

*Full Length Research Paper*

# Genetic diversity in grain quality and nutrition of aromatic rices

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The study was conducted at Bangladesh Rice Research Institute (BRRI), Gazipur in 2005 to assess the genetic divergence of aromatic rices for grain quality and nutrition aspects. Forty genotypes composed of 32 local aromatic, five exotic aromatic and three non-aromatic rice varieties were used. Univariate and multivariate analyses were done. Enormous variations were observed in majority of characters viz. grain length, breadth, kernel weight, milling yield, kernel length, L/B ratio of kernel, volume expansion ratio (VER), protein content, amylose content, elongation ratio (ER) and cooking time. In multivariate analysis, genotypes were grouped into six clusters. In the discriminant function analysis (DFA), function 1 alone absorbed 61.7% of the total variance. The most contributing variables were kernel weight, kernel length and L/B ratio in function 1. The inter-cluster  $D^2$  value was maximum (26.53) between I and VI followed by 21.28 (between I and V). Minimum  $D^2$  value was found (5.90) between II and III. Majority of the local aromatic rice varieties with smaller kernels were included in the cluster I. The cluster III contains Elai, sarwati and sugandha-1 with long-slender kernel and 'very good' appearance. Thus, these varieties can be used in breeding programme for improvement of germplasms in cluster-I.

**Key words:** Aromatic rice, grain quality, genetic diversity.

## INTRODUCTION

Rice is the unique grain that is nearly entirely used as human food, unlike other cereals, which are also used extensively as feed (Swaminathan, 1999). Therefore, evaluation of rice germplasm is an important step for the fulfillment of human demand and options. Aromatic rices constitute a small and special group of rices that is regarded as best in quality and usually used for special dish preparation. Quality of rice may be considered from the view point of size, shape and appearance of grain, milling quality and cooking properties (Dela Cruz and Khush, 2000). The breeders and nutritionists seek rice grain with higher content of protein, vitamins and minerals.

The diversity in crop varieties is essential for agricultural development for increasing food production, poverty alleviation and promoting economic growth. The available diversity in the germplasm also serves as an insurance

against unknown future needs and conditions, thereby contributing to the stability of farming systems at local, national and global levels (Singh et al., 2000). In crop improvement program, genetic variability for agronomic traits as well as quality traits in almost all the crops is important, since this component is transmitted to the next generation (Singh, 1996). Study of genetic divergence among the plant materials is a vital tool to the plant breeders for an efficient choice of parents for plant improvement. Genetically diverse parents are likely to contribute desirable segregants and/or to produce high heterotic crosses. Parents identified on the basis of divergence for any breeding program would be more promising (Arunachalam, 1981). Grouping or classification of genotypes based on suitable scale is quite imperative to understand the usable variability existing among them. For the assessment of variation on multivariate scale, Mahalanobis'  $D^2$ -statistic has proved to be a powerful technique (Murty and Arunachalam, 1966).

Bangladesh has a stock of above 7,000 rice germplasms of which around 100 are aromatic varieties

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(BRRI, 1997; Hamid et al., 1982). Most of our varieties are small and medium grained, unlike long grained rices in the world market. It is true that some small and medium-grained aromatic rices possess excellent aroma and other quality traits like elongation after cooking, taste etc. These could be excellent sources for improving quality in high yielding varieties. The improvement of aromatic rices, requires its collection and evaluation of existing cultivars of Bangladesh. Genetic study of local germplasm of aromatic rices is the pre-requisite for the development of high yielding varieties. Many workers have performed studies on genetic diversity with local and high yielding varieties of rice. But such type of studies on aromatic rices has not yet been done.

In view of the aforesaid discussion, a study was undertaken to determine physico-chemical and cooking qualities of grains and to estimate genetic relationship and genetic distance among the varieties, to classify them in respect of grain quality and nutrition.

## MATERIALS AND METHODS

The experiment was conducted at the farm of Bangladesh Rice Research Institute (BRRI), Gazipur in Transplant Aman (wet season), 2005. A total of forty rice germplasm composed of 32 local aromatic, five exotic and three non-aromatic rice varieties as standard checks, were selected for this research (Table 1). Among the three non-aromatic varieties, BR28 is a modern Boro, BR39 is a modern T. Aman variety and Nizersail is a local improved variety. Among the exotic genotypes Basmati PNR346 was from Pakistan; sarwati and sugandha-1 were from Nepal and Khazar and Neimat were from Iran. The other entries are local having their distribution throughout Bangladesh. Forty rice genotypes formed the treatment variables and were assigned randomly to each unit plot of 5 m × 2 m dimension. Thirty day-old seedlings were transplanted on the 15<sup>th</sup> August in 2005 following random complete block design (RCBD) with three replications. Crop management practices were done accordingly. An area of 5 m<sup>2</sup> was harvested from each plot for analysis.

Physico-chemical properties and nutritional values were determined in the laboratory of grain quality and nutrition division in Bangladesh rice research institute. Milling out turn was determined from duplicate samples of 125 g rough rice. Rough rice samples were dehulled with a Satake laboratory sheller. The sample was poured into the hopper. Then the resulting brown rice was milled in McGill mill number 2 (Adair, 1952). The milled rice sample was collected in a jar and sealed immediately. The rice was allowed to cool before weighing. The weight of the total milled rice was recorded. The moisture content was calibrated to 14%. Milling out turn was calculated with the following formula (Khush et al., 1979).

$$\text{Total milled rice (\%)} = \frac{\text{Weight of milled rice}}{\text{Weight of rough rice}} \times 100$$

Thousand milled rice weight was measured in gram from a randomly selected sample of 1000 milled rice on an electronic balance. The weight was calibrated at 14% moisture content. The process was repeated twice for each replication. volume expansion ratio (VER) was determined by water replacement method. One hundred mL water was poured into a 200 mL measuring cylinder. A sample of 10 g milled rice was poured in the cylinder. The rise of

water level was recorded. Then the rice was cooked. After cooling, the cooked rice was poured into the water of cylinder. Replacement of water volume was recorded. Finally, the ratio was calculated as follows:

$$\text{VER} = \frac{\text{Volume of cooked rice}}{\text{Volume of raw rice}}$$

A sample of twenty-five whole milled kernels were soaked in distilled water for 30 min for the determination of elongation ratio (ER). Each sample was cooked in a water bath at 98°C for 10 min (Azeed and Shafi, 1966). The cooked rice was then transferred to a petridish lined with filter paper. Ten cooked whole rice were measured by a digital vernier caliper.

$$\text{ER} = \frac{\text{Average length of cooked rice grains (mm)}}{\text{Average length of raw rice grains (mm)}}$$

Cooking time was estimated in the method described by Juliano et al. (1969). Five grams of whole milled rice were kept in vigorously boiling water in a beaker. After 10 min of boiling, sample was tested every minute with pressing between two glass plates. The grains were considered cooked when 90% of the grains no longer had opaque or uncooked center. The analysis was done in duplicate. Micro Kjeldahl method was used for the determination of nitrogen (AOAC, 1970). Then protein content was calculated as the following formula:

$$\text{Crude protein (\%)} = \% \text{ N} \times 5.95$$

Amylose content was determined following the procedure of Juliano (1971). For data analyses different statistical tools were used. Genotypic coefficient of variation was estimated according to Burton (1952). The ANOVA was done by IRRISTAT Windows 4.01. Genetic divergence among the genotypes was assessed by using D<sup>2</sup> statistic (Mahalanobis, 1936) extended by Rao (1952). Discriminant function analysis (DFA) was done for conformity of the results and to verify precision level of clustering (Huberty, 1994). These analyses were performed under software SPSS v11.0.

## RESULTS AND DISCUSSION

### Variability of characters in univariate analysis

The traits associated with grain quality and nutrition of forty rice genotypes are shown in Table 1. Data showed highly significant variations among the genotypes for all the traits in the ANOVA (F probability value < 0.01). Maximum grain length was recorded 12.16 mm in Elai, followed by 11.68 mm in Neimat and minimum was 5.81 mm in Rajbhog. Kernel length of Neimat was the highest (7.87 mm) followed by Elai (6.75 mm). Aromatic variety with kernel length 6.0 mm and above is considered widely acceptable size (Kaul, 1970). The breadth of grain ranged from 2.24 to 3.60 mm. A very wide range of thousand kernel weight was observed. It was highest for Gandho kasturi and lowest for Rajbhog. Varieties with small kernels are highly preferred for the preparation of *polao*, *firni* and other dishes in Bangladesh. Milling yield of a variety is another important issue especially for rice millers and traders. Maximum milling yield was computed



Table 1. Contd.

Genotype code and name		Volume expansion ratio	Protein content (%)	Amylose content (%)	Elongation ratio	Cooking time (min)
1	Badsha bhog	4.01	8.30	23.01	1.60	15.50
2	Baoi jhak	4.10	10.70	24.11	1.60	14.99
3	Basmati Tapl-90	4.30	8.90	24.91	1.50	19.03
4	Basmati PNR 346	3.90	9.20	23.40	1.50	18.36
5	Begun bichi	4.10	8.29	22.79	1.60	15.50
6	Benaful	3.60	8.13	22.80	1.51	19.00
7	Bhog ganjia	4.10	7.64	23.30	1.41	16.00
8	BR28	4.50	8.60	27.05	1.46	17.99
9	BR38	4.10	8.49	23.06	1.41	17.01
10	BR39	3.89	8.30	26.20	1.46	20.00
11	Chinigura	4.30	7.12	26.40	1.70	16.03
12	Chinikani	4.31	7.99	23.73	1.70	15.51
13	Darshal	4.29	8.70	23.74	1.80	17.03
14	Doiar guro	4.29	8.88	23.40	1.71	16.02
15	Elai	4.10	7.70	27.00	1.50	16.00
16	Gandho kasturi	4.41	7.40	25.96	1.90	22.03
17	Gandhoraj	3.89	9.28	23.50	1.60	15.05
18	Hatisail	4.01	9.18	22.80	1.70	15.94
19	Jamai sohagi	3.70	8.59	23.40	1.50	14.50
20	Jata katari	3.70	7.68	23.13	1.40	14.50
21	Jesso balam	4.30	8.81	22.77	1.71	14.50
22	Jira katari	4.00	8.32	23.53	1.70	14.50
23	Kalijira Tapl-73	3.71	8.61	25.04	1.41	15.50
24	Kalomai	4.31	8.80	23.77	1.41	16.00
25	Kamini soru	3.70	9.01	22.43	1.60	14.00
26	Kataribhog	3.99	8.30	22.80	1.71	14.01
27	Khazar	3.90	9.93	21.10	1.30	17.03
28	Laljira Tapl-130	3.69	8.40	22.40	1.67	13.02
29	Niemat	3.70	9.68	26.41	1.50	17.98
30	Nizersail	4.10	7.90	25.50	1.55	16.01
31	Philippine katari	3.90	8.40	23.00	1.40	15.03
32	Premful	4.11	9.88	21.51	1.60	14.50
33	Radhuni pagal	4.00	8.90	23.40	1.51	15.50
34	Rajbhog	3.90	9.20	23.41	1.61	14.03
35	Sai bail	3.89	9.20	22.52	1.70	14.98
36	Sakkor khora	4.00	8.20	24.40	1.60	18.50
37	Sarwati	3.90	8.61	19.83	1.30	15.00
38	Sugandha-1	3.70	8.40	18.81	1.70	16.00
39	Tilkapur	3.60	8.73	22.60	1.51	15.50
40	Ukni madhu	4.00	8.81	23.32	1.41	15.00
Minimum		3.6	7.12	18.81	1.3	13.02
Maximum		4.5	10.7	27.05	1.9	22.03
GCV(%)		5.8	8.14	7.34	8.77	11.44
SE		0.04	0.07	0.14	0.01	0.15
F prob. Value		<0.01	<0.01	<0.01	<0.01	<0.01

for BR38 and Sakkor khora and minimum for Basmati PNR346. Length/breadth (L/B) ratio of kernel was found

to vary over a wide range (1.95 to 4.51). Maximum and minimum L/B ratios were observed in Elai and Laljira,

**Table 2.** Tests of equality of group means of 11 variables with probability values by DFA.

Variable	Wilks' Lambda	F value	df1	df2	Level of significant
Grain length (mm)	0.16	37.06	5	34	0.00
Grain breadth (mm)	0.32	14.22	5	34	0.00
1000-kernel weight (g)	0.10	61.52	5	34	0.00
Milling yield (%)	0.46	8.14	5	34	0.00
Length of kernel (mm)	0.15	39.34	5	34	0.00
L/B ratio in kernel	0.20	26.89	5	34	0.00
Volume expansion ratio	0.73	2.58	5	34	0.04
Protein content (%)	0.87	0.98	5	34	0.45
Amylose content (%)	0.41	9.92	5	34	0.00
Elongation ratio	0.68	3.24	5	34	0.02
Cooking time (min)	0.33	14.01	5	34	0.00

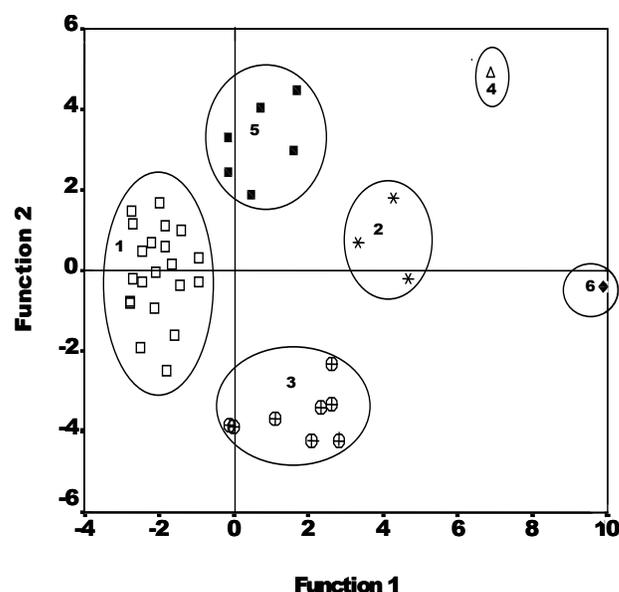
respectively. The volume expansion ratio (VER) ranged from 3.6 to 4.5 and elongation ratio (ER) varied from 1.41 to 1.90. Lower VER is preferred than higher VER. On the other hand, higher ER is preferred than lower ER for quality of cooked rice (Singh et al., 2000).

According to the classification of Kumar and Khush (1986), eight varieties were grouped into high (> 25%) and 30 into intermediate amylose (20 – 25%) groups. Only two exotic cultivars, Sarwati and Sugandha-1, had low amylose content (< 20%). Cooked rice becomes moist and sticky due to low amylose content. Intermediate amylose contents in rice varieties are preferred in most of the rice growing areas of the world since cooked rice becomes soft. Bangladeshi people normally prefer high amylose rice. However, in case of aromatic rice, intermediate amylose varieties are also equally accepted. Protein content was found maximum 10.70% in Baij jhak and minimum 7.12% in Chinigura. A considerable variation was found also in cooking time (Table 1).

Genotypic coefficient of variation was also high for all the 11 characters (Table 1). Highest genotypic coefficient of variation (GCV) was recorded for thousand kernel weight followed by length/breadth ratio and kernel length. High GCV for thousand grain weight and other grain quality traits was reported by several workers (Rather et al., 1998; Kumar et al., 1998). On the other hand, lowest value of GCV was observed for milling yield.

### Clustering of genotypes by multivariate analysis

Univariate analysis of data showed enormous variations in all the 11 characters, which however was difficult to explain with respect to the effect of each variable for individual genotype. Therefore, multivariate analysis was done to make homogenous groups of genotypes on the basis of the characters evaluated. The forty rice genotypes were grouped into six clusters (Figure 1). Test of equality of group means (Table 2) indicated the impact of each variable for separating the groups. The table contains Wilk's lambda, F statistic and significance level.



**Figure 1.** Graphic illustration of discriminant analysis of 6 clusters of 40 rice genotypes based on grain quality characters.

Wilk's lambda is the ratio of the within-group sum of squares to the total sum of squares. It may range from 0 to 1. Small values indicate strong group differences. Values close to unity indicate no group differences. Of the 11 characters, volume expansion ratio, protein content and elongation ratio had the higher values for Wilks lambda and lower level of significance ( $p > 0.01$ ). It indicated that these characters could have little influence on clustering. On the other hand, lower Wilk's lambda values with higher level of significance ( $p < 0.01$ ) for the remaining eight variables indicated their significant contribution in clustering pattern. Mean values of different characters under four functions are presented in Table 3.

A step-wise DFA was carried out to determine the discriminatory functions (principal components) contributed in separating the forty genotypes into six distinct

**Table 3.** Cluster means of seven discriminating variables, identified by DFA, representing different functions.

Cluster #	Function 1			Function 2		Function 3	Function 4
	Thousand kernel weight (g)	Length (mm)	L/B ratio	Elongation Ratio	Amylose content (%)	Cooking time (min)	Milling yield (%)
I	8.43	3.84	2.35	1.60	23.20	14.88	70.12
II	16.12	6.46	3.73	1.49	25.84	19.01	68.91
III	13.19	5.70	3.48	1.48	22.57	16.10	66.49
IV	21.66	5.81	2.26	1.51	22.80	19.00	72.35
V	12.15	4.66	2.62	1.54	24.80	17.01	71.99
VI	24.55	5.87	2.32	1.90	25.96	22.03	66.50

**Table 4.** Contribution by each of discriminant functions\* in grouping of 40 rice varieties into six clusters through step-wise DFA.

Canonical function	Eigenvalue	% of variance	Cumulative (%)	Canonical correlation
1	12.69	61.70	61.70	0.96
2	4.71	22.90	84.60	0.91
3	1.94	9.40	94.10	0.81
4	0.87	4.30	98.30	0.68
5	0.34	1.70	100.00	0.51

First 5 canonical discriminant functions were used in the analysis.

**Table 5.** Standardized canonical discriminant function coefficients with respect to the variables that mostly contributed in the clustering of rice varieties.

Variable	Function				
	1	2	3	4	5
Thousand kernel weight (g)	1.82	-1.00	0.58	-0.34	-0.30
Length of kernel (mm)	-1.71	1.08	-1.01	0.95	-0.81
L/B ratio in kernel	1.37	0.73	0.61	-0.59	0.76
Elongation ratio	0.15	0.75	0.21	-0.64	0.06
Amylose content (%)	-0.18	0.54	0.45	-0.17	-0.25
Cooking time (min)	0.06	-0.02	-0.36	0.16	1.29
Milling yield (%)	-0.05	0.28	0.32	0.86	-0.11

clusters. The contribution of each of five canonical discriminant functions for explaining the variance along with its corresponding eigenvalue and canonical correlation coefficient was presented in Table 4. It was found that the function 1 (the first principal component) accounted for 61.7% of the total variance and the function 2 explained 22.9% of the total variance. In the cumulative effect, functions 1 and 2 absorbed 84.6% of the total dispersion. Since canonical correlation values for functions 1 and 2 were very close to unity, this result indicated strong association between discriminant scores and the groups. Canonical functions have been reported to describe the DFA by several authors (Vicente et al., 1998; Singh et al., 1996).

The traits mostly contributed to the discriminant func-

tions along with their standard coefficients under each function have been presented in the Table 5. The coefficient of thousand kernel weight, kernel length and L/B ratio were highest in function 1. Coefficients with large absolute values corresponding to variables should have greater discriminating ability. Therefore, these three characters were the major contributors to function 1 for explaining 61.7% of the total dispersion. Largest absolute values for elongation ratio and amylose content under function 2 indicated that these two traits were mainly responsible for explaining 22.9% of the total variance.

The orientation of six clusters in different positions in comparison of origin of X and Y ordinate, has been presented in the Figure 1. The genotypes were clustered on the basis of higher values of discriminating variables

contributing in functions 1 and 2 (Table 4). In addition, one character under function 3 and one in function 4 were put down, as these two also were included in the step-wise DFA. Moreover, functions 1 and 2 are considered for the construction of understandable two-dimension graph, as these two can account for more than 80% of the total dispersion (Huberty, 1994). Since functions 1 and 2 had been accountable for more than 84% of total variance (Table 4) and therefore played the deciding role on the placement of clusters in the graphical illustration.

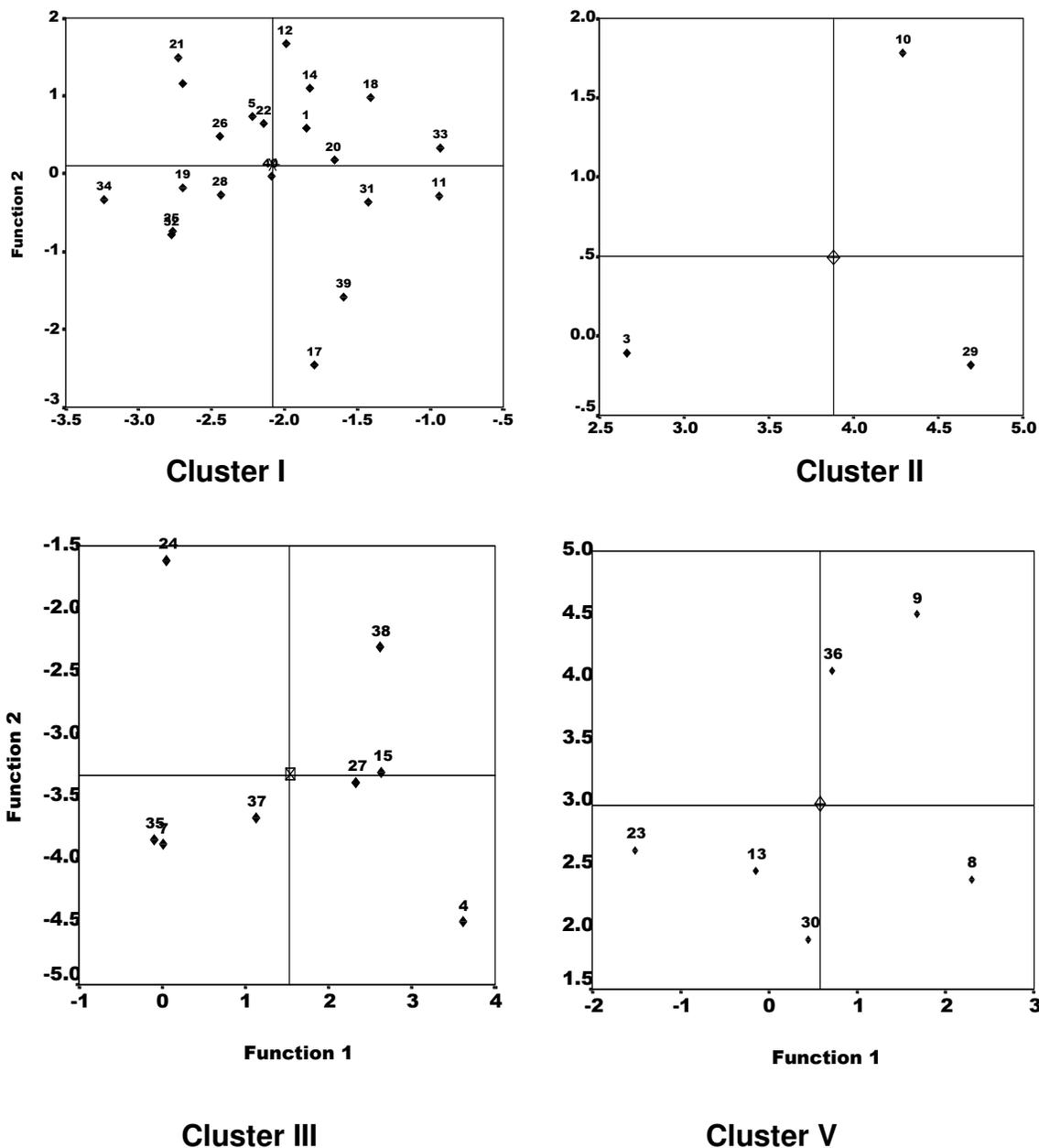
The monogenotypic cluster IV was placed at a distant position from origin containing larger positive values for both functions 1 and 2. The other monogenotypic cluster VI contains extremely high value for thousand kernel weight, the most dominating character under function 1. This single variable decided the position of cluster VI at an extreme right point of X ordinate representing function 1 (Figure 1). The influence of the variables under function 2 was negligible that is, they failed to push down or uplift this cluster in the direction of Y ordinate. Those variables which contributed more in function 1 played more dominant role on the clusters I and II, but had lower influence on the clusters III and V. Consequently, the clusters I and II placed at the considerable distances from the point of origin in the direction of X ordinate. Higher values for governing characters under function 1 placed cluster II to the positive side and low values for those characters placed cluster I to the negative side of X ordinate. The cluster I is characterized by lowest kernel weight, smallest kernel length and lower L/B ratio under function 1. Function 2 governed by moderately low elongation ratio and moderately high amylose content created smaller effects on these two clusters. Figure 1 also showed that the cluster I was distributed on both the sides of Y ordinate. It is an indication of greater dispersion of genotypes within the group. The cluster III and V are situated on the negative and positive side, respectively of the Y ordinate representing function 2. These two clusters keep very closeness with the Y ordinate, indicating minimum influence of function 1 on them. The lowest values for elongation ratio and amylose contents under function 2 decided the position of cluster III to the negative side of Y ordinate and moderate higher values for these variables pushed the cluster V to the positive side. It should be noted that the graphical illustration could not provide any indication about the best cluster, rather it indicated the effect of variables contributing to function 1 and function 2 on the genotypes under each cluster. Therefore, the selection of groups would be dependent mainly on the objectives of the plant breeders in the breeding programme.

In further step-wise DFA, an effort has been made to explore genotypes that better represent their respective clusters. Each cluster was magnified in a separate graph and two reference lines for X and Y ordinates were drawn

through the group centroid (Figure 2). The clusters I, II, III and V were presented in the figure, and the clusters IV and VI did not require such an interpretation because of their monogenotypic nature. The relative positions of genotypes indicated the cumulative response of the variables representing function 1 (thousand-kernel weight, kernel length and L/B ratio) and function 2 (elongation ratio and amylose content). Group centroid of each cluster represented the optimum values of function 1 and 2, resulted from the cumulative effects of all genotypes oriented under the cluster, based on their responses to the discriminatory variables. The placement of a genotype at or very near to the group centroid indicated the absence or minimum deviation of the genotype in response to discriminatory variables and thus was considered as the most representative of the group.

Intra-cluster and inter-cluster distances (Mahalanobis  $D^2$ ) were calculated from the step-wise DFA. The clusters IV and VI were monogenotypic. Therefore, intra-cluster distance for each of these two was zero (Table 6). There were marked variations among intra-cluster distances. The highest intra-cluster distance was computed for cluster I (composed of 20 genotypes) and the lowest was for cluster II (composed of three genotypes). The intra-cluster distances in all the six clusters were lower than the inter-cluster distances (Table 6). It indicated a wider genetic diversity among the genotypes of different groups than those in the same group; that is, within group genotypes were closely related. Accordingly, the six clusters had significant distances from each other at  $P_{0.001}$  level. The inter-cluster  $D^2$  value was the maximum between I and VI followed by between I and V. While, the  $D^2$  value was the minimum between the clusters II and V followed by that between IV and VI. These values indicated that the genotypes belonging to cluster I, were extremely diverged from those of cluster VI. Several researchers performed  $D^2$  analysis to identify the distinct clusters on the basis of different quality characters in rice (Ratho, 1984; Wu and Huang, 1988; Prathepha, 1995; Soni et al., 1999; Kandhola and Panwar, 1999; Sharma et al., 2002; Chauhan and Singh, 2003). Importantly, the genotypes belonging to the highly diverged clusters should be used in hybridization programme for obtaining a wide spectrum of variations in the breeding population (Chawdhury, 1994). On the other hand, Bashir (2002) suggested the selection of genotypes belonging to moderate diversity in order to exploit benefits of heterosis. Above all, the selection of genotypes is dependent on the objectives of the breeding programme. Expectations for different combinations of quality characters along with yield and yield attributes should be considered as selection criteria for desired improvement.

In the present study, majority of the local aromatic rice varieties is included in cluster I, which were characterized by lodging susceptible plant with smaller grain size. The cluster III contains several potential varieties viz. Elai,



**Figure 2.** Graphic illustration of genotypes under each group by discriminant function analysis based on grain quality characters.

**Table 6.** Intra- and inter clusteral Mahalanobis distances ( $D^2$ ) between six clusters based on 11 grain quality and nutrition related parameters of 40 rice varieties.

Cluster #	I	II	III	IV	V	VI
I	3.79					
II	12.05***	1.94				
III	17.20***	15.90***	3.76			
IV	16.06***	9.11***	9.15***	0.00		
V	21.28***	6.15***	9.84***	13.99***	3.46	–
VI	26.53***	14.87***	14.89***	7.96***	15.10***	0.00

Sarwati and Sugandha-1 with long-slender kernel and 'very good' appearance (Figure 1 and Table 1). Elai and Sarwati are the varieties with medium plant height and can successfully be used in crossing for the grain type improvement of germplasms in cluster I.

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