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RESEARCH ARTICLE

AGRO-MORPHOLOGICAL CHARACTERIZATION AND MORPHOLOGY BASED GENETIC DIVERSITY ANALYSIS OF LANDRACES OF RICE VARIETY (*Oryza sativa* L.) OF BANKURA DISTRICT OF WEST BENGAL

***Anjan Kumar Sinha and Mishra, P. K.**

Department of Botany, Vinoba Bhave University, Hazaribag, Jharkhand-825301

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ABSTRACT

An investigation was conducted to determine the agro-morphic characterization and relationship between 20 landraces of rice cultivars of Bankura District of West Bengal. Characterization for 20 qualitative and 13 quantitative morphological characters with 82 agro-morphic descriptors was carried out. Most traits were polymorphic except coleoptiles colour, present of leaf collar, shape of ligule and present of secondary branching in panicle. For Cluster analysis of qualitative traits the cultivars were grouped into five clusters based on similarity coefficient of Jaccard. Pearson correlation matrix, Principal Component Analysis (Pearson –n type), the un-weighted variable pair group method of the average linkage cluster analysis (UPGMA-Person) were used to analyzed quantitative data. This analysis enabled assessment of major characters of landraces variety which have a great impact to the diversity of landraces. Using UPGMA four cluster groups were obtained from 13 quantitative agro-morphological characters. The first three principal components explained about 79.05% of the total variation among the 13 characters. The results of PCA suggested that characters such as leaf length and width ratio, plant height, grain width, decorticated grain width, 50% flowering and maturity time were the principal discriminatory characteristics of landraces of rice variety. Germplasm consist of these characters are better choice for hybridization program. The present study indicated that morphological traits were useful for preliminary evaluation for crop improvement program and can be used for assessing genetic diversity among morphologically distinguishable rice landraces.

INTRODUCTION

Agro-morphological characterization of germplasm variety is fundamental in order to provide information for plant breeding programs (Lin, 1991). The landraces are valuable as they possess a huge treasure of genetic material which may prove valuable in future crop development and improvement programs. Our information about them is incomplete and is therefore urgent to collect and conserve these landraces of rice (Sinha and Mishra 2012). Green revolution has considerably improved production of food grains in our country and its role in achieving status of self sufficiency in food grain is beyond any doubt (Srivastava and Jaffe 1993). But high yielding varieties, which are the back bone of green revolution, have indirectly stimulated erosion of landraces and wild varieties of rice (Fowler and Moony 1990). Presently more than 90% of rice cultivation is being done using high yielding variety only. Obviously landraces are disappearing fast (Holden *et al.,* 1993; Durning 1990; Matson *et al.,* 1997). Importance of landraces can never be denied in agriculture system, because improvement in existing variety depends upon desirable genes which are possibly present in landraces and wild varieties only (Shiva 1991, Holden *et al.,* 1993). Landraces offer a valuable gene pool for future breeding program (Richharia 1979, Patran 2000). In present era of overpopulation *Ex-Situ* conservation is the best strategy to conserve these landraces (Lipton and Longhurt, 1989) because marginal and poor farmers who are the main keepers of traditional variety of rice are more interested in high production but not in genetic diversity.

***Corresponding author:** *Anjan Kumar Sinha,*
Department of Botany, Vinoba Bhave University, Hazaribag, Jharkhand-825301

Several workers reported the use of agro-morphological markers in the characterization of rice diversity. Chakravorty *et al*. (2013) studied multivariate analysis of 51 landraces of rice of West Bengal based upon 18 agro-morphological traits. Assessment of genetic diversity is very important in rice breeding from the standpoint of selection, conservation of different land races variety of rice and proper utilization (Mohammadi-Najad 2008). Evaluation and characterization of existing land races of rice is important due to increasing needs of varietal improvement. Yawen *et al.* (2003) studied the genetic diversity of 5285 accessions of land races of rice in China and found considerable morphological variation among accession. Patra and Dhua (2003) analyzed the agro-morphological diversity of upland rice. The main objective of the present study was to characterize twenty landraces of rice of Bankura district of West Bengal using twelve agro-morphological characters to provide useful information to facilitate the choice of genitors for rice plant breeding programs.

MATERIALS AND METHODS

Extensive survey was conducted in remote villages of Bankura District for seeds collection. 20 traditional rice landraces (folk rice) varieties (Table1) collected from the different parts of this district were assessed in this study. Collected Seeds of local landraces were characterized at lowland condition during the kharif session of 2010, 2011 and 2012 at Vill- Ranbahal, PO- Amarkanan District of Bankura. Rice variety were planted on small study plots with suitable and uniform spacing in between two plants (20cm in a row and 25cm in a Colum) in control condition without giving any synthetic agrochemical manure. Ten mature plants form the inner rows were

Table 1. List of various landraces of rice with their code and source of collection

Code	Name of the cultivars	Place of collection	Code	Name of the cultivars	Place of collection
V01	CHOTODIDI	Northern region	V11	MALABATI	Southern region
V02	FULKHAR	Northern region	V12	PATNAI-23	Western region
V03	MIHIDANA	Southern region	V13	JAMAINADU	Northern region
V04	BHADOI	Western region	V14	LANGAL MURA	Northern region
V05	KALOBAYAR	Eastern region	V15	MARICHSAL	Southern region
V06	BYAMAJHUPI	Southern region	V16	BADAMSARU	Eastern region
V07	SINDURMUKHI	Western region	V17	DANGAPATNAI	Southern region
V08	MUKTA	Western region	V18	TALMUGUR	Western region
V09	DUDHARSAR	Eastern region	V19	URI	Central region
V10	BHURI	Southern region	V20	JHARA	Central region

Table 2. Qualitative Character along with their descriptors of 20 landraces of rice variety of Bankura District

Qualitative traits	Descriptors	Evaluation phase
Coleoptiles colour (CC)	1.colourless; 2.green; 3. purple	First leaf through coleoptile
Basal leaf sheath colour (BS)	1.green; 2.light purple; 3. Purple line; 4. Purple	Booting stage (early)
Pubescence of leaf (PB)	1. absent; 2. Weak; 3.medium; 4.strong; 5.very strong.	Booting stage (early)
Leaf auricle (LA)	1.absent; 2.present.	Booting stage (early
Leaf collar (LC)	1.present; 2.absent.	Booting stage (early)
Leaf ligule (LLi)	1.absent; 2.present.	Booting stage (early)
Shape of ligule (LS)	1.truncate; 2.acute; 3.split.	Booting stage (early)
Culm attitude (CA)	1.erect; 2.semi-erect; 3.open; 4.spreading.	Booting stage (early)
Flag leaf attitude (FLA)	1.erect; 2.semi-eredt; 3.horizontal; 4.deflexed.	Beginning of anthesis
Lemma-Anthocyanin colouration of keel (LAC)	1.absent; 2.weak; 3.medium; 4.strong; 5.very strong.	Anthesis half way
Anthocyanin colour of internode (IC)	1.absent; 2.present.	Milk development
Anthocyanin colour of node (NC)	1.absent; 2.present;	Milk development
Panicle curvature (PX)	1.straight; 2.semi-straight; 3.drooping; 4.deflexed.	Ripening (terminal spikelet ripened).
Panicle attitude of branching (PAB)	1.erect; 2.erect-semierect; 3.semierect; 4.spreading	Ripening
Panicle exertion (PE)	1. partly exerted; 2.exerted; 3.well exerted.	Ripening
Panicle secondary branching (PSB)	1. absent; 2.present.	Ripening
Panicle secondary branching (SB)	1. weak; 2.strong; 3.clustered.	Ripening
Awning (AW)	1. present; 2.absent.	Ripening
Colour of awn (AC)	1.yellowish white; 2.yellowish brown; 3.brown; 4.raddish brown; 5.light red; 6.red;7.light purple; 8.purple; 9.black.	Ripening
Grain lemma and palea colour (LP)	1.straw; 2.gold and gold furrows on straw; 3.brownfurrows on straw; 4.reddish to light purple; 5.purple furrows of straw; 6.purple; 7.black	Ripening
Decorticated grain colour (DC)	1.white; 2.light brown; 3.dark brown; 4.light red; 5.red; 6.purple	Caryopsis hard
Grain aroma (DA)	1.present; 2.absent	Caryopsis hard
Sterile lemma colour (SLC)	1.straw; 2.gold; 3.red; 4.purple.	Caryopsis hard

Table 3. Quantitative Characters along with their descriptors of 20 landraces of rice variety of Bankura District

Quantitative characters	Observed phenotypic class	Evaluation phase
Leaf length	Arithmetic means of the five random sample	Booting stage(early)
Leaf width	Arithmetic means of the five random sample	Booting stage(early)
Leaf length width ratio	Arithmetic means of the five random sample	Booting stage(early)
Plant height	Arithmetic means of the five random sample	Milk development
Panicle length	Arithmetic means of the five random sample	Milk development
1000 grain weight	Arithmetic means of the five random sample	Caryopsis hard
Grain length	Arithmetic means of the five random sample	Caryopsis hard
Grain width	Arithmetic means of the five random sample	Caryopsis hard
Decorticated grain length	Arithmetic means of the five random sample	Caryopsis hard
Decorticated length width	Arithmetic means of the five random sample	Caryopsis hard
50% flowering	No. of days from plant germination until 50% of the plants in each plot flowered;	After flowering
Total maturity	Number of days until 50% of the panicles were mature	After maturation

tagged at random at each plot for the data collection. Data were collected for 23 qualitative and 14 quantitative traits on the particular stage of the rice plant following National guidelines for the conduct of Test for Distinctness, Uniformity and Stability of Rice (*Oryza sativa* L.), INDIA. ITG/01 Date: 03/09/2003, Annexure-I i.e. DUS test. Plant morphological DUS descriptors have been the universally undisputed descriptors applied for DUS testing of crop varieties. The observation of various characteristics were recorded at different growth stage with appropriate procedures as per the DUS test guide line of PPV & FR Act, 2001. The mean values of the data obtained from the three consecutive years are used for the various statistical analyses. A total of 23 qualitative (Table 2) and 13 quantitative characters (Table 3) were used in this observation. The qualitative traits were transformed into binary data considering the presence or absence (1/0) of each descriptors code for a particular characters. The Jaccard similarity coefficient was estimated for the qualitative traits. UPGMA (Un-weighted pair group method with arithmetic mean) was the clustering methods used for both qualitative and quantitative data. The Phenotypic and genotypic correlation coefficient values were calculated and computed by Pearson's Correlation Coefficient method and the data were analyzed using higher clustering methods for similarity/distance measure. Cluster analysis and PCA analysis (Person- n) was done to yield a dendrogram depicting the morphological relatedness of the 20 landraces of rice cultivars. The software XLSTST version 2013.04.05 was used for all test.

RESULTS AND DISCUSSIONS

Qualitative characters

Qualitative characters are considered as the most important characters to identify a particular plant variety. Qualitative character are mostly genetically controlled thus they are less independent to the environmental response. Polymorphism was found in 16 out of 20 qualitative traits. The non polymorphic traits were the coleoptile colour, presence of leaf collar, shape of ligule and present of secondary branching in panicle. Character basal leaf sheath colour 65% variety shows green, 5% variety shows light purple, 20% shows purple line and 5% variety shows purple colouration. Regarding the leaf characteristics pubescence of leaf surface (PB) showing higher variability where 55% variety shows medium pubescence, 20% strong, 20% weak and 5% variety shows very strong pubescence. For the character leaf auricles (LA), anthocyanin colouration of internode (IC), awning of panicle (AW) and decorticated grain aroma (DA) two alternative forms of characters were observed. Presence (75%) and absence (25%) genotypes were observed for auricles (LA), in case of anthocyanin colouration of internode (IC) 20% variety shows present and 80% variety shows without colouration. 80% of the variety consist of awn and 20% variety lack awn (AN) while presence (10%) and absence (90%) of the aroma in decorticated grain were found (DA).

Character culm attitude (CA) 70% variety shows erect, 20% shows semi erect and 5% variety shows open and spreading culm attitude respectively. The characters showing higher variability were flag leaf attitude, FLA (90% erect, 5% semi-erect, 5% horizontal), anthocyanin colouration of keel of lemma, LAC (75% absent or very week, 10% medium, 10% strong, 5% very strong), panicle curvature of main axis, PX (50% straight, 10% semi-straight, 35% drooping, 5% deflexed), panicle attitude of branching, PAB (55% erect, 25% erect to semi erect, 10% semi-erect to spreading, 10% spreading), panicle exertion, PE (25% partly exerted, 55% mostly exerted, 20% well exerted), panicle secondary branching, SB (20% weak, 70% strong, 10% very strong), lemma and palea colour of grain , LP (65% straw, 5% gold and gold furrows on straw, 20% brown furrows on straw, 10% black), and decorticated colour of grain, DC (50% white, 25% light brown, 5% dark brown, 10% light red, 5% red, 5% purple) . Character sterile lemma colour of grain (SLC) showing lowest variability (5% purple and 95% straw). The cluster analysis for the qualitative traits using the Jaccard similarity coefficient classified the accession into five classes (Figure 1). Thirteen varieties together form Class I (65%), two varieties consist of Class II (10%), only one variety form Class III (5%) and Class IV (5%) and Class V consist of three variety (15%) (Figure 2).

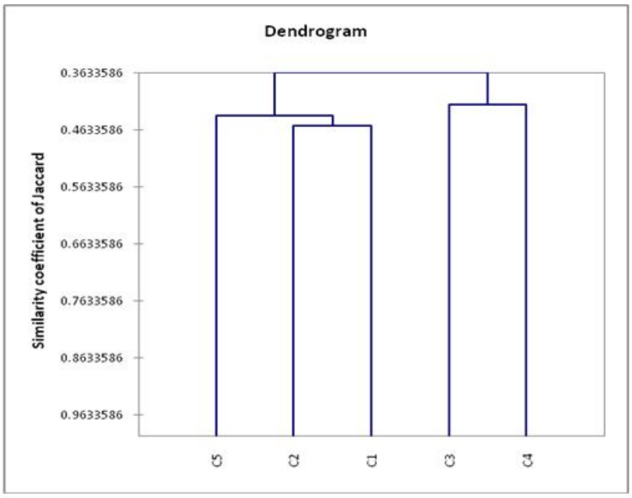

Figure 1. Dendrogram of similarity class by the the UPGMA methods based on Jaccard similarity coefficient estimated from 23 qualitative ago-morphological traits analyzed in 20 landraces of rice vareity

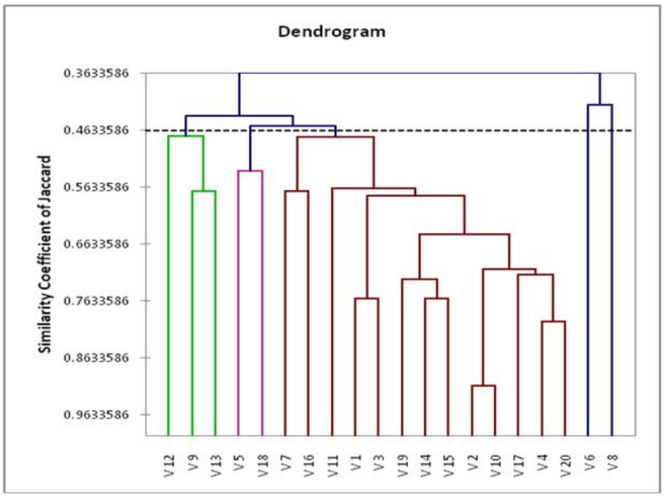

Figure 2. Dendrograme of similarity patterns by the UPGMA methods based on Jaccard similarity coefficient estimated from 23 qualitative ago-morphological traits analyzed in 20 landraces of rice vareity

Central object of the class I is variety Mukta, central object of class II is variety Bhadoi, central object of class III is variety Kalobayar, central object of class IV is Uri variety and central object of class V is Malabati variety. Maximum distance (4.899) was found in betwee the variety 8 (Uri) and the variety Bhadoi of class-II and minimum distance (3.464) was observe between the variety Malabati of class-V and the variety Mukta of class-I.

Quantitative analysis

Correlation analysis of Phenotypic Traits (Pearson's coefficient). Pearson's corellation (r) is a measure of the strength of association between the two characters. Assesment of coefficient of correlation for pair of characters in the landraces of rice are presented in Table 4. Among the 14 different quantitative agro-morphological characters studied, the highest correlation corresponded to the 50% flowering date (50% FLW) and the time of maturity (TM) (r= 0.95). Characters presenting highly significant correlation with these two characteristics were Grain length (GL) and decorticated grain length (DL) (r=0.95), grain width (GW) and the decorticated grain width (DW) (r= 0.85), 50% flowering (50% FLW) and the hight of plant (HT) (r= 0.83), time of maturity (TM) and hight of plant (HT) (r= 0.77), grain length (GL) and decorticated grain length width ratio (DL/DW) (r= 0.74), decorticated grain length width ratio (DL/DW) and decorticated grain length (DL) (r=0.69), grain length width ratio (GL/GW) and grain length (GL) (r=0.67). Character decorticated grain length width ratio (DL/DW) negatively correlated with the decorticated grain width (DW) (r= -0.74), character grain length width ratio (GL/GW) negatively correlated with the character decorticated grain width (DW) (r= -0.71) and character grain width (GW) (r= -0.74) respectively. Leaf width (LW) is highly correlated with the decorticated grain width (DW) (r= 0.64), showing the effect of leaf configaration in the production of photosynthates.

Principle component analysis

Principal componant analysis (PCA) clearly indicate the genetic variation of the landraces (Table 5). It measures the important characters which have a greater impact to the total variables and each coefficient of proper vectors indicated the degree of contribution of every original variable with which each principal component is associated (Sanni *et al*., 2010). According to Clifford and Stephenson (1975) and Guei *et al*. (2005) first three principal component are often the most important in reflecting the variation patterns among the differnt landraces and the characters associated with these are most important in differentiating various landraces. Raji (2002) was chosen to determine the cutoff limit for the coefficients of the proper vectors. According to this criterion coefficients greater than 0.3 (regardless the direction positive or

Table 4. Pearson correlation matrix of 14 quantitative characters used in characterization of landraces of rice

Variables	LL	LW	LL/LW	HT	PL	GWt	GL	GW	GL/GW	DL	DW	DL/DW	50% FLW	TM
LW	0.406													
LL/LW	0.671*	-0.389												
HT	0.493	0.514*	0.106											
PL	0.388	0.318	0.164	0.301										
GWt	-0.141	0.312	-0.402	0.215	-0.374									
GL	0.175	-0.028	0.174	0.063	-0.176	0.522*								
GW	0.061	0.592*	-0.367	0.289	-0.021	0.652*	-0.030							
GL/GW	0.079	-0.402	0.355	-0.127	-0.081	-0.139	0.679*	-0.743*						
DL	0.199	0.016	0.183	0.073	-0.175	0.537*	0.954**	0.117	0.550					
DW	0.133	0.647*	-0.359	0.279	0.044	0.470*	-0.144	0.859**	-0.717*	-0.041				
DL/DW	0.048	-0.426	0.366	-0.117	-0.144	0.008	0.744*	-0.562*	0.908**	0.690*	-0.741*			
50% FLW	0.456*	0.447*	0.097	0.839**	0.313	-0.033	-0.056	0.049	-0.035	-0.048	0.188	-0.125		
TM	0.594*	0.503*	0.187	0.777**	0.346	-0.124	-0.086	0.000	-0.017	-0.097	0.192	-0.153	0.958**	

Values in bold are different from 0 with a significance level alpha=0.005 (*) and alpha 1E-4 =0.01 (**).

LL- Leaf length, LW- Leaf width, LL/LW- Leaf length & width ratio, HT- Plant height, PL- Panicle length, Gwt- 1000 grain weight, GL- Grain length, GW-Grain width, GL/GW- grain length & width ratio, DL- Decorticated grain length, DW- Decorticated grain width, DL/DW- Decorticated grain length width ratio, 50% FLA- 50% flowering of Plant, TM- Maturity time.

Table 5. Coefficient and vector association with the first 3 principal componant

	F1	F2	F3
Eigenvalue	4.653	3.500	2.915
Variability contribution (%)	33.238	24.997	20.822
Cumulative variance contribution %	33.238	58.235	79.057
		Coefficient vector	
Leaf length (LL)	-0.105	0.413	-0.062
Leaf width (LW)	-0.356	0.144	0.149
Leaf length width ratio (LL/LW)	0.167	0.294	-0.184
Plant height (HT)	-0.252	0.367	0.035
Panicle length (PL)	-0.131	0.199	-0.237
1000 grain weight (GWt)	-0.104	-0.030	0.534
Grain length (GL)	0.216	0.242	0.432
Grain width (GW)	-0.341	-0.101	0.323
Grain length width ratio (GL/GW)	0.381	0.250	0.049
Decorticated grain length (DL)	0.177	0.229	0.455
Decorticated grain width (DW)	-0.386	-0.075	0.216
Decorticated grain length width ratio(DL/DW)	0.390	0.225	0.143
50% flowering (50% FLW)	-0.227	0.382	-0.099
Time of maturity (TM)	-0.228	0.401	-0.144

negative) as having a large enough effect to be considered important, while traits having a coefficient less than 0.3 were considred not to have important effects on the overall variation observed in the present study. The first principal componant accounted for more than 33% of the total variation. Leaf width (-0.35), grain width (-0.34), grain length width ratio (0.38), decorticated grain width (-0.38) and decorticated grain length width ratio(-0.39) were the variable mostly contributed in first componant among them Leaf width, grain width, decorticated grain width variables were negatively contributed. Thus the first componant identified yield component variables presenting positive and negative contribution to the variables. Second principal componant accounted for the more than 23% of the total variation. Characters highly and positively correlated were plant height (0.36), 50% flowering (0.38) and the total maturation period (0.40). As a result, second component differentiated those accessions that the 50% flowering duration and the maturation pereiod was earlier in the session and had long leaf. Third principal componant accounted for more than 20% of the total variation. This component consist of 100 grain weight (0.53), grain length (0.43), grain width (0.32) and decorticated grain length (0.45). Thus thrid componant registered thouse component which have high yield-componant value. Takeda (1990) and Caldo *et al*. (1996) also reported that grain size – as indicated by weight, volume of length and maturity time, 50% flowering, plant height, leaf length were the major factors contributing to the variations of parental line.

Cluster analysis

According to the Pearson's corellation coeffiient of degree of divergence twenty landraces of rice were grouped into four cluster (Table 6) (Figure 3 & 4). The maximum number (6) of variety were included in the cluster I and the minimum number (1) of variety included in the cluster IV. Cluster II and III consist of four variety each. Variety of each cluster were obtain form the different region of the district that menas the clustering pattern did not follow the geographical distribution and genotypes of the same cluster were found form different portion of the district. This observation agree with the observation of Choudhury *et al*.(1999), Candra *et al*. (2007). According to the Shanmugam and Rangasamy(1982) grouping of materials of same origin into different clusters was an indication of broad genetic base of the genotypes belonging to that origin. The inter cluster distance (Table 7) was maximum in between cluster I and IV (135.74) and minimum in between cluster I and III (28.57). Hence the genotypes of cluster I is more divergent then the cluster IV. The range of inter-cluster values ranged from 28.37 to 135.74 indicates wide range of diversity. Chossing of genotypes belonging to distant clusters were expected due to execute maximum heterosis in crossing and to be used in hybridization programme for obtaing a wide spectrum of variation among the segrigation (S.M. Hosan *et.al* 2010). Medium cluster distance were observed for cluster I and cluster II (53.57) and in between II and cluster III(51.69) and cluster II and cluster IV (93.72). Crossing involving parents form the medium

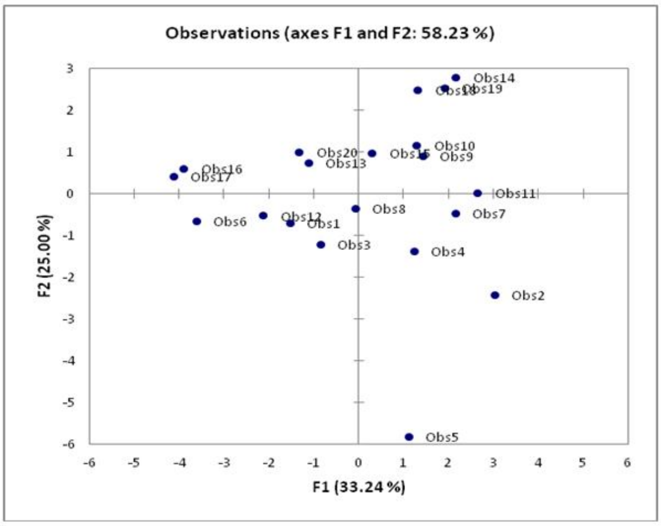

Figure 3. 2D scatter diagram of 20 landraces of rice used in the experiment from 14 quantitative agro-morphological traits based on Person (n) PCA scores

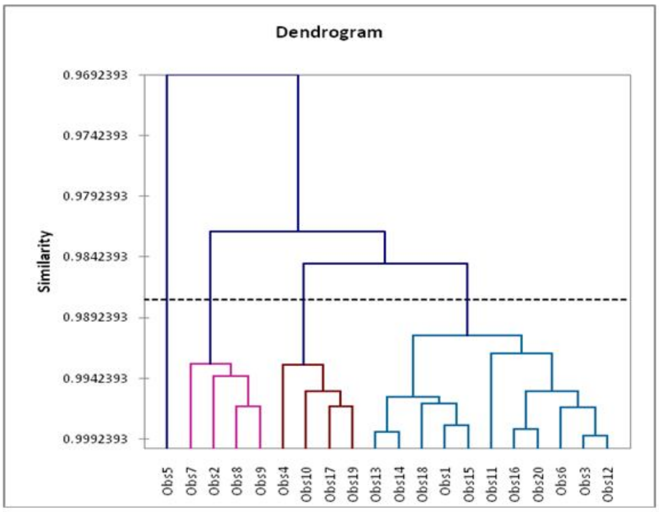

Figure 4. Dendrogram of 20 landraces of rice cultivars of Bankura District derived by UPGMA Person correlation coefficient from 12 morphological traits. Codes of cultivars are showed in Table 1

divergent clusters also exhibit significantn heterotic segregants for yield and some of its components (Mian and Bhal, 1981). It was also observed that the inter cluster distance is minimum in between cluster I and IV followed by that in between cluster IV and III indicating that the genotypes of these cluster are very close to each other. The results of the various qualitative and quantitative statistical analyses

Table 6. Distribution of 20 rice genotypes in different clusters

Cluster no	Genotype serial no	Total number of genotypes	Names of genotypes
I	1,3,6,11,12,13,14,15,16,18,20	11	Chotodidi, Jahra(sada), Kalobayar, Duderswar, Bhuri, Malabati, Patnai-23, Jamaynadu, Langalmura, Badamsaru, Talmugur dhan.
II	2,7,8,9	4	Fulkhar, Bymajhupi, Uri, Sindurmukhi
III	4,10,17,19	4	Mihidana, Mukta, Marichsal, Danga patnai
IV	5	1	Bhadoi

Table 7. Distance between four classes of 20 rice genotypes (based on UPGA Person corellation coefficient)

Cluster	I	II	III	IV
I	0			
II	53.570	0		
III	28.576	51.698	0	
IV	135.744	93.729	131.380	0

showed a broad spectrum of phenotypic diversity presence among the landraces of rice variety. PCA analysis clearly indicate that leaf length, leaf width, leaf length width ratio, Plant height, 50% flowering, maturity time and decorticated grain width and grain width characters are the important characters which have a strong effect in plant variation. Cluster analysis based on quantitative characters suggested that the landraces variety could be grouped. From the cluster analysis we may conclude that Badamsaru, Patnai-23, Kalobayar, Uri, Bhadoi, Fulkhar and Byamajhupi variety are useful to breeders that may be used as parents as because these varieties possess vast genetic difference.

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