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RESEARCH ARTICLE

A STUDY ON MORPHO-QUALITATIVE AND NUMERICAL TAXONOMY OF INDIGENOUS WILD AND CULTIVATED RICE GERMPLASMS OF MANIPUR

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ABSTRACT

Rice is life for Asians and Indians in particular. The studies of rice germplasms are essential for identification of rice cultivator for producing new hybrid varieties. Thirty-seven rice germplasms consisting of thirty-five indigenous rice including three wild rice varieties *Oryza rufipogon* local collection I, II and III from Manipur and two wild rice *O. rufipogon* and *O. nivara* were studied during the present investigation. Morpho-qualitative and numerical taxonomic analyses were used to classify rice cultivars on the basis of phenotypic traits. Dendrogram was generated for the Euclidian distance, phenotypically all the cultivars were classified into different groups based on morpho-qualitative characters. Possession of primary green colour is considered the most advanced form and thus occupy top position in the evolutionary sequence of the all the 37 germplasms. Anthocyanin pigmentation, in the present studies, varied from completely non-pigmented (green) to pigmented in, as many as five different plant parts. Distribution of anthocyanin pigmentation in different organs of rice plant was very variable. The rice germplasms were classified into different groups according to the combinations of anthocyanin pigmentation. The result indicated that agro-morphological traits were helpful for characterization which can be used as a broad spectrum approach to assess genetic diversity among morphologically distinguishable rice cultivars.

INTRODUCTION

Rice (*Oryza sativa* Linn) a member of the genus *Oryza* in the natural order Gramineae (Poaceae) is one of the major food crops among the various population of the world. It is the staple food for one third of the world's population. It is one of the most important crops in the world, next to wheat in total cultivated area, and to corn in production. In addition rice is also an ideal model plant for studying grass genetics and genome organization owing to its diploid genetics and comparatively small genome size of 430Mb (Causse *et al.,* 1994; Kurata *et al.,* 1994), considerable level of genetic polymorphism (Tanksley 1989; Wang et al.1992; McCouch *et al.,* 1988), large amount of well conserved genetically diverse material, ease of use of widely collected and well matched wild species (Pervaiz *et al.,* 2010; Rabbani *et al.,* 2010). Although world rice production has doubled in the past 30 years due to the introduction of superior varieties and better cultivation practices, however it is still unsatisfactory to meet the ever increasing global demands (Fischer *et al.,* 2000; Sasaki and Burr, 2000). From 2001 to 2025, it is estimated that the demand for rice in the world would increase by 1% per annum, so the present average yield has to be increase considerably in order to meet up the rising needs (Maclean *et al.,* 2002). Rice occupies 2.96 million hectares that is about 12% of the total cultivated area. Its production was 6.95 million tones and 2347 kg yield per hectares (Omer Farooq, 2009). In the perspective of global biodiversity loss, Manipur lost several indigenous rice varieties. According to traditional classification more than 50 rice cultivars were cultivated before the introduction of high yielding varieties breed in Manipur. In order to have an extensive breeding program for high yields and response to inputs, the knowledge of genetic diversity in the crop is essential. For efficient use of germplasms in breeding program the germplasms need to be systematically characterized with the traits which have high degree of heritability so that the cultivars can be identified with reasonable degree of certainty under varied environmental conditions. Systematic evaluation of local rice germplasms of both *O. sativa* cultivars and wild species for morphological and agronomic characters, and genetic diversity are urgently the need of hour as far as the improvement of local germplasms is contemplated. In this context, it is essential not only to conserve the indigenous cultivated and wild rice varieties but also to explore the gene pool of aromatic rice for breeding purposes of well adapted better quality and high yielding varieties in the country (Rabbani *et al.,* 2008; Pervaiz *et al.,* 2009). Conservation of biodiversity in the wake of depleting natural resources has assumed considerable significance and worldwide importance. Land races, traditionally grown primitive cultivars and wild relatives of cultivated plants are the basic raw materials that

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not only sustained the present day crop improvement program but are also required to meet the aspirations of future generations to face unforeseen challenges of both biotic and abiotic stresses.

In favour of this reason, estimates of genetic diversity and the relationships among wild and cultivated rice germplasms are very practical for facilitating the resourceful germplasms collection and management. Several tools are available for studying the variability and relationships between cultivars including Isozymes, storage protein study and molecular markers linked to particular traits. Moreover for the classification and estimation of the germplasms, morphological evaluation is preliminary step (Smith & Smith 1989; Smith *et al.* 1991). The identification of genetic variability in any character concerned with yield synthesis provides scope for improvement and breeding new aromatic rice cultivars with desire traits. Keeping in view, the present study was established to estimate genetic diversity in rice cultivars using morpho-qualitative and numerical taxonomic techniques.

MATERIALS AND METHODS

The experimental material consisted of thirty-five indigenous rice germplasms of Manipur and two wild rice *Oryza rufipogon* and *Oryza nivara* procured from IRRI, Philippines. The field experiment was conducted at the experimental farms of Manipur University, Imphal and Central Agricultural University Iroisemba, Imphal. The experiment was carried out in the experimental farm in the randomized block design with three replications. The inter-row and intra-row spacing were maintained at 20 cm. The experimental data on various quantitative and qualitative characters were recorded as per criteria laid down in the Standard Evaluation System for rice IRDP and Descriptions for Rice published jointly by International Rice Institute and International Board for Plant Genetic Resources (1980). For morpho-qualitative characters, data were recorded through visual observation and each observation was recorded by a code number. Nine qualitative characters were taken for the study. They are leaf blade colour, leaf blade pubescence, basal leaf sheath colour, leaf angle, ligule colour, collar colour, auricle colour, flag leaf angle and ligule shape. Quantitative characters used for numerical taxonomic study were flag leaf length, flag leaf width, ligule length, culm diameter, panicle length, culm length, ear-bearing tillers/plant, days to 50% flowering, grain length, 100 grain weight, spikelet/panicle, grain yield/plant.

Agglomeration Schedule Dendogram

A visual representation of the steps in a hierarchical clustering solution that shows the clusters being combined and the values of the distance coefficients at each step. Connected vertical lines designate joined cases. The dendogram does not plot actual distances but rescales them to numbers between 0 and 25. This preserves the ratio of the distances between steps. The scale displayed at the top of the figure corresponds to the rescaled distances. Dendogram were prepared for each of the different varieties. Considering the most frequent or mean weightage value of each morphological character for each species, the hierarchical clustering analyses were performed for all the varieties.

Hierarchical Model (Sneath and Sokal 1973; Degens 1983)

A special class of log linear model. If a term for the interaction of a set of variable exists, there must be lower-order terms for all possible combinations of these variables. For example, if the term A by B by C is in the model, then the terms A, BC, A by B, A by C and B by C must also be in the model.

Agglomerative Hierarchical Clustering Method

A method for creating clusters in which each case start out as a cluster. At every step, clusters are combined until all cases are members of a single cluster. Once cluster is formed it cannot be split, it can only be combined with other clusters. Hierarchical cluster combines cases into clusters hierarchical, using a memory intensive algorithm that allows examining many different solutions easily. For performing hierarchical cluster analysis first Euclidian distance is measured and clusters are made by several methods using corresponding agglomeration schedule. These schedules are being prepared on the basis of Euclidian dissimilarity distance coefficient matrix. All the statistical computations were performed using a Statistical Software package, SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Evaluation of morpho-agronomic characters of rice germplasms

To facilitate classification of the 37 varieties, 7 major morpho-qualitative trait combinations were taken and classify in 13 groups in the following sequence - degree of colouration in leaf blade (LB), basal leaf sheath colour (BLSC), ligule colour (LC), collar colour (CC), auricle colour (AC), awn colour (AWC) and sterile lemma colour (SLC) as shown in Table 1. A total varieties bearing group 1 with code no. 47, 119, 129, 130, 200, 204, 255 shares character in common in possessing pale green in leaf blade, green in basal leaf sheath, white in ligule colour, pale green colour, auricle being pale green, awn colour being straw and straw colour in sterile lemma and thus form a separate group. The sharing of common character reveals close relationship among the varieties and forms descendents from a common ancestral stock. Group 2 consists of 13 varieties bearing code no. 34, 40, 70, 74, 84, 123, 166, 196, 197, 198, 281, 236 and 5 forms a common group in having green colour in both leaf blade and basal leaf sheath, pale green in both colm and auricle and straw colour in both awn and sterile lemma. Group 3 with 3 varieties bearing code no. 203, 2 and 3 possess dark green colour in leaf blade while the other 6 major characters remain the same with group I and 2 and thus form a deviated group 4. Group 4 varieties bearing code no. 195, 199, 201 and 202 shares 6 characters in common with that of Group 2 but deviate in possessing green colour in colm, thus form a separate group 5. Group 5 consists of two varieties bearing code no. 263 and 237 which also shares 6 characters in common with that of group 1 but also deviate in possessing green colour in colm as that of group 2. Eight individual varieties bearing code no. 31, 32, 33, 74a, 167, 167a, 1 and 4 bears no major character in common and also deviate from each other. Thus they are placed separately as individual group on the basis of their unique individual character. Again, grouping all the sub characters of each major characters to the primary colour, we can group all the 37 varieties into 6 groups i.e., pale green, green and dark green can be place under green category, purple and any

Table 1 First line of classification base on morpho-qualitative trait

Characters	Total no. of varieties	Varieties code
LB1- BLSC1- LC1- CC1- AC1- AWC1- SLC1	7	47, 119, 129, 130,200,204,205
LB2- BLSC1 -LC1-CC1- AC1- AWC1- SLC1	13	34, 40, 70, 74, 84, 123, 166 196, 197, 198, 218, 236, 5
LB3- BLSC1- LC1- CC1- AC1- AWC1- SLC1	3	203, 2, 3
LB2- BLSC1- LC1- CC2- AC1 -AWC1- SLC1	4	4,195, 199, 201, 202
LB1- BLSC1- LC1- CC2- AC1 -AWC1- SLC1	2	263, 237
LB2- BLSC2- LC3- CC3- AC2 -AWC5- SLC4	1	31
LB1- BLSC2- LC1- CC3 -AC2 -AWC5- SLC31	1	32
LB2- BLSC3- LC3- CC3- AC2- AWC6- SLC4	1	33
LB2- BLSC2- LC1- CC3- AC2- AWC1- SLC1	1	74a
LB5- BLSC4- LC2- CC3- AC2- AWC1 -SLC1	1	167
LB2- BLSC3- LC1- CC3- AC2- AWC1- SLC3	1	167a
LB3 -BLSC2- LC1- CC1- AC1- AWC1- SLC1	1	1
LB3- BLSC1- LC1- CC3- AC2- AWC1- SLC1	1	4

variation in purple colouration can be grouped as purple group etc. Thus in the second grouping of germplasms, a collection of 29 germplasms form a group on the possessing green leaf blade, green basal leaf surface, white ligule, green colm colour, green auricle, straw awn and sterile lemma as shown in Table 2. In Table 1, serial No. 1-5 forms a group comprising of 29 germplasms. Serial No. 6 and 8 can be combined together to form a group which comprises of code no. 31 and 33. Code no. 32, 74a and 167a in serial No. 7, 9 and 11 forms another group. There are three individual single groups comprising of one variety each i.e. serial No. 10, 12 and 13.

thus it follows next to the first group 13 and 7 germplasms in phylogeny, occupying third position. Germplasms 31, 32 and 74a also shares almost all the characters in common except with 31 which deviates in having purple in LC thus are related in some way and very close. Similarly 167, 167a also share almost all the character but are distinctly separated in possessing green LB in 167a whereas it is purple tip or margin in 167. Again germplasms 31, 33 and 167 shares almost all the characters but 167 has total purple in LB, BLS, LC whereas 31 and 33 have green in LB with the remaining characters as purple. Germplasms 32 and 74a are advanced over 167a as the

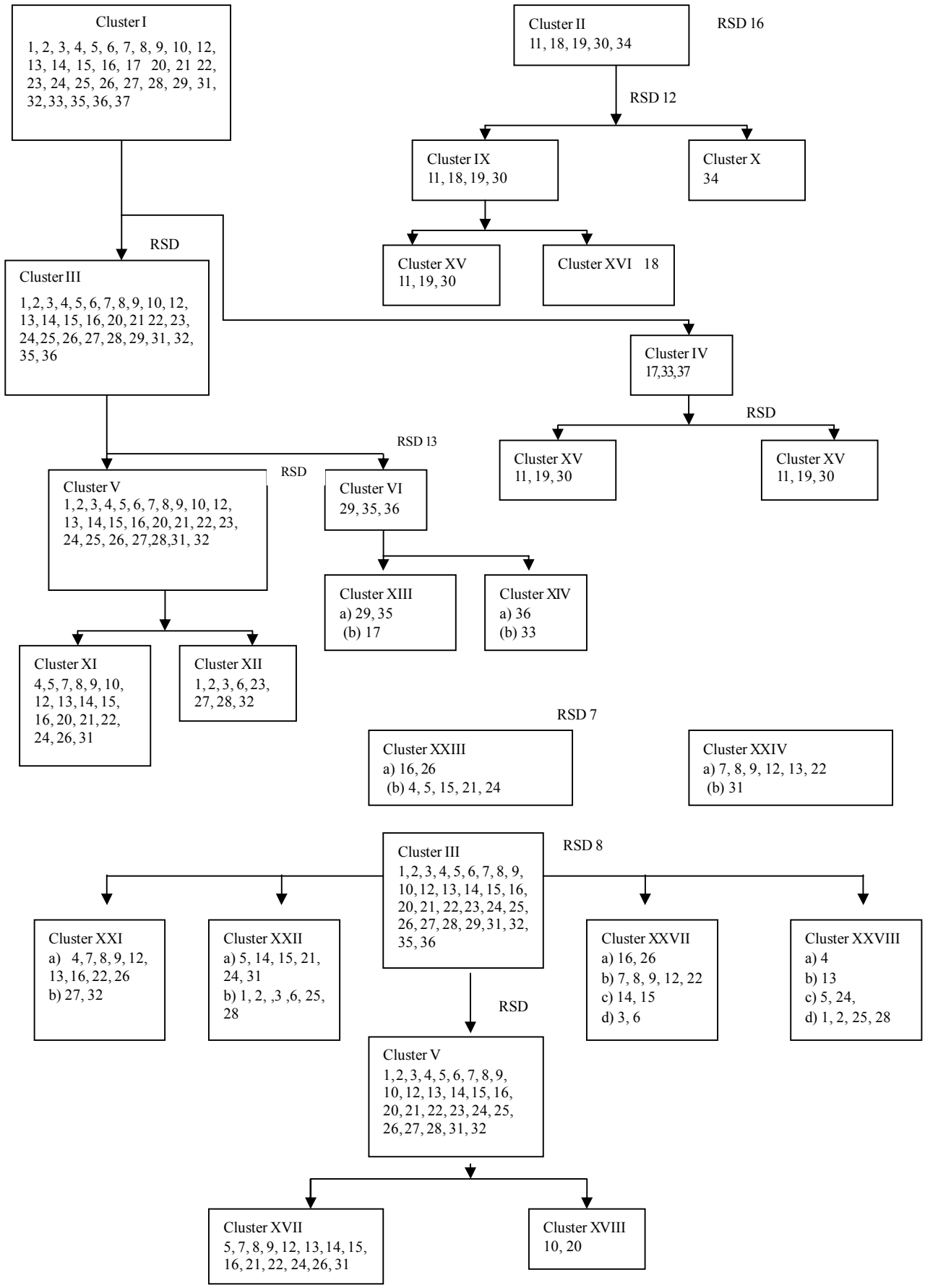
Table 2 Second line of classification based on morpho-qualitative trait

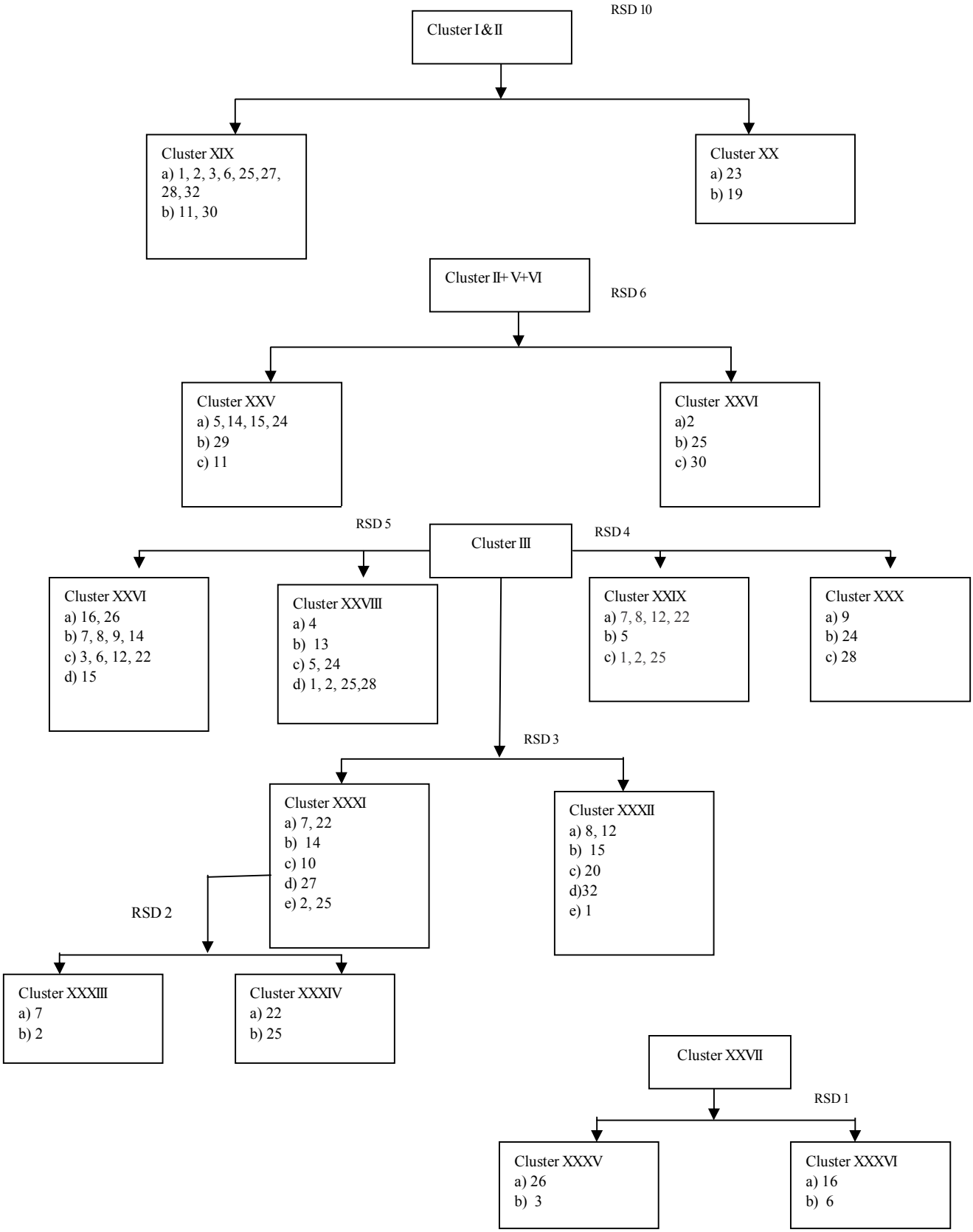
Sl.No	Characters	Total no. of varieties	Varieties code
1	LB- BLSE- LC- CC- AC- AWC- SLC	29	34, 40, 47, 70, 74, 84, 119, 123, 129, 130, 166, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 218, 236, 237, 255, 263, 2, 3, 5
2	LB- BLSC- LC- CC- AC- AWC- SLC	2	31,33
3	LB- BLSC- LC- CC- AC- AWC- SLC	1	167
4	LB- BLSC- LC- CC –AC- AWC- SLC	1	1
5	LB- BLSC- LC- CC- AC- AWC- SLC	1	1
6	LB- BLSC- LC- CC- AC- AWC- SLC	1	4

By comparing the outcomes of 1st and 2nd line classification, phylogeny deviates from ancestral line, precedent and descendent can be determined in some way. Possession of primary green colour can be considered as most advanced form and thus occupy top position in the evolutionary sequence of the all the 37 germplasms. Thus the 13 varieties at serial No. 2 of the first line classification shown in Table 1 occupied top position while that of serial No. 1 comprising a total of 7 germplasms sharing all the characters in common with that of 13 germplasms. But, it only deviates in having pale green in leaf blade whereas in the 13 germplasms leaf blade is green. Thus these 7 germplasms are one step behind the 13 varieties in phylogeny. The same is the case in regard of serial No. 4 and 5 comprising 4 germplasms and 2 germplasms each. Germplasms at serial No. 3 comprising of 3 germplasms possess dark green in LB while the other characters remain the same. As such these germplasms from serial No. 1 to 5 originates from a common ancestral pool with slight deviation in leaf blade coloration either in response to geographical location or environmental adaptation. Germplasm 203, 34 and 35 at serial No. 3 shares all the character in common expect in having dark green in LB,

3 germplasms shares almost all the characters in common except in BLSC of 167a, it is light purple. Possessing of light purple in the whole basal leaf sheath can be considered primitive to the reduction into a single purple line as found in 32 and 74a. Thus 32 and 74a are grouped together and 167a is left aside. Presuming all the characters having purple as one, we can safely group 32,74a and 167a as one group. Again 167 and 167a share almost all the characters with 167 deviate in possessing purple tip or margin in LB. Possession of purple margin or tip can be considered advanced form since all or one purple line blotch in the LB is reduced in space or confined to only a limited space in the LB. Thus 167 is advance over 32, 74a and 167a. Thus is placed one step above 167a. Germplasms 33, 34 and 35 have all the characters but in germplasms 1 deviates in having green in BLS and purple AC whereas 34 and 35 have green in BLS and pale green in AC. These three germplasms are kept in the same line of phylogeny but germplasms 33 deviates away occupying one distinct position.

Table 3 Hierarchical cluster analysis grouping of rice germplasms




RSD 10
Cluster I & II
Cluster XIX
a) 1, 2, 3, 6, 25, 27, 28, 32
b) 11, 30
Cluster XX
a) 23
b) 19
Cluster II+ V+VI
RSD 6
Cluster XXV
a) 5, 14, 15, 24
b) 29
c) 11
Cluster XXVI
a)2
b) 25
c) 30
RSD 5
Cluster III
RSD 4
Cluster XXVI
a) 16, 26
b) 7, 8, 9, 14
c) 3, 6, 12, 22
d) 15
Cluster XXVIII
a) 4
b) 13
c) 5, 24
d) 1, 2, 25,28
Cluster XXIX
a) 7, 8, 12, 22
b) 5
c) 1, 2, 25
Cluster XXX
a) 9
b) 24
c) 28
RSD 3
Cluster XXXI
a) 7, 22
b) 14
c) 10
d) 27
e) 2, 25
Cluster XXXII
a) 8, 12
b) 15
c) 20
d)32
e) 1
RSD 2
Cluster XXXIII
a) 7
b) 2
Cluster XXXIV
a) 22
b) 25
Cluster XXVII
RSD 1
Cluster XXXV
a) 26
b) 3
Cluster XXXVI
a) 16
b) 6

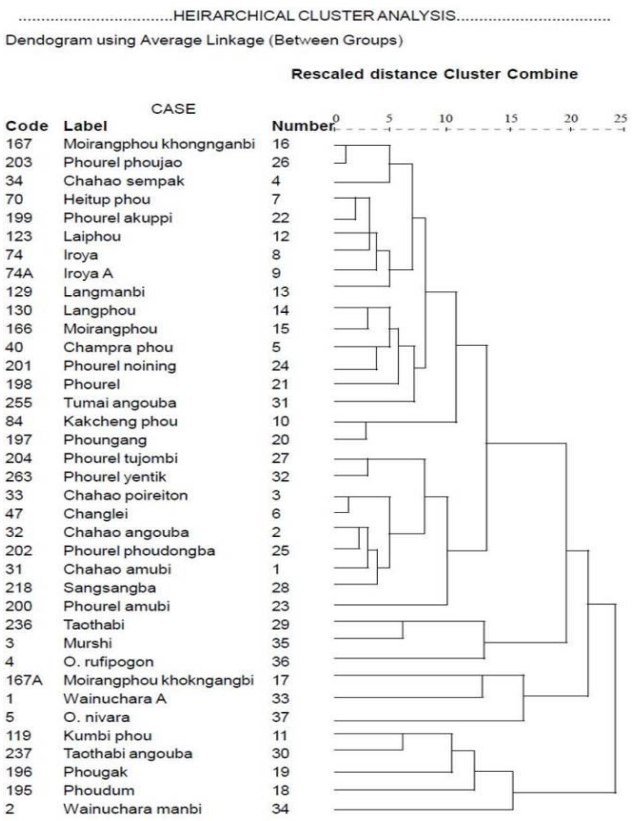


Figure 1 Dendogram of hierarchical cluster analysis

Anthocyanin Pigmentation

Anthocyanin Pigmentation, in the present studies, varied from completely non-pigmented (green) to pigmented in, as many as five different plant parts. Distribution of anthocyanin pigmentation in different organs of rice plant was very variable. 37 rice germplasms were classified into different groups according to the combinations of anthocyanin pigmentation in different plant parts. Such a grouping of cultivars according to the presence of anthocyanin pigmentation in different plant parts as a means of classification of cultivars was reported by Hector (1922), Jones (1929) and Ramiah (1945, 1953). The earliest classification of rice germplasms was based on the occurrence of pigmentation and other morphological characters like culm, leaf, panicle, grain etc. (Hector *et al.,* 1934; Alam, 1935; Kashi Ram and IKbote, 1936). Characterization of a cultivar according to the pattern of anthocyanin pigmentation in its plant parts could further be supplemented with easily identifiable morpho-qualitative traits so that the cultivar could be identified with certainly at the phenotypic level itself from the other cultivars. These morpho-qualitative traits not only utilized for classification and identification of cultivars but also of great use in searching desirable plant types for breeding programmes. Characterization of morpho-qualitative traits in other rice germplasms and character based on their genetic divergence in the present study were reported by Padmanabhan (1971), Asthana and Majumdar (1981)**.**

Numerical taxonomy

Grouping of germplasms into different clusters by hierarchical cluster analysis grouping are shown in Figure 1 and Table 3. Clustering of 37 germplasms with 32 germplasms in cluster I and V germplasms in cluster II, cluster II is again divided into two sub-clusters, sub cluster IX comprising 4 germplasms and sub-cluster X comprising only one germplasms into two sub-cluster, sub-cluster XV comprising 3 germplasms and cluster XVI having only one germplasms shows presence of high genetic diversity among the germplasms. Cluster I having 32 germplasms may be due to high upper limit of arbitrary value which, in turn, warranted sub-clustering of cluster I. Cluster I and Cluster II was further grouped into different clusters thereby indicating existence of sufficient genetic divergence among the germplasms. The random clustering pattern of germplasms from different districts of Manipur indicated that the genetic diversity of the germplasms is not necessarily related with the distribution of germplasms in different districts of Manipur. The genetic diversity among the germplasms in the present study may be resulted from genetic drift and selection that cause greater genetic diversity than geographical distribution as suggested by Murty and Arunachalam (1966). Wainucharamanbi (2) Phoudum 195, *O. nivara* (7), Tumai angouba (255) Phourel amubi (200), Phougak (196) Taothabi (29), Kumbi phou (119), Chahao angouba (32), Phourel phoudongba (202) Taothabi angouba (237), Chahao sempak (34) Langmanbi (129), Champra phou (40), Heitup phou (70) Langphou (130), Kakcheng phou (84), Phourel tujombi (204), Moirangphou (116) Phoungang (197), Phourel yentik (263), Phoungang (197), Phourel phoujao(203), Chahao poireiton (33), Moirangphou khokngangbi (167) and Changlei (47) in cluster X cluster XVI, cluster VIII, cluster XXIV, occupies its separate identity by making monogenetic groups i.e. cluster X, cluster XVI, cluster VIII while forming clusters. These results may be due to its different genetic makeup from that of others. Since the cluster I consisted of 32 germplasms with appreciably high value of cluster distance, presence of high heterogeneity among the germplasms is expected. Further sub-clustering of cluster I is an approach towards the effective selection of desired parents for hybridization programme within the cluster.

CONCLUSION

The genetic diversity among the germplasms in the present study may be resulted from genetic drift and selection that cause greater genetic diversity than geographical distribution. Numerical taxonomic results show different genetic makeup with appreciably high value of cluster distance and presence of high heterogeneity among the germplasms. Further, sub-clustering of cluster 1 is an approach towards the effective selection of desired parents for hybridisation programme within the cluster.

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