RESEARCH ARTICLE

Morphological variation in selected rice (*Oryza sativa* L.) germplasm of Sri Lanka

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Abstract: Genetic improvement of rice for increased grain yield has changed plant height and canopy architecture of the rice (Oryza sativa L.). Under such circumstances, selection based on grain yield only can lead to the loss of other important characteristics of the low-yielding traditional rice varieties. In this context, morphological variation among traditional and improved Sri Lankan rice varieties using important vegetative and reproductive characteristics was studied. Principal component (PC) and cluster analyses were used to assess the patterns of morphological variation. The first five PCs explained over 90% of the total variation. PC1 and PC4 represented vegetative characteristics (i.e. plant height, leaf dry weight, leaf area, stem dry weight), and PC2, PC3 and PC5 represented reproductive characteristics (i.e. number of filled and total grains panicle-1, number of panicles m-2, number of tillers hill-1 and hundred grain weight). PC1 was found to be not important to explain the variability of grain yield among rice varieties in the presence of PC2-PC5. Based on both vegetative and reproductive characteristics, two clusters in each of the improved and traditional variety groups were identified and the existence of those clusters were proved by Wilks' lambda under multivariate analysis of variance (MANOVA). Traditional varieties had a comparatively higher variation in their vegetative and reproductive characteristics. The implications of the results for rice improvement and germplasm conservation are also discussed.

Keywords: Improved and traditional varieties, morphological variation, multivariate methods, rice, vegetative and reproductive characteristics.

INTRODUCTION

Knowledge on the genetic diversity and population structure of germplasm collections is important for crop improvement. Due to the importance of rice (*Oryza sativa* L.) as a major world crop, its origin and diversity has attracted great interest (Thomson *et al.*, 2007).

Rice is grown in tropics under a wide range of physical environments such as, different elevations, soils and hydrological regimes.

In Sri Lanka, approximately 75% of the rice lands are located within the inland valley systems with varying form and size, and the balance 25% are located in coastal plains and associated flood plains (Panabokke, 1996). Out of total cultivated extent around 99% of the area is cultivated with new improved varieties (NIVs). The remaining area is cultivated with traditional rice varieties with low yield (less than 2.5 t ha⁻¹). The average rice yield in Sri Lanka has increased from around 1.3 t ha-1 in the 1950s to 3.1 t ha⁻¹ by the late 1980s (IRRI, 1991). At present the national average rice yield is 4.2 t ha⁻¹, which is 50-60% of the genetic potential (6-7.5 t ha⁻¹) of NIVs recommended for cultivation in Sri Lanka (Dhanapala, 2000; Central Bank of Sri Lanka, 2008). Although NIVs produce comparatively higher yields, local and export market demand for traditional rice varieties is still higher because of their grain qualities, such as high fibre content, despite the lower production (Wickramasinghe and Noda, 2008). Furthermore, farmer's perceptions (Efisue et al., 2008), improvement of system sustainability (Abeyratne, 1956) and the higher adaptability to problem soils (Mandal et al., 1999) further increased interest towards traditional rice varieties.

While the overall population structure of global rice germplasm has been well characterized, more detailed analyses on a regional or country-specific basis have only been recently begun (Thomson *et al.*, 2007). One such example is Sri Lanka; a country with a wealth of biodiversity that is largely untapped. A long history of traditional rice production across diverse environments in Sri Lanka has led to a diverse array of traditional

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rice varieties. However, the relative importance and influence of yield related traits on grain yield have changed over time as a result of rice improvement. Moreover, yield potential of a variety is a hypothetical concept determined by a complex series of interactions with the components of the environment it is exposed to. Although recommendations of crop varieties are usually done on the basis of grain yield (t ha-1), there are other important traits related to grain quality and/or agronomy, which are not related to grain yield (Samita et al., 2005). Under such circumstances, selection based on yield can lead to the loss of these important characters. Therefore, classification using multiple morphological characteristics is important to identify adaptation of a variety and to improve the evaluation of varieties for potential adaptation (Lin and Binns, 1985; Lin et al., 1986; Souza and Sorrells, 1991). At present, there is still a lack of information on morphological diversity, how the diversity has changed with rice improvement and its impact on grain yield of traditional and improved rice varieties in Sri Lanka.

Grain yield of rice is basically determined by the genotype, climatic and edaphic environment, and management (Slafer, 1994; Richards, 2000). Therefore, one way to assess the diversity among rice varieties including both traditional and NIVs is to conduct experiments in favourable environments and assess the diversity. Under this background yield related morphological and physiological traits of sorghum (Sorghum bicolor L.) have also been identified (Ayana & Bekele, 1999). However, there are no reports on such performance comparisons of traditional and improved rice varieties in Sri Lanka.

In the current study yield related vegetative and reproductive characteristics of several traditional and NIVs in Sri Lanka were investigated, under uniform management. The objectives of the study were to (i) determine the extent and patterns of distribution of morphological variation of 13 quantitative traits in 22 rice varieties, (ii) identify important yield related traits and, (iii) identify groups of varieties with similar quantitative characteristics.

METHODS AND MATERIALS

Plant materials: The traditional rice varieties used in this study were Seeragasamba, Wedaheenati, Pachcheperumal, Kaluheenati, Kalubalawee, Rathnawalu, Rathel, Dikwee, Godawee, Suwanda samba and Suduru samba while H4, Bg94-1, Bg250, Bg300, Bg305, Bg352, Bg359, Bg360, Bg450, At303 and Ld356 represented NIVs. Traditional rice varieties used in the research

study are grown in certain parts of the country and the Rice Research and Development Institute (RRDI) at Batalagoda (Bg) maintains and uses these varieties for research and breeding purposes. The NIVs used here are widely cultivated representing different maturity groups ranging from $2\frac{1}{2}$ - $4\frac{1}{2}$ months.

Land preparation and experimental design: Field experiments were conducted during September to February (Maha), 2005/06 cropping season at the University Experimental Station at Maha-Illuppallama (latitude 8.117° N and longitude 80.467° E with a height above MSL 137-m). Average ambient temperature during the cropping season was 29 °C and the soil was reddish brown earth (RBE). The soil was puddled and levelled, and plots of size 4 m long and 2 m wide were arranged in a randomized complete block design with 3 replications. Seedlings were raised in a Dapog nursery for 10 d and 3 seedlings hill-1, 2-3 cm apart, were transplanted in field plots with 20 × 15 cm inter and intra row spacing, respectively. Guard rows of 0.5 m of minimum width were maintained around each plot with a variety. Excess seedlings were thinnedout, at one to two weeks after transplanting, to achieve a uniform density of 2 seedlings hill-1 (67 seedlings m-2). Manual weeding was done at regular intervals and the competition from weeds was kept minimal. Approximately 5 cm of permanent standing water level was maintained throughout the experiment. Fertilizer was applied at the time of field planting (basal dressing), tillering and heading stages (top dressings) according to the Department of Agriculture recommendations. Long-age class (4 - 4.5 months) NIVs fertilizer recommendation was used for traditional varieties, as there were no special recommendations.

Data collection: Data were collected at heading and at harvest of each variety. Plant height (height from the base of the culm of the plant to the tip of the furthest leaf in cm), leaf area hill-1 (cm²), leaf blade dry weight (DW) hill-1 (g) and stem (including stalk and leaf sheathes) DW hill-1 (g) were collected at the time of heading for each variety. Number of tillers hill-1, number of panicles m² (panicle density), number of filled and unfilled grains panicle-1, hundred-grain weight (g), stem DW hill-1 (g), leaf blade DW hill-1 (g) and number of total grains panicle-1 were collected at the time of harvest for each variety. Plant age (days) at harvest was measured as the days from time of planting to time of maturity.

On each occasion sampling was done using 9 selected plants (3×3 adjoining rows and columns representing a square) within the inner rows of each plot. For destructive sampling, plants were dug and washed to remove adhering soil. Within each hill the number of tillers was counted and then the sample was subdivided

into stem, leaf blade and panicle. Leaf blades were used to determine the leaf area using a LiCor LI-3000 portable area meter, which was equipped with LI-3050A transparent belt conveyer accessory (LiCor Biosciences Nebraska, USA). Plant parts were kept in a cold-water bath to minimize wilting and respiration during sampling and separation process (Samonte et al., 1998). Partitioned plant samples were stored in a ventilated drying oven at 70 °C for a minimum of 48 h or until a constant weight was reached for DW measurements. Panicles were hand threshed. Filled, unfilled and total grain number panicle-1 was counted after allowing seeds to float or sink in a water container. As heading and maturity of rice varieties did not coincide due to the time of planting being same for all the varieties, estimates were done at different time periods corresponding to their maturity.

Statistical analyses: The data were standardized to a mean zero and a variance of unity to avoid differences in scales used for recording data on the different characters (Sneath and Sokal, 1973) before undertaking a series of multivariate analyses using the appropriate procedures in SAS computer programme (SAS Institute, 1998).

Principal component (PC) analysis was performed with correlation matrix using PROC PRINCOMP to define the patterns of variation between all the explanatory variables excluding the grain yield. Grouping of variables into PCs were noted and thereby the dimension of the data set reduced. In order to find the relationships between those PCs and the grain yield, regression analysis was performed with the "stepwise" selection approach and the selected model is presented.

Then, the 22 varieties were clustered using PROC CLUSTER of SAS, which grouped and sorted the closely related varieties into clusters, using the first five PC scores of varieties. Measure of dissimilarity was the Euclidean distance and the clustering method was COMPLETE linkage. The number of clusters was determined in such a way that a cluster should contain at least two varieties so as to allow subsequent statistical analysis. The relationships among the clusters were assessed by measuring the intercluster distances using Mahalanobis distance. Once the clusters of rice varieties were identified significance of those clusters were determined using the multivariate analysis of variance (MANOVA) procedure in SAS.

Table 1: Means of different characteristics of different rice varieties at the time of heading

Variety	Plant height (cm)	Stem DW (g plant ⁻¹)	Leaf DW (g plant ¹)	Leaf area (cm²plant¹)
Dikwee	133.3	22.2	7.1	1715
Godawee	140.8	30.1	11.1	2358
Kalubalawee	124.5	13.5	5.7	1468
Kaluheenati	123.8	21.9	6.9	1590
Pachcheperumal	119.5	14.3	4.8	1150
Rathel	116.8	15.4	6.5	1955
Rathnawalu	147.5	17.1	5.5	1300
Seeraga samba	118.3	9.9	4.3	1203
Suduru samba	156.0	28.2	8.2	1573
Suwanda samba	137.0	26.5	10.1	1995
Wedaheenati	114.0	12.1	5.1	1400
H4	127.8	10.3	5.1	1298
At303	86.8	13.5	4.5	1023
Bg94-1	83.3	15.3	5.1	1568
Bg250	94.8	8.4	4.0	1033
Bg300	95.5	13.5	5.4	1490
Bg305	95.0	27.0	7.2	1858
Bg352	92.8	18.5	6.5	1770
Bg359	85.8	17.0	5.0	1150
Bg360	96.0	21.0	7.9	2048
Bg450	96.5	21.2	7.0	1605
Ld356	78.3	10.0	3.9	995
LSD(α=0.05)	17.9	9.2	3.1	667

n = 6

RESULTS

Morphological characteristics at heading and maturity

Plant height, stem DW, leaf DW and leaf area of each variety measured at the time of heading are given in Table 1. A wide variation of these characteristics across species was found. All traditional varieties were taller than 114 cm at heading while that of NIVs was less than 100 cm. Godawee, Suduru and Suwanda samba had very high values of stem DW, leaf DW and leaf area while those of Bg250 and Ld356 were the lowest. Characteristics measured at the time of maturity are given in Table 2 for each variety. The traditional varieties needed more than 115 days for their maturity while all the NIVs needed less than 105 days, except for Bg450 where it needed 125 days. Apart from the age, all other characteristics were similar between traditional and NIVs groups. However, within each group for each variable a huge variability was present (Table 2).

Extent and pattern of distribution of morphological variation

The first five PCs explained over 90% of the total variation of the 13 quantitative traits studied (Table 3). The PC1 and PC2 accounted for 35% and 23% of the total variation, respectively. All the explanatory variables, except for the number of unfilled grains panicle⁻¹ and plant age, loaded within the first five PCs (Table 4). Therefore, PC1 and PC4 explained the insights of vegetative (leaf and stem) characteristics, while PC2, PC3 and PC5 explained reproductive (yield components) characteristics.

Principal component plot of PC1 and PC2 is given in Figure 1. Traditional varieties obtained higher, positive PC1 scores, while NIVs obtained low, negative PC1 scores, which reflect the differences in plant height, leaf DW, leaf area and stem DW at harvest (Table 4). Among traditional varieties Godawee and Suwanda samba had PC1 scores over 4, while Seeraga samba and Pachcheperumal had negative scores. All NIVs obtained

Table 2: Means of different characteristics of different rice varieties at the time of harvesting

Variety	Age (days)	Number of tillers (hill-1)	Stem DW (g plant ⁻¹)	Leaf DW (g plant ⁻¹)	Number of panicles (m ⁻²)	Number of filled grains (panicle-1)	Number of unfilled grains (panicle-1)	Hundred grain wt (g)	Number of total grains (panicle ⁻¹)
Dikwee	125	19	9.9	3.2	157	166	18	2.0	185
Godawee	130	18	23.0	8.4	160	117	6	2.8	123
Kalubalawee	125	15	12.2	4.1	140	102	6	2.6	108
Kaluheenati	125	20	18.6	6.0	216	81	10	2.5	91
Pachcheperumal	115	15	11.3	4.2	164	155	7	2.9	162
Rathel	130	16	18.3	6.2	159	117	38	2.9	155
Rathnawalu	125	12	16.1	3.7	103	129	5	2.9	134
Seeraga Samba	130	11	13.3	4.9	144	142	30	1.2	172
Suduru	126	10	15.8	6.0	133	120	16	2.7	136
Suwanda Samba	125	17	22.6	7.9	199	155	15	2.7	170
Wedaheenati	130	16	17.9	6.0	203	91	3	2.5	94
H4	120	9	15.1	5.5	201	122	23	3.0	144
At303	100	13	9.5	4.4	184	142	15	2.9	156
Bg94-1	105	29	10.1	3.7	225	116	5	2.9	121
Bg250	85	15	12.1	4.5	204	85	27	2.7	112
Bg300	95	17	10.5	5.0	191	181	41	2.7	223
Bg305	100	19	9.4	4.6	176	165	25	2.4	190
Bg352	105	17	15.0	5.6	169	144	31	2.6	175
Bg359	105	18	12.5	5.2	187	151	8	2.8	159
Bg360	105	23	14.3	6.0	300	144	13	1.5	158
Bg450	125	19	15.8	5.8	215	227	38	1.3	264
Ld356	105	15	9.9	3.3	272	139	16	2.2	156
LSD(α=0.05)	13	2.9	6.4	1.4	66	45	9	1.1	16

low PC1 scores while, short-medium duration NIVs such as Bg250, Bg300, At303 and Ld356 had the lowest PC2 scores.

Table 3: Eigen values and cumulative percentage variance explained by first five principal components (PCs)

PC	Eigen value	Cumulative % variance explained
1	4.59	35.3
2	3.06	58.9
3	2.04	74.6
4	1.06	82.7
5	0.97	90.2

Table 4: Loading of vegetative and reproductive characteristics into principal components (PCs) with scores

PC	Variable	PC score
1	Plant height (cm)	0.34
	Leaf blade DW at heading (g)	0.42
	Leaf area at heading (cm ²)	0.37
	Stem DW at harvesting (g)	0.40
2	Number of filled grains panicle-1	0.47
	Total grains panicle-1	0.49
3	Number of tillers hill-1	0.55
	Number of panicles m ⁻²	0.48
4	Stem DW at heading (g)	0.39
5	Leaf blade DW at harvesting (g)	0.39
	Hundred grain weight (g)	0.54
6	Number of unfilled grains panicle-1	-0.53
7	Plant age (days)	0.47

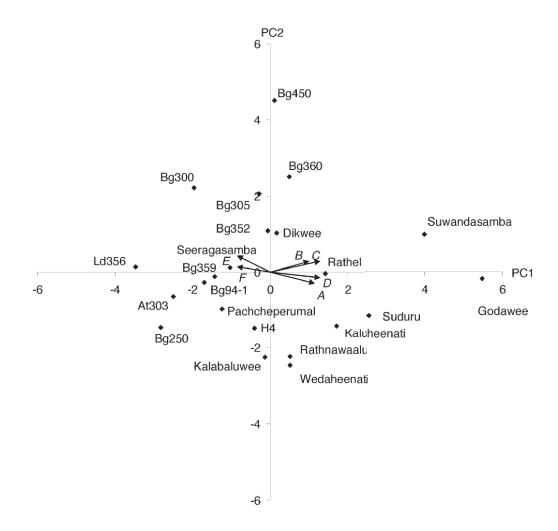


Figure 1: Score plot of PC1 versus PC2 of rice varieties based on vegetative and reproductive characteristics. The vectors represent the variable loadings; *A*- plant height, *B*- leaf area at heading, *C*- leaf DW at heading, *D*- stem DW at harvesting, *E*- total grains panicle⁻¹ and, *F*- filled grains panicle⁻¹.

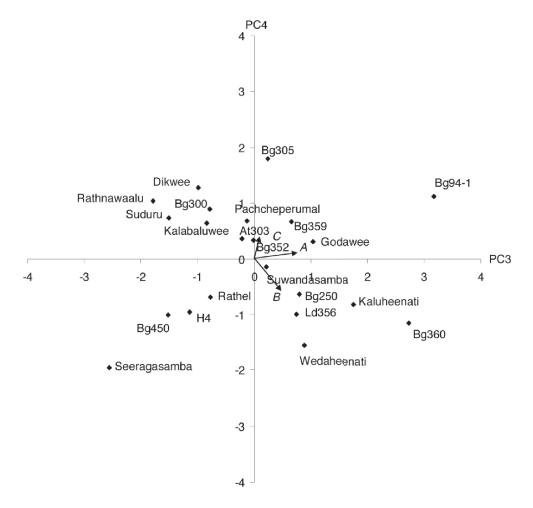


Figure 2: Score plot of PC3 versus PC4 of rice varieties based on vegetative and reproductive characteristics. The vectors represent the variable loadings; *A*- number of tillers hill⁻¹, *B*- number of panicles m⁻² and, *C*- stem DW at heading.

Extreme positive PC scores of PC2 were obtained by Bg450 and then Bg360, Bg300 and Bg305 (Figure 1), reflecting the higher number of total as well as filled grains panicle⁻¹ (Table 4). Among traditional rice varieties Dikwee and Suwanda-samba had high positive PC2 scores. In the opposite direction most of the traditional varieties such as Rathnawalu, Kalubalawee and Wedaheenati had PC2 scores even less than -2. Apparently, the PC2 differentiated varieties with respect to the number of total or filled grains panicle⁻¹ (Table 4).

When PC3 and PC4 were plotted, Bg360 and Bg94-1 had relatively high positive PC3 scores compared with other improved varieties while Kaluheenati, Wedaheenati and Godawee had higher scores compared with other traditional varieties (Figure 2). Furthermore, Seeraga samba, Suduru and Rathnawalu obtained negative PC3 scores among traditional varieties, while Bg450 had the

lowest scores compared with other NIVs. With respect to PC4, Dikwee, Rathnawalu and Pachcheperumal had higher scores among the traditional rice varieties, while Wedaheenati and Seeraga samba obtained very low scores. On the other hand Bg305 had higher PC4 scores while Bg450 obtained lower PC4 scores compared with other NIVs. PC4 differentiated varieties based on stem DW at heading (Table 4) and both traditional varieties and NIVs had a higher variability among them (Figure 2).

PC5 mainly represented the leaf DW at final harvest and the hundred grain weight. NIVs such as Bg250, Bg300 and Bg352 obtained higher scores while mediumlong-age class NIVs such as Bg360 and Bg450 had lower scores. Among traditional varieties, Dikwee had the lowest PC5 scores, while Rathel had highest positive scores (data not shown).

Important yield related traits

Regression analysis of grain yield with PC scores as explanatory variables, across all the rice varieties, revealed a significant relationship ($F_{(4,18)}$ =7.22, p<0.001). Among the first five PCs, only PC2-PC5 were found to be important to explain the variability of grain yield. The intercept of the model was not significant. Summary of the reduced final model is given in Table 5. The model explained 61% of the total variability of grain yield (R^2 =0.61). As PCs were independent ($cov\ PC_i\ PC_j$ =0) interaction terms were not included. Therefore, the grain yield of rice varieties increased with the increase of PC2, PC3 and PC5 (reproductive characteristics) and decreased with the increase of PC4 (stem DW at heading) (Table 5).

Table 5: Parameter estimates and the significance of principal components (PCs) during the regression analysis

PC	Estimate±s.e.	t	p> t
PC2	0.16 ± 0.07	2.1	0.04
PC3	0.37 ± 0.10	3.6	0.00
PC4	-0.32 ± 0.14	-2.2	0.04
PC5	0.40 ± 0.15	2.7	0.01

Grouping of varieties

The 22 rice varieties were grouped into 4 clusters (Figure 3) at a normalized maximum distance of 1.47. Number of varieties per cluster varied from 2 varieties in last cluster to 9 varieties in the second cluster. Clusters I and III contained NIVs (formed at a normalized maximum distance of 0.90 and 1.15, respectively). Furthermore, cluster I contained NIVs from three major rice breeding centres of Sri Lanka (At-Ambalanthota, Bg-Batalagoda and Ld-Labuduwa) while cluster III contained NIVs only from Batalagoda. Traditional variety Pachcheperumal clustered with the NIVs within the cluster I at a distance of 0.41. Clusters II and IV contained traditional rice varieties (formed at a normalized maximum distance of 1.19 and 0.46 respectively). The old improved rice variety H4 grouped with the traditional variety Rathel under the cluster II at a distance of 0.52. The two traditional rice varieties, Suwanda samba and Godawee grouped separately from other traditional variety cluster and also clustered at last with other clusters at a normalized maximum distance of 1.97. The varieties first formed into a group were At303 and Bg359 at a normalized maximum distance of 0.33. The shortest age improved variety, Bg250, clustered with other improved rice varieties in cluster I at a normalized maximum distance of 0.60. A

clear differentiation of rice varieties into two traditional and two improved variety clusters were apparent.

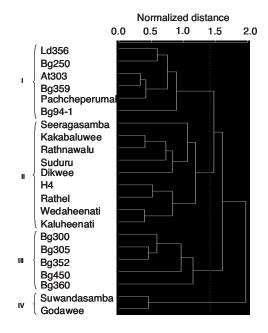


Figure 3: Dendrogram of rice varieties obtained through complete linkage cluster analysis based on 13 vegetative and reproductive characteristics. Dashed line indicates the normalized distance where clusters were identified and I-IV denotes the clusters.

Significance of clusters of varieties

Subsequent MANOVA analysis of the four rice variety clusters as groups against the 13 variables studied produced significant Wilks' lambda statistics of 0.00093 ($F_{42,15.598}$ =3.53, p<0.005). Furthermore, first three characteristic roots were 18.4, 11.7 and 3.3 explaining 55%, 35% and 10% of the total variability, respectively. Therefore, four groups of rice varieties generated through the cluster analysis, considering vegetative and reproductive characteristics, are truly different.

DISCUSSION

Results of the study revealed that the vegetative and reproductive characteristics can be effectively used to group rice varieties and visualize the existence of natural groups. Although rice varieties are broadly categorized into traditional and improved, a wide variability in age, vegetative structures, yield components and grain yield were still observable within those groups (i.e. out of 13 variables studied only the plant height and plant age at

maturity were different between traditional and NIVs while all other characteristics were similar and highly variable within each group) (Tables 1 and 2). Traditional rice varieties used in the study could be grouped into two clusters based on both vegetative and reproductive characteristics. However, most of the traditional varieties were grouped into cluster II. Varieties from cluster II had lower scores for the PC1 and PC2, representing lower leaf and stem masses during both heading and maturity stages, less total and filled grains panicle-1 compared to traditional varieties from cluster IV. Comparatively lower stem and leaf DW reflect smaller plant vegetative structures, which would result in a lower potential grain yield, unless those varieties have acquired efficient and adaptive physiological mechanisms such as ideal canopy architecture and/or dry matter partitioning to produce higher grain yield. Therefore, traditional varieties used in the experiment could easily be differentiated into two clusters, basically by PC1 and PC2.

NIVs from cluster I had low PC1 and PC2 scores and were lower than that of the NIVs from cluster III. Therefore, varieties from cluster I were characterized by lower leaf and stem DW, at both heading and maturity stages, as well as lower number of grains panicle-1. Higher PC2 scores for the NIVs from cluster III revealed their potential to produce bigger panicles with a large number of grains. Therefore, rice varieties widely grown in the country, with higher yield potential, are represented by cluster III. Furthermore, most of the NIVs had lower scores for PC1 than traditional varieties reflecting the changed architecture and structure (i.e. ideal plant type) achieved through rice improvement. Also, NIVs from cluster I had positive scores close to one for the PC3 indicating their lower variability of tiller and panicle density compared to the NIVs from cluster III.

When important yield related traits were studied, PC1 was not important to explain the variability of grain yield in the presence of other PC's. As explained earlier, PC1 was reduced and PC2 was increased with rice improvement. Therefore, the regression model contained only the yield related characteristics of rice (PC2-PC5) (Table 5). Similar relationships of changes in plant vegetative characteristics and grain yield of rice through path analysis approach has also been reported (Suriyagoda *et al.*, 2006).

The greater discrimination power of rice varieties in to distinct groups observed through the cluster analysis and subsequent MANOVA clearly indicates the greater importance of morphological factors (both vegetative and reproductive) in discriminating Sri Lankan rice varieties. This information may have several implications such as determining varieties to be used in rice improvement programmes and in maintaining germplasm collections. When crossing varieties belonging to different clusters of wider Mahalanobis distance, opportunities for transgressive segregation could be maximised. Therefore, there is a higher probability that unrelated genotypes from distant clusters would contribute unique desirable alleles at different loci (Kanwal et al., 1983; Shamsuddin, 1985; Peeters and Martinelli, 1989; Souza and Sorrells, 1991; Beer et al., 1993). When considering germplasm collections a representative collection should consist of the genetic diversity of Sri Lankan rice varieties with a minimum repetitiveness. This is particularly important since the size of the gene banks of major crops continue to grow both nationally and internationally (Brown, 1989a, b; Frankel and Brown, 1984). Therefore, data from the present study together with data from genetic characterization can be helpful, at least partly, to select core collections for rice. Also this would help to define strategies for further collection.

In Sri Lanka the highest potential for rice productivity is found in the low country, dry and intermediate zones, where solar radiation and other climatic parameters are conducive and the edaphic environment is relatively favourable (Dhanapala, 2000). Since the present experiment was conducted in this major rice growing region results could be widely applicable.

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