditional basmati rices are accepted as Basmati. There is a strong need that the germplasm of this cash crop be collected preserved and characterized in detail. Indian rice varieties have been developed tradionally by selection, hybridization and back crossing with locally adapted high yielding lines. An important source for the introduction of new traits is the existence of genetically diverse pool of rice germplasm available in country, but lying unexplored. Many farmers in India still grow local germplasm under different names and they also bring some varieties from distant places and start cultivating them with local names. Present study was undertaken to evaluate variability and relatedness in forty five genotypes based on twenty agro-morphological traits.

Materials and Methods

Field performance of all the forty five germplasm was evaluated at Seed Production Centre (S.P.C) at G.B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India, during *kharif* 2004 in two replications, using Randomized Block Design (RBD) under organic farming system. Nursery sowing was done in June 2004. Seedlings of each germplasm were transplanted in a 20 m^2 (5 x 4 m^2) plot. Transplanting was done after one month of sowing. During transplanting plant-to-plant distance was 15 cm and row-to-row distance was 20 cm. Observations for twenty traits were recorded during crop season taking data of five plants each in two replications for forty five genotypes.

The mean values of two replications were used for statistical analysis. The analysis of genetic divergence using D^2-statistics of Mahalanobis (1936) as described by Rao (1952) and grouping of genotypes were done in to different clusters following Tocher's method (Rao 1952).The agro-morphological traits data was converted in to binary data and Dendrogram was prepared by Unweighted Pair Group Method Arithmetic Averages (UPGMA) based study using Jaccard Coefficient (Software Applied Math's 3.5 version). For cluster analysis Sequential Agglomerative Hierarchical Nestee (SAHN) was done.

Results and Discussions

Analysis of variance

The analysis of variance revealed the presence of significant variability among genotypes for all the characters studied. (Table 1)

Clustering pattern

All genotypes were grouped in 10 clusters using twenty agro-morphological traits. The clustering pattern is presented in Table 2. Twenty genotypes were accommodated in Cluster I, while cluster II accommodated 8 genotypes .There were 5 genotypes in cluster III and Cluster IV , cluster V, cluster VI, cluster VII and cluster VIII accommodated 2 genotypes each, while Cluster IX and X accommodated only one genotype each.

Intra and inter cluster divergence

The average intra and inter-cluster D^2 values and average genetic distance between and within cluster for agro-morphological characters are presented in Table 3. The maximum intra-cluster divergence was observed for cluster VI (D^2 value of 2760.88, average distance 52.54) and least intra-cluster divergence was recorded for cluster IX and X (D^2 value of 0.00; and average distance of 0.00).

As far as the inter cluster divergence is concerned cluster IX and X were highly divergent, as indicated by maximum D^2 value of 15819.74 and average distance of 125.78 between them. The least inter-cluster divergence was recorded between cluster VIII and II as indicated by lowest D^2 value of 2499.51 and average distance of 50.00.

Cluster means for different characters

The cluster means of various agronomic traits are presented in Table 4. Cluster I consisting of 20 genotypes exhibited highest mean plant height (147.12 cm) and lowest mean for days to maturity (114.97 days) and filled grain/panicle (87.67). Cluster II having 8 genotypes and cluster III with 5 genotypes did not exhibit highest or lowest mean values for any characteristic. Cluster IV consisting of 2 genotypes exhibited highest mean value for panicle length (29.6 cm), panicle weight (3.65 g), filled grain /panicle (155.40) and lowest mean value for number of tillers/hill (9.70) and fertile tillers/hill (6.80). Cluster V, with 2 genotypes exhibited highest mean value for L/B ratio of flag leaf (27.07 cm) and lowest mean value for yield (33.30 q/h).Cluster VI with 2 genotypes exhibited highest mean value for panicle exersion (6.00) and total grains/panicle (170.75) and lowest mean value for plant height (90.40 cm). Cluster VII, with 2 genotypes exhibited highest mean value for flag leaf length (31.57 cm), flag leaf breadth (1.18 cm), ear bearing tillers/hill (9.42), days

Table 1. Analysis of variance for agro-morphological traits

Mean Squares

Source of variation	d.f.	Flag leaf length	Flag leaf breadth	L/B ratio of flag leaf	First leaf length	First leaf breadth	L/B ratio of first leaf	Tillers hill	E.B.T. hill	Panicle type
Replication	1	0.453	0.001	3.122	0.126	0.000	10.750	0.464	0.050	0.711
Treatment	44	22.74**	0.01**	16.72**	45.06**	0.02**	54.94**	12.34**	3.52**	10.32**
Error	44	0.048	0.000	0.215	1.495	0.000	2.266	0.206	0.090	0.711
C.D. at 1%		0.594	0.031	1.249	3.292	0.039	4.053	1.224	0.811	2.270

Table 1. contd.

Mean Squares

Source of variation	Panicle exersion	Days to 50% flowering	Days to Maturity	Plant height	Panicle length	Weight of panicle	Total grains panicle	Filled grains panicle	Unfilled grains panicle	1000 grain weight	Grain yield
Replication	0.000	7.534	44.080	7.086	-0.020	0.084	309.82	582.65	16.043	0.012	8.55
Treatment	5.95**	68.21**	91.30**	75.72**	11.29**	1.066**	2334.3**	1790.4**	110.4**	9.23**	195.5**
Error	0.272	1.965	1.986	1.409	0.215	0.006	15.53	107.54	12.635	0.060	0.47
C.D. at 1%	1.406	3.774	3.795	3.197	1.249	0.220	10.61	27.92	9.570	0.659	1.86

All values are significant at 1% level

Table 2. Clustering pattern of 45 genotypes using agro-morphological traits

Cluster	Genotypes	Total number
I	Basmati 370,Basmati 3034,Dehradun Basmati 3020, Basmati Mohan 381,Hansraj 3086, Basmati 106, Basmati 122, Basmati 376, Basmati 134, Hansraj 3067, Basmati C 622, Hansraj 3074, Hansraj 3077, Basmati Sathi, Basmati1-1 A, Basmati 6129,Type 3, Basmati 107, Taraori Basmati and Basmati 5836.	20
II	Hansraj 3074 (U), BasmatiUzearpka (U), Basmati 217, Basmati124-10, Basmati 375 A, Basmati 43 A, Basmati 5875, Basmati 433.	8
III	Basmati 3317-1, Basmati 3065AR 1409 (U), Basmati 3032AR 575 (U), Dehradun Basmati 3020 (U), Hansraj 3072-1.	5
IV	Tilakchandan 3048, Kalanamak 3121.	2
V	Basmati 3085, Basmati 3065 AR 771(U).	2
VI	Hansraj 3078, Basmati 127.	2
VII	Pokkali (U) & Hansraj 3072-2(U).	2
VIII	Basmati 136 & Basmati sufaid 100.	2
IX	Hansraj 3072-2.	1
X	Basmati Nepal.	1

Table 3. Average intra and inter-cluster D^2 (bold) and D values (agro-morphological traits)

Cluster	1	2	3	4	5	6	7	8	9	10
1	2085.09	3120.07	6923.40	9839.01	3027.61	13149.76	6367.93	2739.39	13985.24	3440.11
	45.66	55.86	83.21	99.19	55.02	114.67	79.80	52.34	118.26	58.65
2		1786.38	4367.21	5518.74	4186.51	7889.13	4570.34	2499.51	9117.61	4741.81
		42.27	66.08	74.96	64.70	88.82	67.60	50.00	95.49	68.86
3			2207.82	4795.20	4832.23	4462.52	4165.84	6370.93	4126.55	8406.91
			46.99	69.25	69.51	66.80	64.54	79.82	64.24	91.69
4				2603.46	9791.54	4515.60	7060.10	7763.13	2917.71	10945.94
				51.02	98.95	67.20	84.02	88.11	54.02	104.62
5					2279.83	10968.51	4711.20	4800.54	11098.80	3672.67
					47.75	104.73	68.64	69.29	105.35	60.60
6						2760.88	4618.93	12649.51	4221.49	12195.22
						52.54	67.96	112.47	64.97	110.43
7							2538.50	7752.23	8411.66	4681.34
							50.38	88.05	91.72	68.42
8								1149.75	11531.82	5306.65
								33.91	107.39	72.85
9									0.00	15819.74
									0.00	125.78
10										0.00
										0.00

to 50 % flowering (107.50 days) and yield (54.62 q/h) and lowest mean value for panicle length (21.07 cm). Cluster VIII, with 2 genotypes exhibited highest mean value for panicle type (7.00) and lowest mean value for L/B ratio for flag leaf (20.50), L/B ratio for first leaf (41.15), panicle exersion (1.00), days to 50 % flowering (87.00 days), weight of panicle (1.57 g), total grains/panicle (89.25), unfilled grains /panicle (10.25). Cluster IX with 1 genotype exhibited highest mean value for L/B ratio of first leaf (58.00), number of tillers/hill (15.10), 1000-grain weight (23.50 g) and lowest mean value for flag leaf length (19.60 cm), flag leaf breadth (0.95 cm), first leaf length (38.00 cm), panicle type (1.00). Cluster X, with 1 genotypes exhibited highest mean value for first leaf breadth (1.05 cm), first leaf length (51.85 cm), days to maturity (140 days), unfilled grains /panicle (46.50) and lowest mean value for 1000 grain weight (14.50 g).

Contribution of different characters towards genetic divergence

Contribution of different agro-morphological traits towards the genetic divergence among indigenous *Basmati* rice germplasm under study is presented in Table 5. It was observed that weight of panicle (32.6 per cent), plant height (21.0 per cent), 1000-grain weight, (18.9 per cent) and flag leaf length (13.9 per cent) were major contributing factors to genetic diversity among 45 aromatic rice genotypes. first leaf breadth (7.9 per cent), panicle length (3.2 per cent), unfilled grains/panicle (1.4 per cent), and tillers/hill (1.1 per cent) were moderate contributing factors to genetic diversity. Least contribution towards genetic diversity was of first leaf length (0.20 per cent), ear bearing tillers/hill (0.40 per cent), days to 50% flowering (0.40 per cent), days to maturity (0.30

Table 4. Cluster mean values for different agro-morphological traits

Cluster No.	Flag leaf length cm	Flag leaf breadth cm	L/B ratio of flag leaf	First leaf length cm	First leaf breadth cm	L/B ratio of first leaf	Tillers hill	E.B.T. hill	Panicle type	Panicle exersion	Days to 50% flowering	Days to to maturity	Plant height cm	Panicle length cm	Weight of panicle (g)	Total grains panicle	Filled grains panicle	Unfilled grains panicle	1000 grain weight (g)	Grain yield (q/ha)
I	28.26	1.08	26.17	45.99	0.92	50.18	12.35	8.03	6.80	1.35	88.85	114.90	147.10	25.41	1.95	98.65	87.67	10.90	20.98	36.02
II	26.43	1.14	23.05	43.98	0.99	44.08	13.63	8.13	6.25	1.20	88.75	116.20	142.20	26.68	2.71	136.70	119.80	16.80	20.84	37.90
III	26.99	1.06	25.81	40.70	0.84	47.50	13.62	8.32	5.00	2.20	90.00	116.00	108.80	27.21	2.50	119.30	103.40	15.90	22.66	38.56
IV	21.12	0.98	21.52	42.89	0.85	48.32	9.70	6.80	3.00	3.50	93.75	127.50	120.50	29.60	3.65	169.00	155.40	13.80	20.35	39.75
V	29.32	1.08	27.07	36.95	0.74	49.87	13.72	8.55	3.00	2.00	95.25	115.50	138.90	24.82	2.37	116.20	100.20	16.20	20.33	33.30
VI	27.60	1.13	24.05	46.05	0.98	46.70	13.00	7.90	4.00	6.00	97.00	121.70	90.40	26.70	3.37	170.70	154.00	16.70	20.53	40.95
VII	31.57	1.18	26.57	46.82	0.95	49.17	14.80	9.42	5.00	5.00	107.50	134.50	108.20	21.07	2.56	169.70	146.50	23.20	21.85	54.62
VIII	22.27	1.08	20.50	41.45	1.01	41.15	13.20	9.35	7.00	1.00	87.00	116.70	142.50	23.75	1.57	89.25	79.00	10.20	21.05	31.63
IX	19.60	0.95	20.60	38.00	0.65	58.00	15.10	8.30	1.00	3.00	98.50	117.00	101.90	28.00	3.39	154.00	92.00	12.00	23.50	50.00
X	29.65	1.18	25.10	51.85	1.01	51.05	12.70	8.80	5.00	5.00	94.50	140.00	135.00	23.25	1.80	158.00	111.50	46.50	14.50	36.55

Table 5. Per cent contribution of agro-morphological traits under study towards genetic divergence

S.No.	Character	Per cent contribution
1	Weight of panicle	32.8
2	Plant height	21.1
3	1000-grain weight	16.8
4	Flag leaf length	13.9
5	First leaf breadth	7.8
6	Panicle length	3.2
7	Unfilled grains/panicle	1.4
8	Tillers/hill	1.1
9	Total grains/panicle	0.5
10	Ear bearing tillers/hill	0.4
11	Days to 50% flowering	0.4
12	Days to maturity	0.3
13	First leaf length	0.2
14	Grain yield	0.1
15	Flag leaf breadth	0.0
16	L/B ratio of flag leaf	0.0
17	L/B ratio of first leaf	0.0
18	Panicle length	0.0
19	Panicle exertion	0.0
20	Filled grains/panicle	0.0

per cent), total grains/panicle (0.50 per cent) and yield (0.10 per cent). Traits like flag leaf breadth, L/B ratio of flag leaf, L/B ratio of first leaf, panicle type, panicle exertion and filled grains/ panicle showed no influence on genetic diversity among 45 rice genotypes.

Dendrogram Clustering

At 15 % similarity all genotypes were grouped in three major groups i.e. A group (6 genotypes), B group (36 genotypes) and C group (03 genotypes). The A major group was sub grouped in to A 1 and A 2 subgroups having 4 and 2 genotypes, respectively at 25 % similarity. The B group was further sub grouped in to B 1 and B 2 subgroups at 20 % similarity consisting of 4 and 32 genotypes, respectively. The B 2 sub group was further classified in to B 2-a and B 2 -b sub-sub groups consisting of 14 and 18 genotypes respectively. The C group comprised of 3 genotypes only.

The concept of "genetic distance" has been of vital utility (Arunachalam 1981). Several measures of distance have been proposed over the past two decades to suit various objective of which Mahalanobis generalized distance (Mahalanobis 1936 and Rao 1952) has occupied a unique place in plant breeding. D^2 statistics has its several advantages over other methods. Firstly, it utilizes the multivariate normal distribution of the natural variation to construct the dispersion matrix, secondly, it recognizes the genotypes and environmental variation, thirdly, relative importance of characters is determined by the percentage of variation accounted by them, thus avoiding bias in choice, and lastly, the set of different populations or genotypes are classified in to distinct groups or clusters based on D^2 values. Usefulness of D^2 statistics as measure of genetic diversity will be dependent on stability over environment. The analysis of variance revealed highly significant differences among all the genotypes for the fourteen quality traits. This indicated the existence of significant amount of variability among the values for the characters studied.

Cluster prepared on the basis of D^2 statistics based on agro-morphological traits grouped, Tilak Chandan 3048 and Kalanamak 3121 in same cluster, was also confirmed by Dendrogram prepared by UPGMA based study using Jaccard Coefficient. All genotypes from usher soil (presented by "U" suffix) were clustered in same groups, i.e. Basmati 3065 1409 (U), Basmati 3032 AR 575 (U), Dehradun basmati 3020 (U) in cluster III and Pokkali (U), Hansraj 3072-2 (U) in cluster V.

Three genotypes i.e. Tilak Chandan 3048, Kalanamak 3121& Basmati Mohan 381 grouped in C group of Dendrogram, further Kalanamak 3121and Basmati Mohan 381 segregated together at 40 % similarity, though these two were groped in different groups by D^2 analysis. The reason was that there was quite similarity in Agro-Morphological traits of Kalanamak 3121& Basmati Mohan 381 with respect to Tilak Chandan 3048 which was as following. Flag leaf length (22.40 cm, 32.40 cm, 31.75 cm) L/B ratio of flag leaf (21.25, 27.0, 28.35) No. of tillers/hill (5.40, 9.40, 8.40) EBT/hill (5.40, 9.40, 8.40) and 1000 grain weight (20.50 gm, 21.30 g, 21.50 g) for Tilak Chandan 3048, Kalanamak 3121& Basmati Mohan 381 genotypes, respectively.

Bindali 3317, a medium grain genotype showed quite

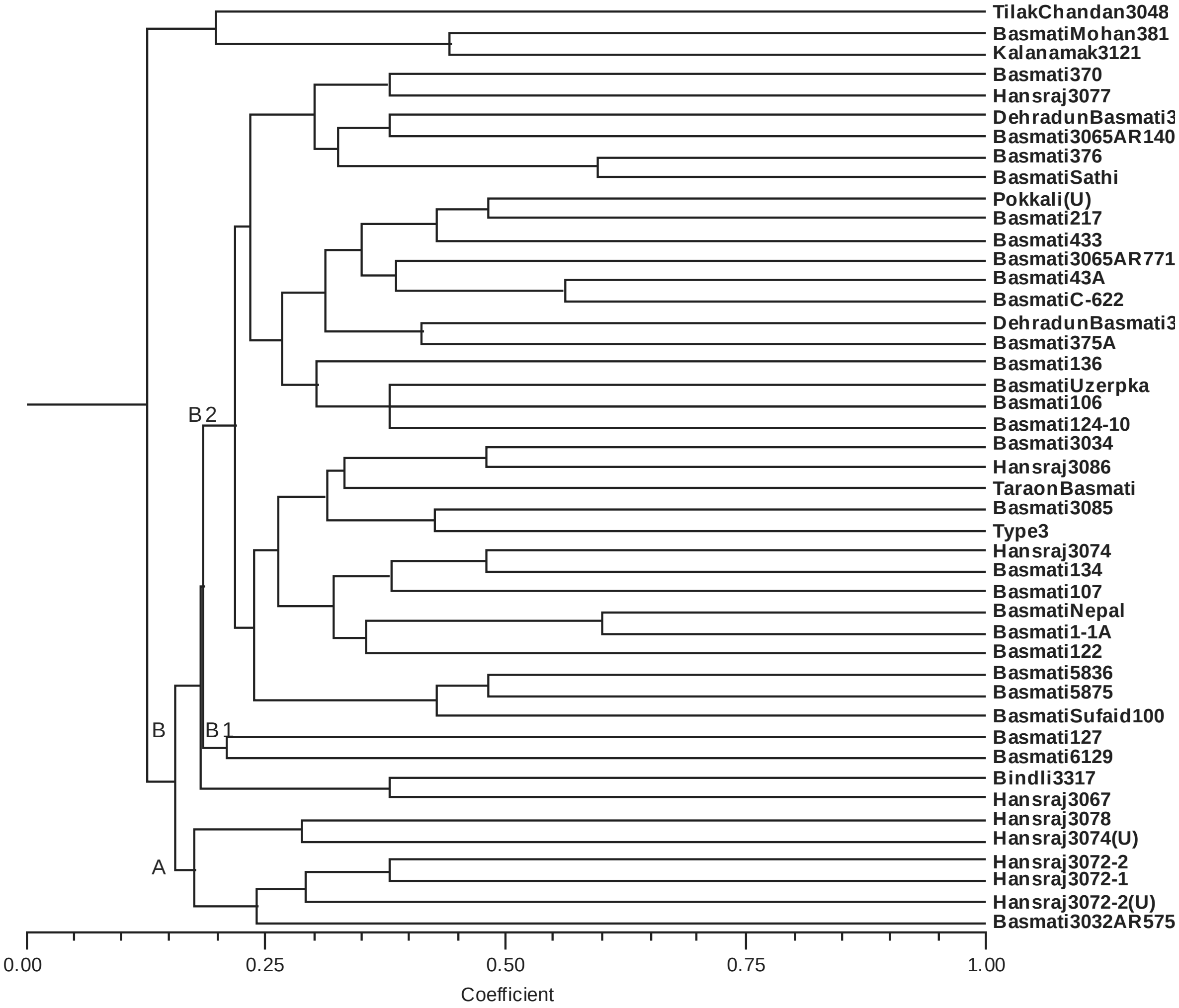

Fig. 1: Agro-Morphological trait based dendrogram

good resemblance to Hansraj 3067 a long grained genotype and segregated in B1 group of Dendrogram due to resemblance in agro-morphological traits as following. Flag leaf length (22. 75 cm, 27.0 cm), L/B ratio of flag leaf (26.50, 26.85), first leaf length (39.0 cm, 40.0 cm), days to 50 % flowering (82.0 days, 82.0 days), days to maturity (106 days, 106 days) and 1000 grain weight (21.0 g, 21.0 g) for Bindli and Hansraj 3067 genotypes, respectively.

Basmati 3032 AR 575 (U) and Hansraj 3072 (U) genotypes clustered together due to resemblance in their adaptation of growing environment as both are adapted for usher soil environment. They showed resemblance in agro-morphological traits as following. Days to 50 % flowering (97.0 days, 98.5 days), days to maturity (117 days, 117 days) and plant height (102 cm, 101.9 cm) for Basmati 3032 AR 575 (U) and Hansraj 3072 (U) genotypes, respectively.

Present study indicated that different indigenous varieties named after aromatic rice varied widely in their agro-morphological characteristics. However, a number of Dehradun Basmati and Hansraj selections showed high degree of similarity indicating that Hansraj which was once grown widely in districts Bijnore, Pilibhit and Bareily of Uttar Pradesh and Udham Singh Nagar of Uttarakhand is *Basmati*. Probably *Basmati* lines grown in these districts were called as Hansraj.

As agro-morphological traits are influenced by environmental factors, hence there is need to use molecular markers like RAPD, and SSR in follow up of this study.

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