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Existing Status and Genetic Divergence of Agro-Morphological Characters in Bangladeshi Fine Rice

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ABSTRACT

A set of 30 rice (*Oryza sativa* L.) cultivars including 28 primitives, one exotic and one improved variety were collected from different agro-ecological zones of Bangladesh. The experiment was conducted in a randomized complete block design with three replications during the wet season (July-December) of 2009 at the Plant Breeding Field Laboratory, Hajee Mohammad Danesh Science and Technology University, Dinajpur. The existing status regarding cultivation and preservation attitude were realized through an oral interview with 20 randomly selected farmers of each location from where a particular cultivar was collected. Four cultivars ('Kalozira', 'Badshabhog', 'Zethakatari', 'Katari') were most preferred by innovative farmers due to their use as food and in different traditional and religious festivals. Cultivars such as 'Shilkamal', 'Binnipakri', 'Darkashail', 'Dudshar', 'Lalchikon' and 'Uknimodhu' were rarely cultivated, hence the need to preserve them. The 30 cultivars were grouped into 7 clusters through principal component analysis and D^2 statistics based on 12 agro-morphological characters. Cluster VI comprising 'BR34' and 'Darkashail' produced the highest yield (4.05 t/ha). Days to maturity was generally high in all 7 clusters and ranged from 112.7-132.3 days; no cultivars matured slowly. Therefore, the development of rapidly maturing varieties by conventional breeding is assumed to be very difficult in fine rice. Among the 12 characters, spikelets/panicle exerted maximum weighting (20.37%) on total genetic divergence while percentage sterility had the least weighting (3.58%). Since highest intercluster distance (651.88) was observed between clusters III ('Malshira', 'Chiconsarna', 'Lalchicon', 'Radhunipagal', 'Nazirshail', 'Lalfota', 'Sumonsarna') and VI ('BR34', 'Darkashail'), parents could be selected from these two clusters to obtain desirable segregants in the F_2 generation. Alternatively, one parent from each cluster could be selected to initiate a diallel cross to assess the best cross combination and to identify those parents with high general combining ability for improvement.

Keywords: breeding exercise, D^2 statistics, farmers' attitude, improvement, land race
Abbreviations: BRRI, Bangladesh Rice Research Institute, **GCA**, general combining ability; **PCA**, principal component analysis

INTRODUCTION

Rice is the world's most important crop and a major source of nutrition for about two-thirds of humans (Vaughan *et al.* 2003) and it is the staple food consumed by more than half of the world population, three billion of which are Asian (Waghmare *et al*. 2008) and more than 90% of the world's rice is grown and consumed in Asia where 60% of the earth's population live (Khush 1997). In Bangladesh, rice is extensively cultivated over a large area covering about 74% of the total human calorie intake; about 10.77 million ha of land is used for rice cultivation, which produces 25.18 million metric tons of rice (Latif *et al.* 2011). For most developing countries, rice is the basis of food security and is intimately associated with traditional cultures and customs in local regions (Lu and Snow 2005).

Fine rice may be aromatic or non-aromatic; when the grain is large, the length will exceed the breadth considerably but when the grain is small, it may be any type either small or slender. Independent of the grain type, all rice grain has a high market price and socio-cultural importance in Bangladesh. Fine rice production has been significantly increased in recent years, reaching the maximum level of quality and fetching a much higher premium (>$1/kg) than coarse rice. The coarse rice grain is bold and large having a lower market price (<$0.5/kg). Bangladesh rice breeders are keen to understand the genetic diversity of fine rice cultivars so as to be able to improve them. Most of the fragrant rice cultivars are traditional but none of them is 'Basmati', which has a tall stature, low yield, insensitive to photoperiod, susceptible to diseases and pests and unresponsive to higher doses of fertilizers (Rani 2012). In Bangladesh, there is a wide range of land races with unique flavor and other attractive grain characteristics such as distinct grain texture, endosperm chalkiness, non-stickiness of cooked rice, etc. (Hosan *et al.* 2010). Thus, there is a wide scope in breeding programmes to improve fine rice to fulfill divergent demands of farmers and consumers regarding food security, changing palatability, nutrition supply, mitigation of risk due to sudden failure of modern or hybrid varieties, good economic return, uses in socio-cultural programs, among others (Hien *et al*. 2007). This study takes one step closer to creating such programmes.

Agriculture relies heavily on the genetic diversity of crop plants. Ever since the very beginning of agriculture (more than 10000 years ago), through the process of domestication and acclimatization of crop plants, a wealth of genetic diversity was selected and partly preserved, although it is estimated that not even 15% of the potential diversity has been utilized. Thousands of valuable allelic variations of economically significant traits remain unattended in nearly all crop plants which could be effectively explored to meet the existing and emerging challenges that threaten world food security (FAO 2002). Estimating genetic diversity and similarity among genotypes of any species has various applications in plant breeding. A population with numerous diverged genotypes plays a key role in the success of any breeding program since the parents involved

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in a particular cross should be genetically divergent. Even though rice is a self pollinated crop, there is every possibility of genetic variation among the parents collected from different eco-geographical regions (Vennila *et al.* 2011). Moreover, estimation of genetic diversity among different genotypes in a crop of interest is the first and foremost process in any plant breeding program (Rajesh *et al.* 2010) and genetically diverse parents produce desirable recombinations in segregating generations (Banumathy *et al.* 2010). Genetic diversity in plant genotypes can be estimated by observing and measuring agro-morphological characters since these characters are important for describing plants and are influenced by consumers' preferences, the socio-economic reality and natural selection (Kurlovich 1998).

By 2050 the world's population is estimated to reach 9.1 billion, 34% higher than today. Nearly all of this population increase will occur in developing countries. Urbanization will continue at an accelerated pace, and about 70% of the world's population will be urban (compared to 49%; FAOSTAT 2009). Income levels will be many multiples of what they are now. In order to feed this larger, more urban and richer population, food production (net of food used for biofuels) must increase by 70%. Annual cereal production will need to rise to about 3 billion tones from 2.1 billion today (FAOSTAT 2009). With an increase in global population and predicted declines in water availability, a decrease in arable land, the constant battle against new emerging pathogens and possible adverse effects from climate change along with the threat of burgeoning biofuel industries to migrate food crops to renewable energy sectors will bring great challenges for rice breeders and agricultural scientists to breed high premium cereals by exploiting currently available fine rice cultivars. The term "fine rice" is very popular among Bangladeshi, and although the origin of the term is unknown, fine rice represents a special group with best qualities related to consumer preference and nutritional properties which will ultimately influence the preparing of meals (Shahidullah *et al.* 2009).

The development of modern rice cultivars over the past three decades concentrated on high yield, short stature, erect leaves, high tillering, sturdy stems, early maturing and fertilizer responsiveness. These high-yielding improved varieties have replaced many traditional cultivars in farmers' fields but the varieties are imperfect in the long run due to reduction of yield potential and susceptibility to pest and diseases. Understandably, if high-yielding varieties share a common ancestor, this could reduce genetic diversity (Caldo *et al.* 1996). Local landraces represent an intermediate stage of domestication between a wild ancestor and modern varieties and serve as reservoirs of genetic variation. Such genetic variation is influenced both by natural processes such as selection and drift, and also by agricultural practices of local farmers (Pusadee *at el.* 2009). Rice scientists have an obligation to conserve divergent landraces; the first step towards achieving this is by studying the existing status and genetic divergence based on agro-morphological characters. Therefore, accurate assessment of genetic divergence is indispensable in fine rice breeding for the following multifarious applications:

1. Analysis of genetic variability in landraces and cultivars (Cox *et al.* 1986).
2. Identification of divergent parental combinations to create segregating progenies with maximum genetic variability for further selection (Barret and Kidwell 1998).
3. Introgressing desirable genes from diverged germplasm into the available genetic base (Thompson *et al.* 1998).

Therefore, this study describes the existing status and genetic divergence of agro-morphological characters in Bangladeshi fine rice.

MATERIALS AND METHODS

The experimental materials comprised 30 fine rice cultivars including one exotic and one improved variety (**Table 1**). The field layout was a randomized block design with three replications. The unit plot size was 2 m × 3 m and spacing was 20 cm × 20 cm. All the evaluated cultivars, except for 'BR34', are photosensitive and cannot be grown in the dry season even with irrigation, so the experiment was conducted in the Plant Breeding Field Laboratory at Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh during the wet season (July-December) of

Table 1 Different fine rice cultivars with their accession numbers and existing status.

Accession no.	Cultivar name	Place of collection	Cultural use	Status
FR 1	Zirashail	Sadar, Dinajpur	Family tradition	Cultivated
FR 2	Najirshail	Sadar, Kurigram	Food and marketing	Occasionally cultivated
FR 3	Philippine katari (exotic)	Birol, Dinajpur	Food and marketing	Cultivated
FR 4	Kalosuru	Sadar, Dinajpur	Festival	Occasionally cultivated
FR 5	Salna	Patnitola, Naogaon	Food and marketing	Cultivated
FR 6	Ranjit	Fulbari, Dinajpur	Food and marketing	Widely cultivated
FR 7	Chiconsarna	Sadar, Naogaon	Festival and marketing	Widely cultivated
FR 8	Paijam	Fulbari, Dinajpur	Food and marketing	Cultivated
FR 9	Shilkamal	Fulbari, Dinajpur	Family tradition	Rarely cultivated
FR 10	Binnipakri	Chirirbondar, Dinajpur	Family tradition	Rarely cultivated
FR 11	Shadakatari	Chirirbondar, Dinajpur	Festival and marketing	Cultivated
FR 12	Shitabhong	Gournadi, Barisal	Festival	Occasionally cultivated
FR 13	Zira	Birol, Dinajpur	Family tradition	Cultivated
FR 14	Kalozira	Sadar, Narail	Food and festival	Cultivated
FR 15	Lalfota	Dacop, Khulna	Family tradition	Occasionally cultivated
FR 16	Darkashail	Sadar, Natore	Family tradition	Rarely cultivated
FR 17	Badshabhog	Chirirbandar, Dinajpur	Food and festival	Cultivated
FR 18	BR 34 (improved)	BRRI, Joydebpur	Food and marketing	Widely cultivated
FR 19	Dudshar	Poba, Rajshahi	Family tradition	Rarely cultivated
FR 20	Begunbichi	Dumki, Patuakhali	Food and marketing	Cultivated
FR 21	Sumonsarna	Raninagar, Naogaon	Food and marketing	Cultivated
FR 22	Moulata	Boda, Panchagarh	Family tradition	Occasionally cultivated
FR 23	Zetha katari	Birol, Dinajpur	Food and festival	Cultivated
FR 24	Lalchicon	Avaynagar, Jossore	Family tradition	Rarely cultivated
FR 25	Malshira	Fulbari, Dinajpur	Family tradition	Occasionally cultivated
FR 26	Chinigura	Ulipur, Kurigram	Festival and marketing	Cultivated
FR 27	Rajshahisharna	Sadar, Rajshahi	Food and marketing	Cultivated
FR 28	Uknimodhu	Chilmari, Kurigram	Family tradition	Rarely cultivated
FR 29	Radhunipagal	Birol, Dinajpur	Festival and marketing	Cultivated
FR 30	Katari	Sadar, Dinajpur	Food and festival	Cultivated

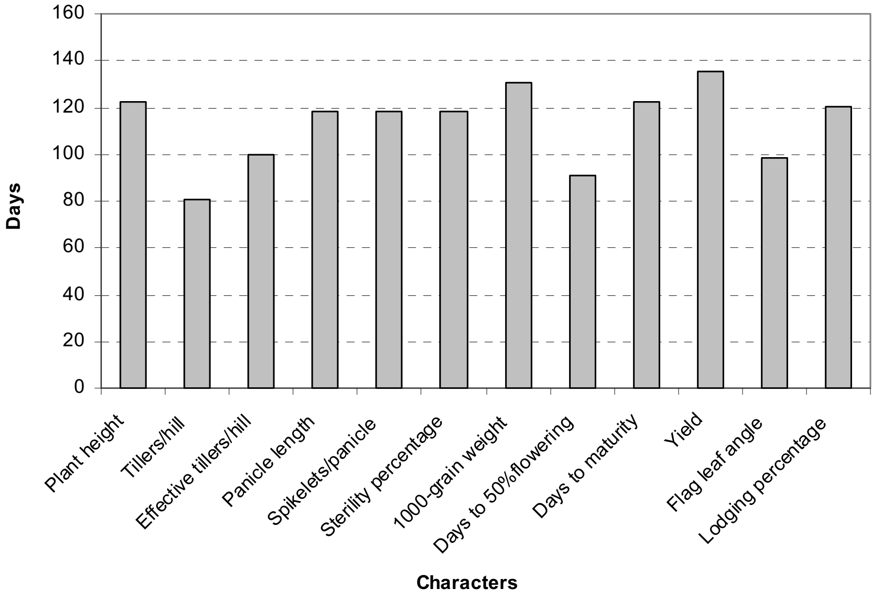

Fig. 1 Mean number of days required to assess 12 characters of fine rice.

2009. Recommended agronomic practices and plant protection measures followed the recommendations of the BRRI rice production manual to raise a healthy crop for a good harvest. The following parameters were observed: plant height (cm) (122.5 days after sowing (DAS)), number of tillers/hill (80.56 DAS), number of effective tillers/hill (100 DAS), panicle length (cm) (118.6 DAS), number of spikelets/panicle (118.6 DAS), sterility percentage (118.6 DAS), 1000-grain weight (g) (130.5 DAS), days to 50% flowering (91.15 DAS), days to maturity (122.5 DAS), yield (t/ha) (135.5 DAS), flag leaf angle (°) (98.4 DAS) and lodging percentage (120.3 DAS). The number of days required to assess all characters is depicted in **Fig. 1**.

Sterility percentage =
(total number of sterile grains)/(total number of grains) × 100

The existing status regarding cultivation and farmers' presservation attitude of the collected cultivars were realized through an oral interview from randomly selected farmers in each location of collection. 20 randomly selected farmers were asked to provide reliable information about the cultivar collected from their locality.

Initially, data were subjected to principal component analysis to group the cultivars into different clusters using non-hierarchical classification (Rao 1964) and then computed from the covariance or correlation matrix and genotype scores obtained from the first stage which has the properly accounting for maximum variance and succeeding components with latent roots greater than unity (Jeger *et al*. 1983). Genetic divergence based on 12 characters was computed using GENSTAT 5.5 software program and the cultivars were clustered by using hierarchical classification through a covariance matrix. Wilk's criterion is a simultaneous test of differences between mean values of a number of correlated variables. The intra- and intercluster distances were measured following the D^2 statistics proposed by Mahalanobis (1936):

$$P^{D^2} = Wij(\bar{x}^1 i - \bar{x}^2 i)(\bar{x}^1 j - \bar{x}^2 j)$$

where
P^{D^2} = genetic divergence between two cultivars;
Wij = the inverse of estimated variance and covariance matrix;
xi and xj = multiple measurements available for each cultivar.

A measure for group distance based on multiple characters was performed by clustering the cultivars according to Singh and Chaudhary (2010). The various steps involved in the estimation of D^2 values are:

a) Collection of data;
b) Test of significance;
c) Transformation of correlated variables;
d) Computation of D^2 values.

RESULTS AND DISCUSSION

The status and preservation attitude of the cultivars in this study were assessed by an oral interview of 20 randomly selected farmers *in loco* for a particular cultivar from where it was collected. Out of 30 cultivars screened, 3 were widely cultivated, 15 were cultivated, 6 were occasional and another 6 were rarely cultivated (**Table 1**). Therefore, 'Shilkamal' 'Binnipakri', 'Darkashail', 'Dudshar', 'Lalchicon' and 'Uknimodhu' were under rare cultivation and should be conserved at least by community-based farmers. Among the cultivars, none was cultivated only for marketing but 'Kalosoru' and 'Shitabhog' were occasionally cultivated for traditional and religious festivals. 'Nazirshail', 'Philippinekatari', 'Salna', 'Paijam', 'BR34', 'Begunbichi', 'Sumansarna' and 'Rajshahisarna' were cultivated for food and economic returns; therefore, these cultivars are beyond the risk of erosion. Overall, 'Kalozira', 'Badshabhog', 'Zethakatari' and 'Katari' are very popular, irrespective of farmers' categories because these are predominantly popular both for food and festivals.

The sum total effects of 12 characters were tested by Wilk's criterion, indicating highly significant differences among the cultivars for the characters (Ahmed *et al.* 2010).

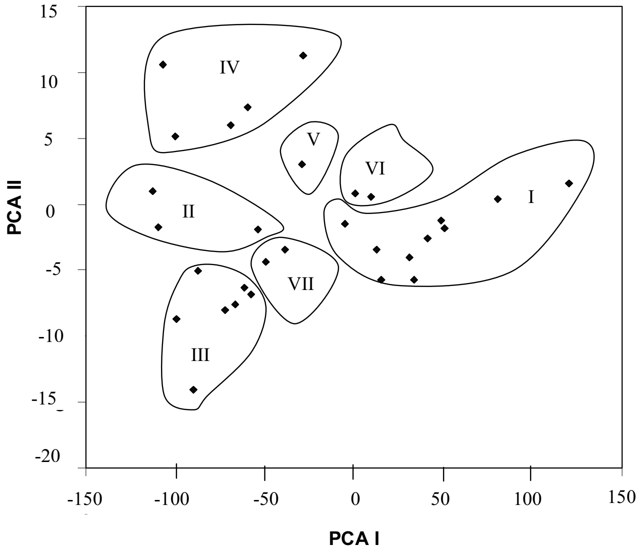

Fig. 2 Distribution of 30 cultivars in a two dimensional scattered diagram based on PCA scores superimposed with clusters (GENSTAT 5.5).

Based on PCA score I and PCA score II obtained from PCA, a 2-D scattered diagram was developed. The positions of the 30 cultivars in the generated scattered diagram were apparently distributed into seven groups (**Fig. 2**). The PCA could be performed on some selected morphological traits in rice (Ashfaq *et al.* 2012). Cluster I accommodated the maximum number of cultivars (10), cluster III had 7, cluster IV had 5, cluster II had 3, clusters VI and VII each had 2 and cluster V had only one cultivar (**Table 2**). The D^2 values (**Table 3**) revealed the highest intracluster distance ($D^2 = 101.29$) in cluster VI followed by cluster I ($D^2 = 90.35$). The cultivars in cluster III were genetically distant from the cultivars of cluster VI as the highest intercluster distance ($D^2 = 651.88$) was measured between these two clusters. However, as an alternative option, the cultivars under clusters I and III were suitable for hybridization, supported by the high intercluster distance ($D^2 = 536.18$). The highest intercluster D^2 value indicates the greatest genetic distance between two clusters concerned (Subudhi *et al.* 2009). The minimum intercluster distance ($D^2 = 38.65$) was estimated between clusters III and V followed by clusters II and V and clusters II and VI (**Table 3**), indicating that cultivars of clusters III and V were mostly related. Hybridization from two or more related clusters does not produce sufficient variability in the segregating generations (Bose and Pradan 2005).

The cluster mean for each of 12 characters has been displayed and the highest effective number of tillers/hill (12.72) was recorded from cluster II but the highest yield (4.05 t/ha) was from cluster VI (**Table 4**), which was comprised of 'BR34' and 'Darkashail', suggesting that the cultivars in clusters II and VI have excellent genetic worth. Therefore, a hybridization program is essential between these two clusters for increasing the number of effective tillers/hill and yield (t/ha) but narrow genetic diversity ensures narrow adaptation and susceptible to diseases and pests; hence, this improvement would not be sustainable over a wide range of environments. Days to maturity among the 7 clusters did not remarkably differ and ranged from 122.85-128.30 days and the range of characters was high; hence, development of short-duration cultivars in fine rice is very difficult through conventional breeding. The relative importance of the characters contributing towards total genetic divergence can be extrapolated from the coefficients of the first two canonical vectors (Z_1 and Z_2). Spikelets/ panicle alone had noticeable contribution (20.37%) towards genetic divergence among the characters studied (**Table 5**). Plant height and 1000-grain weight also showed intermediate contribution towards divergence. Maturity time, plant height and number of tillers contributed the most to genetic divergence in rice (Maurya and Singh 2012). The minimum contribution towards genetic divergence was by sterility percentage (3.58%) followed by flag leaf angle (5.26%) and lodging percentage (5.77%). Three characters viz., plant height, biological yield and flag leaf width appear to be the major sources of genetic divergence in rice (Ganta and Jyothula 2010). The relative importance of quantitative characters in inter-varietal divergence in rice was also measured by other authors (Pravin *et al.* 2003; Pradhan and Mani 2005). Though morphological variation might not always reflect the real genetic variation due to a genotype × environment interaction and phenotypic plasticity, unknown polygenetically controlled morphological and agronomical characters have yet to be studied for precise discrimination

Table 5 Mean ranges and contribution towards genetic divergence in fine rice.

Characters	Mean range	Relative contribution towards diversity (%)
Plant height (cm)	100.7 – 151.3	11.85
Tillers/hill	11.33 – 21.00	9.67
Effective tillers/hill	5.40 – 16.00	7.45
Panicle length (cm)	20.60 – 30.73	9.41
Spikelets/panicle	121.00 – 187.00	20.37
Sterility percentage	6.15 – 19.04	3.58
1000-grain weight (g)	13.02 – 30.71	13.20
Days to 50% flowering	79.00 – 103.30	8.29
Days to maturity	112.70 – 132.30	10.08
Yield (t/ha)	2.03 – 5.0 4	6.92
Flag leaf angle (0°)	16.00 – 36.30	5.26
Lodging percentage	0.00 – 34.33	5.77

Table 2 Clustering pattern of 30 fine rice cultivars.

Cluster	Number of cultivars	Cultivars
I	10	Katari, Zira, Badshabhog, Zethakatari, Philippinekatari, Zirashail, Chinigura, Shadakatari, Dudshar, Salna
II	3	Kalozira, Shilkamal, Shitabhog
III	7	Malshira, Chiconsarna, Lalchicon, Radhunipagal, Nazirshail, Lalfota, Sumonsarna
IV	5	Paijam, Ranjit, Kalosoru, Rajshahisharna, Begunbichi
V	1	Moulata
VI	2	BR 34, Darkashail
VII	2	Binnipakri, Uknimodhu

Table 3 Average intra and inter-cluster distances (D^2) for 30 fine rice cultivars based on 12 characters.

Clusters	I	II	III	IV	V	VI	VII
I	90.35	140.25	536.18	99.63	85.70	208.56	78.00
II		29.60	450.42	240.12	53.66	68.16	130.19
III			80.05	488.62	38.65	651.88	200.27
IV				54.28	116.98	86.47	206.36
V					0.00	150.32	292.48
VI						101.29	95.07
VII							23.37

Table 4 Means of yield and yield-contributing characters of fine rice clusters.

Cluster	Plant height (cm)	No. tillers/hill	Effective No. tillers/hill	Panicle length (cm)	No. spikelets/ panicle	Sterility (%)	1000-grain weight (g)	Yield (t/ha)	Days to 50% flowering	Days to maturity	Flag leaf angle (°)	Lodging (%)
I	133.91	17.56	10.54	27.32	159.76	19.83	16.48	3.38	92.99	125.23	25.07	12.93
II	120.10	17.41	12.72	25.39	154.90	13.42	15.15	3.64	92.11	125.43	25.10	12.22
III	113.78	17.38	10.00	26.72	161.37	10.56	17.08	3.26	92.28	124.53	22.83	10.95
IV	137.44	16.83	8.98	25.25	170.82	12.06	18.53	2.99	91.53	123.54	22.06	14.00
V	126.30	16.57	8.10	25.27	163.00	9.41	17.11	2.50	97.00	128.30	18.30	18.33
VI	139.80	18.10	12.32	26.92	186.25	10.69	19.29	4.05	96.65	126.65	25.20	10.85
VII	144.80	17.92	7.00	29.35	166.85	18.14	17.59	2.34	89.89	122.85	35.60	21.67

of closely related cultivars and in predicting their genetic relationship (Beyene *et al.* 2005). Independent of this, traditional varieties with high genetic variation overwhelmingly support the improvement of rice (Rekha *et al.* 2011). Despite these limitations, morphological characters are useful for preliminary evaluation because it is fast and simple and can be used as a general approach for assessing genetic divergence among morphologically distinguishable fine rice cultivars.

Many cultivars ('Zirashail', 'Shilkamal', 'Binnipakri', 'Zira', 'Lalfota', 'Darkashail', 'Dudshar', 'Moulata', 'Malshira' and 'Uknimodhu') are cultivated only for family interest. These cultivars may become extinct if the concerned families would divert their interest towards modern cultivars or other crop varieties. Besides, rich farmers prefer those cultivars which have socio-economic potential. That is why this class of farmers are interested to cultivate 'Chiconsarna', 'Sadakatari', 'Chinigura' and 'Radhunipagal'. The Asian primitive cultivars have distinct groups and a number of alleles responsible as the key of domestication (Kovach *at el.* 2009). The grouping pattern shown by PCA supports the results obtained by D^2 statistics in which 30 cultivars were distributed into 7 clusters. Sabesan *at el.* (2009) reported that genotypes from more than one place of origin were grouped into one cluster and genotypes from one state were grouped in more than one cluster. Hence, parents could be selected from the highest genetically distant groups taking into consideration certain areas for desirable traits, which could be made useful for improvement through intervarietal hybridization (Joshi *et al.* 2008). Highly diverged cultivars are able to produce a broad spectrum of variability in the segregating generation, enabling further selection and improvement (Chandra *et al.* 2007). The distinct groups of the evaluated fine rice cultivars in this study could be integrated into a rice breeding program for the development of improved varieties (Maji and Shaibu 2012). The information about the nature and magnitude of genetic divergence existing in the available germplasm of a particular crop is essential for selection of diverged parents, which, upon hybridization, may provide a wide spectrum of genetic recombination for quantitatively inherited traits (Pratap *at el.* 2011). Moreover, the hybrids developed from these cultivars within the limit of cross compatibility may show high heterotic effects or even desirable transgressive segregants in the F_2 generation. Ecological diversity did not reflect the genetic diversity among the cultivars all the time. The mode of distribution of genotypes from different geographical regions into various clusters was random, indicating no relationship between geographical distribution and genetic divergence among the cultivars (Pratikh *at el.* 2011). Generally, geographical diversity has been considered as a measure of genetic diversity but this was an inferential criterion and it miht not be so effective in quantifying or differentiating different populations (Waghmare *et al.* 2008). Effects of individual genotypes on ecological processes and patterns are a prerequisite for ecological consequences of genetic diversity, but these do not address genetic diversity *per se* (Whitham *et al.* 2006). However, an effective breeding program could be launched if parents are selected with high genetic distance, maximum contribution of a particular character towards total genetic divergence and cluster means for the yield-accelerating characters if considered (Falconer 1960). In this situation, a diallel crossing program would be driven by selecting one parent from each of the clusters (Jagadev *et al.* 1991) to measure general combining ability (GCA) of the selected parents.

Improvement of the commercial status and preservation of such indigenous fine rice cultivars would be linked to the conservation of a long heritage of India (Das *et al.* 2012). Therefore, cultivars collected for this study indicate a source of diversity that should be taken into account in a breeding exercise for the sustainable improvement of fine rice.

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