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A MULTIVARIATE ANALYSIS OF RICE GENETIC RESOURCES

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Abstract

Fifty rice genotypes originating from hybridization and induced mutagenesis along with some commercial varieties were subjected to diversity analysis for selection of desirable genotypes and subsequent use in the future breeding program. The data were analyzed by Principle Component Analysis (PCA) and Mahalanobis's generalized distance (D^2). Two principal components presented 65% information of the raw data of the yield related traits. Cluster analysis (D^2) exhibited six distinct clusters with the range of 2 genotypes in cluster VI to 13 in cluster I and III each. Regularly, inter-cluster distance was larger than the intra-cluster distance suggesting wider genetic diversity among the genotypes. Maximum inter-cluster distance was observed between cluster I and VI (79.81) followed by cluster I and V (71.90). The highest intra-cluster distance (14.41) was observed in cluster V where as lowest intra-cluster distance was observed in cluster VI (5.87). Relatively high yielding, short stature genotypes with less number of productive tillers per plant were grouped into cluster I and II to get the highest divergence of these from clusters V and VI. The genotypes in cluster I and II showed high inter-cluster distance with those in cluster IV, V and VI indicating the possibility to obtain heterotic progenies, by attempting the crosses, among the genotypes of these groups.

Introduction

Breeding and adoption of rice cultivars with enhanced yield potential is a common objective in irrigated ecosystem of Basmati rice in Pakistan. A number of varieties of rice with Basmati and coarse background have been developed and adapted in the irrigated ecosystem of the country. In Pakistan, six Basmati rice cultivars viz, Basmati 370, Basmati Pak, Basmati 385, Super Basmati, Shaheen Basmati and Basmati 2000 have been approved for general cultivation (Tabasum *et al*., 2011). Kashmir Basmati was originated as a radiation induced mutant from the world's finest quality Basmati rice variety Bas-370 (Iqbal *et al*., 2010).In spite of the concerted efforts, there is considerable yield gap between national and international yield. Modern rice varieties have generally higher yield potential than traditional cultivars when grown under controlled conditions with relatively stable and sufficient inputs (Khush, 1995). Although traditional varieties have low yield but they are believed to be more locally adapted and suited to real socio-cultural constraints (Bellon *et al.,* 1998). Therefore, the yield performances of these genotypes need to be improved. For yield improvement, genetic diversity needs to be investigated for successful utilization of genotypes in breeding programs.

Information about genetic diversity facilitates the selection of parental genotypes from random populations. Briefly, accurate assessment of the levels and patterns of genetic diversity can serve for the analysis of genetic variability in germplasm (Smith, 1984; Cox *et al.,* 1986), identification of diverse parental combinations to create segregating progenies with maximum genetic variability for further selection (Barrett & Kidwell, 1998) and introgression of desirable genes from wild germplasm into the adapted high yielding germplasm resource (Thompson *et al.,* 1998). Such information is particularly useful to assess the potential of heterotic combinations before attempting crosses and hence saving time and resources (Hallauer & Miranda, 1988). Analysis of genetic diversity in germplasm collections can facilitate reliable classification of accessions and identification of subsets of core accessions with possible utility for specific breeding purposes. Significant emphasis is being paid to comprehensive analysis of genetic diversity in numerous field crops for long-term success of breeding program and maximum exploitation of the genetic resources (Belaj *et al.,* 2002). If the structure of the genetic diversity is known within a large collection of germplasm which may be of great help to make decisions on management procedures and breeding strategies to be used in breeding programs. With the development of advanced biometrical techniques such as multivariate analysis, quantification of degree of divergence among the biological populations and assessing the relative contribution of different components to the total divergence at intra- and inter-cluster levels have now become possible. Such a study also permits to select the genetically diverse parents to obtain the desirable recombinant in the segregating populations upon crossing. In the hybridization programs, inclusion of more diverse parents has been observed to increase the chances for obtaining stronger heterosis and giving broad spectrum of variability in segregating generations (Joshi & Dhawan, 1966; Ananda & Murty, 1968). Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan has considerable number of accessions/advance lines in the gene bank derived from various sources such as National Uniform Rice Yield Trial (NURYT), induced mutation and hybridization program but the information on the aspect of genetic diversity was scanty. The present study was, therefore, undertaken to assess the extent of genetic diversity in 50 rice genotypes, which will help to select prospective parents to develop transgressive segregates.

Materials and Methods

Study included 50 rice genotypes, collected from the National Uniform Rice yield Trial (NURYT) of the country and the breeding program of the rice group at NIAB Pakistan - which has developed several basmati and non basmati rice genotypes through the induced mutation by gamma rays of radioactive isotope Co^{60} (Cheema *et al*., 2003; Rashid *et al.,* 2003). The material was planted at the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Punjab (183 meter above mean sea level, 31^{0} 24$^{/}$ N and 730 ^{0} 05 E$^{/)}$ during the kharif season 2004 and 2005 in a Randomized Complete Block Design (RCBD) with three replications. Thirty days old seedlings from each entry were transplanted using single seedling per hill in 15m^{2} plot maintaining 20cm plant to row spacing. Standard cultural practices and plant protection measures used in the irrigated ecosystem of rice in Punjab province were observed. All the fertilizers, except nitrogen, were applied at transplanting of the seedlings. Nitrogen was applied in three equal splits *i.e.* a basel dose, 25-30 days after transplanting (DAT) and 45-50 DAT. Inter-cultural operations and pest control measures were done as and when necessary. Ten plants from each replication of each genotype were randomly selected for recording data on plant height (cm), productive tillers/plant, panicle length (cm), panicle fertility (%), days to 100% flowering and yield (kg ha^{-1}). Yield was recorded on plot basis and then was converted to kg ha^{-1}. The data were analyzed following PCA and Mahalanobis's (1936) generalized distance (D^{2}) extended by Rao (1952). The statistical analysis was carried out using computer software Minitab Version 13.2 (2000).

Results and Discussion

Depending upon the range of diversity, all the genotypes were grouped into six clusters (Table 1, Fig. 1). Significant differences among the genotypes for all the characters suggested the presence of significant variation among the genotypes for all the traits under study (Table 2). The distribution pattern revealed maximum number of genotypes (13 genotypes) in each of the cluster I and III whilst cluster VI included minimum number of genotypes (2 genotypes). Cluster II, IV and V included 10, 8 and 4 genotypes respectively. Limited number of the genotypes i.e. 4 and 2 were grouped in clusters V and VI respectively. The limited number of genotypes in these clusters was probably due to small number of characters included in this study. In this connection, Huidong & Shiliang, (1987) have reported that based on hierarchical and dynamic clustering, the frequency of the cultivars in a given cluster is increased by increasing the number of traits under study.

Table 1. Distribution of rice genotypes to different clusters as determined by multivariate analysis.

Cluster	Serial No. of the mutants hybrids/ advance lines	Frequency	Name of genotypes
I	1, 18, 13, 10, 11, 2, 5, 4, 12, 15, 7, 23, 3	13	RH1, RD-25, RH13, RH10, RH11, RH2, RH5, RH4, RH12, RH14a, RH7, KSK-406, RH3,
II	8, 14, 16, 20, 17, 6, 19, 26, 21, 22	10	RH8, RH14, RH15, KSK-202, DIK-1, RH6, GPP-101, KS-282, KSK-401, KSK-402
III	9, 44, 42, 43, 24, 25, 27, 29, 28, 47, 30, 39, 40,	13	RH-9, EF-40-05, EF-60-05, EF-103-05, 99723, PK7797-1, DM-7-99, DM-15-04, DM-3-99, EF-50-05, DM-34-04, EF-9a-05, EF-9b-05
IV	31, 32, 41, 45, 46, 33, 37, 34,	8	EF-15-04, EF-59-04, EF-59-05, EF-49a-05, EF-49b-05, EF-119-02, Super Basmati, EF-54-02
V	35, 36, 38, 50,	4	Basmati-370, Basmati-385, Basmati-2000, Basmati-Pak
VI	48, 49	2	EF-27-05, EF-50-05

Table 2. Mean squares for six characters of rice genotypes.

Characters	Sources of variation		
	Replications	Genotypes	Error
Plant height	106.13*	569.87* *	27.31
Productive tillers/ plant	9.70*	21.16* *	2.91
Panicle length	0.19 $^{n .s}$	18.38* *	0.64
Fertility %age	24.62 $^{n. s}$	56.89* *	10.95
Days to flowering	3.19*	181.64* *	0.87
Yield kg ha $^{-1}$	416.7 $^{n. s}$	18993749.15* *	272943.98

n.s. = Non-significant; * = Significant; * * = Highly significant; Df: Degree of freedom

The inter-cluster distances in all the cases were greater than the intra-cluster distances suggesting wider diversity among the genotypes of the distant groups (Table 3). The similar results regarding inter and intra-cluster distances have been described by Islam *et al*., (2003) in rain fed low land rice and Rasul & Okubo (2002) in teasel gourd. The intra-cluster degree of diversity was maximum in cluster V *i*.e. 14.41 and minimum in cluster VI (5.87), indicating that the genotypes in cluster V were more heterogeneous and those in cluster VI were comparatively more closely related. The range of intra-cluster values indicated generally heterogeneous nature of the genotypes within the clusters. Previously, Iftkharudaulla *et al.*, (2002) examined the range of intra-cluster distances in irrigated Boro rice and reported homogeneous nature of the genotypes within the clusters, contrary to the present findings, which is probably due to the limited number of the characters included in the present study.

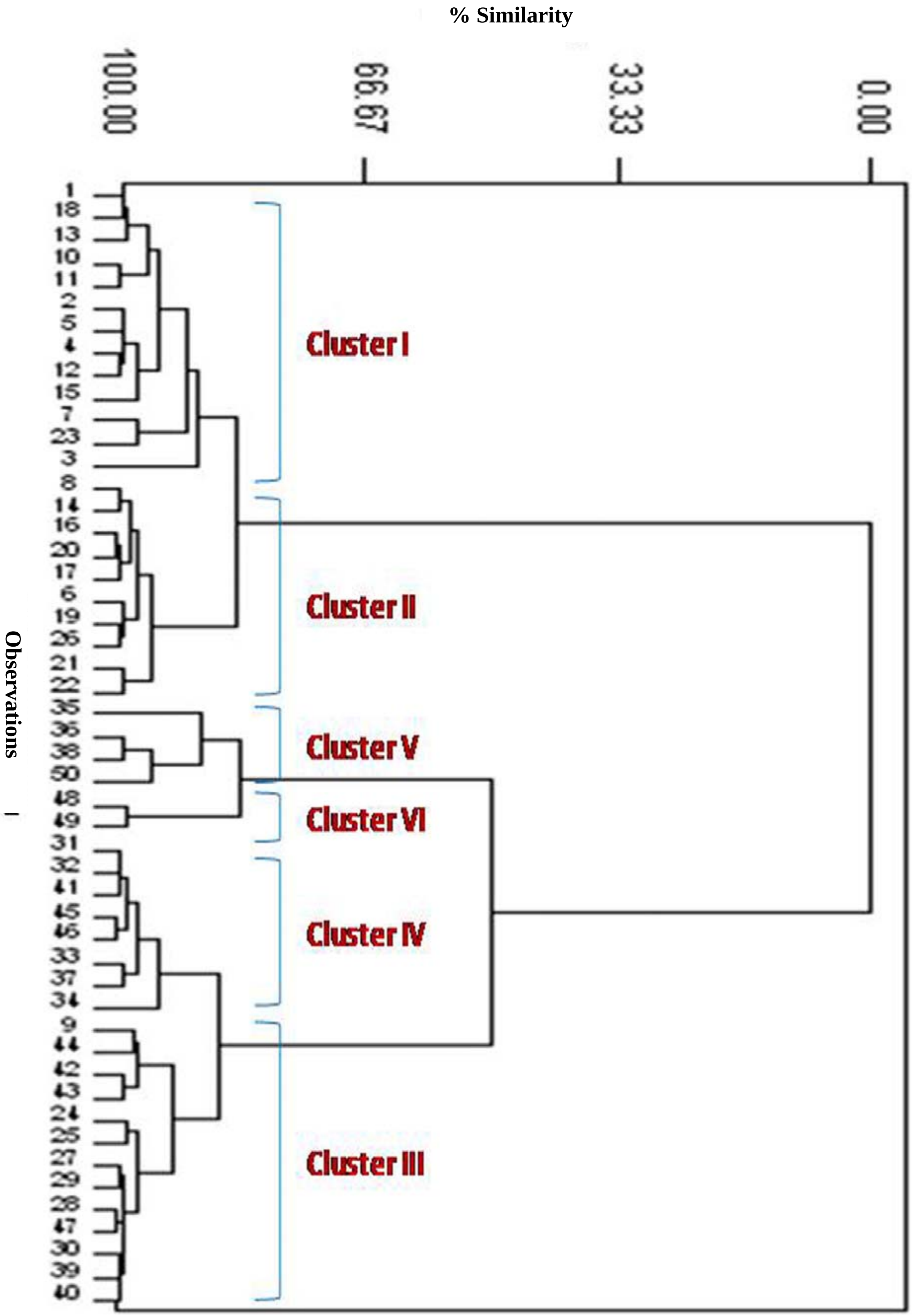

Fig. 1. Phenogram of rice genotypes based on quantitative characters.

Table 3. Average intra and inter-cluster distance (D^2) for rice genotypes.

Cluster	I	II	III	IV	V	VI
I	(11.74)	18.87	46.16	52.08	71.90	79.81
II		(8.66)	38.31	46.64	71.40	74.47
III			(9.25)	14.88	39.12	37.28
IV				(8.18)	27.73	31.81
V					(14.41)	27.16
VI						(5.87)

Values in bold illustrate the intra-cluster distance and others show inter cluster distance

Regarding the inter-cluster distance, cluster I showed maximum genetic distance from cluster VI (79.81) followed by the genetic distance between cluster II and VI (74.47). The genetic distance of the cluster V with respect to the cluster I and II was also high and almost equal *i.e.* 71.90 and 71.40 respectively which reflects wider diversity among these clusters. Minimum distance was found between the genotypes of the cluster III and IV (14.88) followed by the genetic distance between clusters I and II (18.87).

Mean performance of different clusters for the characters studied (Table 4) reflected that all the high yielding genotypes with improved panicle fertility percentage were grouped into cluster I whereas cluster VI included low yielding genotypes with low fertility percentage indicating maximum contribution of these characters towards the divergence between cluster I and VI. On the other hand cluster I was found divergent from the cluster V mainly due to plant height, days to flowering and grain yield indicating maximum contribution of these characters towards the divergence. In fact, except grain yield, the intra-cluster mean performance of cluster I for these characters was lower than in cluster V. Similarly, cluster II was divergent from cluster V and VI mainly for plant height, panicle length and days to flowering. Intra-cluster mean performance for these characters was observed to be the lower or the lowest in cluster II but higher or the highest in cluster V indicating the role of these characters towards the divergence between cluster II and V. In this context, Jgadev *et al.,* (1991) reported that the characters contributing maximum towards the divergence should be given greater emphasis for deciding the type of cluster for the purpose of further selection and the choice of parents for hybridization.

Table 4. Cluster means for different parameters for rice genotypes.

Traits	Cluster means					
	I	II	III	IV	V	VI
Plant height	135.75	122.34	140.06	144.85	168.20	155.90
Productive tillers per plant	13.37	13.93	15.74	16.34	14.40	22.03
Panicle length	29.85	28.43	29.84	30.85	34.73	28.78
Fertility %age	86.37	90.86	90.98	92.82	90.54	82.12
Days to flowering	109.00	104.30	103.69	116.75	124.67	110.33
Yield kg ha^{-1}	9965	8818	5429	4947	3777	2305

The contribution of different characters towards the divergence is presented in (Table 5). The basic objective of PCA is to minimize the dimensionality of large number of interrelated variables in a given data set and retaining maximum information about the genetic variation. The principle components, which are basically transformed set of variables, maintain most of the variation present in the germplasm (Joliffe, 2005). In the present study, PCA revealed that vector I and II are responsible for about 65% of the total variation in the data. Additionally, values in both vectors (vector I and II) were positive for plant height, panicle length and days to flowering. Such results indicated that these characters contributed maximum towards the divergence. It is, therefore, obvious that greater divergence among the genotypes in the present study was attributed to plant height, panicle length and days to flowering and will offer a good scope for the improvement of yield through rational selection of the parents. The major contribution of these characters towards the divergence was well confirmed by their cluster means where the ranges varied indicating major role of these characters to differentiate at inter-cluster level. In the present study, yield and five yield related characters were included to compute the PCA which indicated that up to 65% of the information about variation is retained by two components. However, in a previous report Qing-Lil *et al.,* (2009) showed that 90.71% of the genetic information, about the drought related characters in foxtail millet, was offered by 6 principle components.

Table 5. Relative contribution of characters to the total divergence in rice genotypes.

Traits	Latent Vectors	
	PCI	PCII
Plant height (cm)	0.549	0.133
Productive tillers per plant	0.294	-0.556
Panicle length (cm)	0.405	0.511
Fertility %age	0.069	-0.486
Days to flowering	0.427	0.228
Yield kg ha^{-1}	-0.511	0.345
% Variance	43.00	22.00

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The means for plant height, panicle length and days to flowering in cluster II were observed as 122.34cm, 28.34cm and 104.3 days respectively whereas in cluster V as 168.2cm 34.73cm and 124.67 days respectively, reflecting a considerable difference in means. This was also confirmed by relatively high inter-cluster distance (71.40) between these two clusters. The cluster mean for grain yield was 9965kg ha^{-1} and 8818 kg ha^{-1} for cluster I and II respectively as compared to 2305kg ha^{-1} for cluster VI with a difference of about four times lesser and it was also confirmed by the largest inter-cluster distance of cluster I and II with cluster VI showing values as 79.81 and 74.47 respectively (Table 3).

It is assumed that maximum amount of heterosis will be manifested in cross combinations involving the parents belonging to the most divergent clusters. The objective of a successful breeding program is not only high heterosis but also to achieve high level of yield by reducing the life span of a variety so that it can be better fitted in the prevailing cropping pattern. In the present study, relatively high genetic distance existed between cluster I and VI, II and VI followed by I and V as well as II and V. Several reports showed that the parents separated by the medium magnitude of divergence also show high heterosis (Ghaderi *et al.,* 1979; Arunachalam *et al.,* 1984; Mian & Bahl, 1989). Therefore, parents from these clusters also have a potential for trait improvement. There are reports that selection of parents for hybridization should be from two clusters having wider inter-cluster distance to get maximum variability in the segregating generations and subsequent selection of ideotypes (Pradhan & Ray, 1990; Rahman *et al.,* 1997; Bose & Pradhan, 2005). Moreover, it has been reported that the crosses attempted between, distantly related, *Brassica species* yielded superior progenies than the crosses between the species of the same origin (Lefort-Buson *et al.,* 1987). Out of the 6 different procedures, commonly used for cluster analysis, Mahalanobis' D^2 approach has also shown the same potential for selection of the parents in breeding programs (Bhatt 1970; Ariyo, 1987). Keeping in view these reports, it appears that the crosses among the genotypes belonging to cluster I and II with those of cluster IV, V and VI would exhibit higher heterosis for improvement of desirable characters in segregating populations. So, the genotypes located in these clusters may be selected for future breeding program.

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