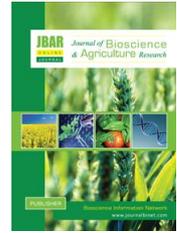


Published with Open Access at **Journal BiNET**

Vol. 09, Issue 01: 759-767

Journal of Bioscience and Agriculture ResearchHome page: www.journalbinet.com/jbar-journal.html

Genetic diversity for reproductive traits of CMS restorer lines of rice (*Oryza sativa* L.)

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ABSTRACT

An experiment was conducted to study genetic divergence in 29 CMS restorer lines of rice. The study was carried out at the experimental farm, Dept. of Genetics and Plant Breeding, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur during January, 2014 to June, 2014. Genetic divergence was estimated using D^2 and principal component analysis. PCA showed 71.2 % variation against first Eigen values. From the scattered diagram it was revealed that the genotypes Acc-7386/ SHAKTI-1R, Acc-7394/ ACI-1R, Acc-7407/ CHINA-2R, Acc-7404/ HB-8R and Acc-7409/ 1R-509R were distantly located. Nonhierarchical clustering using co-variance matrix grouped 29 CMS restorer lines of rice into five different clusters. PCO showed that the highest inter genotype distance (2.422) was observed between the genotypes Acc-7386/ SHAKTI-1R and Acc-7394/ ACI-1R. The lowest distance (0.458) was observed between the genotypes Acc-7396/ LP-106R and Acc-7397/ LP-108R. The genotypes of cluster II exhibited the highest mean value for 11 out of 21 characters studied followed by cluster V exhibited the highest mean value for 5 out of 21 characters studied. The genotypes of cluster I exhibited the lowest mean value for 8 out of 21 characters studied followed by cluster IV exhibited the lowest mean value for 6 out of 21 characters studied. There were marked variations in intra-cluster distances, which ranged from 0.000 to 1.066. Considering magnitude of genetic distance, contribution of different characters towards the total divergence, magnitude of cluster means for different characters and performance of the genotypes Acc-7386/ SHAKTI-1R, Acc-7394/ ACI-1R, Acc-7407/ CHINA-2R, Acc-7404/ HB-8R and Acc-7409/ 1R-509R might be selected for further hybrid breeding program.

Key Words: Eigen value, Cluster means, Divergence, Non-hierarchical clustering and D^2 analysis

Cite article: Jesmin, R. & Mian, M. A. K. (2016). Genetic diversity for reproductive traits of CMS restorer lines of rice (*Oryza sativa* L.). *Journal of Bioscience and Agriculture Research*, 09(01), 759-767.



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I. Introduction

Rice (*Oryza sativa* L.) is a self-pollinated cereal food plant, extensively cultivated in warm climates. It is generally considered as a semi aquatic annual plant, although it could survive as perennial in the tropics and subtropics. Rice is a cereal crop belonging to the family Gramineae (Poaceae) having

chromosome numbers $2n=24$ under the order Cyperales and class monocotyledon. It is self-pollinated crop. The genus *Oryza* includes a total of 22 to 27 species out of which only two are cultivated. The two cultivated species are *Oryza sativa* (Asian rice) and *O. glaberrima* (African rice). It consumed exclusively by humans, as it is a staple food for about 50% of the global population (Ramkumar et al., 2010). Rice is the staple food for at least 63% of planet inhabitants and contributes on an average 20% of apparent calorie intake of the world population (Calpe and Prakash, 2007). Per capita daily rice consumptions is about 450g in Bangladesh and it provides about 75% of the calories and 55% of Proteins in the average daily diet of the Bangladeshi people (Bhuiyan et al., 2002).

Worldwide, rice is cultivated on 166 million hectares and 745 million tons of rough rice is produced at an average productivity of 4.48 tons per hectare and Asia contributes up to 88% and 91% of the world's rice area and production, respectively (FAO, 2015). Bangladesh is the fourth largest producer and consumer of rice in the world with an annual production of about 33.5 million tones. Our total cultivable land is 14.85 million hectare whereas rice covered about 77 % of total cropped area (Julfiquar et al., 2009). Area under T.aman is the highest which is about 50.35% of total rice land and contributes 40.96% of the total rice production (BBS, 2012). Hybrids may offer to break through the yield ceiling of semi dwarf rice began in 1964. The discovery of CMS line in rice (Athwal and Virmani, 1972) suggested that breeding could develop a commercially viable F1 hybrid. The study on reproductive traits is one of the criteria of parent selection in three line hybrid system.

Asia is traditionally rich in the diversity of rice including the wild progenitors of cultivated rice. The indigenous rice germplasm of Bangladesh is enriched with wide genetic diversity and valuable gene system for yield attributes and adaptability. The Himalayan foot hills including parts of Bangladesh are considered to be the secondary center of diversity of the genus *Oryza* (Morishima, 1984). Genetic divergence analysis is one of the criteria of parent selection. In three line system (A/B/R) of heterosis breeding in rice, genetic diversity of the lines is very important.

Genetic diversity can be evaluated with morphological and reproductive traits. The D^2 technique is based on multivariate analysis developed by Mahalanobis (1936) had been found to be a useful tool in quantifying the degree of divergence in germplasm. Multivariate analysis with D^2 technique measures the amount of genetic diversity in a given population in respect of several characters and assesses relative contribution of different components to the total divergence (Zahan et al., 2008). This analysis provides a measurement of relative contribution of different components on diversity both at intra and inter-cluster level and genotypes drawn from widely divergent clusters are likely to produce heterotic combinations and wide variability in segregating generation (Ghafoor et al., 2001).

Considering the above idea in mind the present investigation was undertaken with the following objectives, to study the genetic diversity of reproductive traits among the CMS restorer lines and to identify the superior R line by comparing the reproductive traits.

II. Materials and Methods

Experimental site and design: The experiment was conducted at the experimental field of the Dept. of Genetics and Plant Breeding, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Salna, Gazipur-1706, during January, 2014 to June, 2014. The experimental site is located at the center of Madhupur Tract ($24^{\circ}09'$ N latitude and $90^{\circ}26'$ E longitude) having an elevation of 8.4m from sea level. The soil type of experimental field belongs to the shallow Red Brown Terrace type under Salna Series of Madhupur Tract (Brammer, 1971). The field is under Agro Ecological Zone (AEZ) 28 which is characterized by silty clay with pH value of 6.5. The climate of the experimental site is subtropical characterized by heavy rainfall during May to September and scanty during the rest of the year. The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. The whole experimental area was divided into three blocks, representing three replications. Twenty nine treatments were distributed in the experimental unit of each replication through randomization by using the IRRISTAT program.

Genotypes: The experiment was conducted using 29 CMS restorer lines. The seeds of all varieties were collected from genetic resources center (GRC), Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU) mentioned in (Table 01).

Collection of data : Data on phenology such as days to 10% flowering (DFF), days to 50% flowering (DF), days to maturity (DM), plant height (PH), effective tillers per plant (ETP), total tillers per plant (TTP), Panicle exertion rate (%) (PER) were recorded in time. At heading stage anther length (AL), anther breadth (AB), filament length (FL), Pollen sterility (%) (PS), Pollen fertility (%) (PF) were recorded. After maturity the selected ten hills of each genotype of each plot were harvested to record yield and yield component like Panicle length (PL), number of primary branches per panicle (NPBP), number of secondary branches per panicle (NSBP), filled grains per panicle (FGP), unfilled grains per panicle (UGP), Grain length-breadth ratio, 1000 grain weight (GW) and grain yield per plant (GYP).

Statistical analysis: Genetic diversity was analyzed using GENSTAT 5.13 software program (copyright 1987, Lawes agricultural Trust, Rothamsted Experimental Station, UK). Genetic diversity analysis involves several steps, i.e., estimation of distance between the varieties clustering and analysis of inter-cluster distances. Therefore, more than one multivariate technique are required to represent the results more clearly and it is obvious from the results of many researches (Bashar, 2002; Uddin, 2001; Junned et al., 1988; Ariyo, 1987; Patil et al., 1987; Anand and Rawat, 1984; Dani and Murthy, 1985).

Table 01. Sources of 29 CMS restorer lines of rice genotypes with their origin

Sl.	Genotypes/Accessions number	Source	Sl.	Genotypes/Accessions number	Source
01	Acc-7385/ HB-9R	BSMRAU	16	Acc-7400/ MOYNA-R	BSMRAU
02	Acc-7386/ SHAKTI-1R	BSMRAU	17	Acc-7401/ SL-8R	BSMRAU
03	Acc-7387/ BHD-1R	BSMRAU	18	Acc-7402/ BU-1R	BSMRAU
04	Acc-7388/ BHD-2R	BSMRAU	19	Acc-7403/ BU-7R	BSMRAU
05	Acc-7389/ BHD-3R	BSMRAU	20	Acc-7404/ HB-8R	BSMRAU
06	Acc-7390/ HERA-2R	BSMRAU	21	Acc-7405/ BU-3R	BSMRAU
07	Acc-7391/ HERA-5R	BSMRAU	22	Acc-7406/ BU-2R	BSMRAU
08	Acc-7392/ HERA-10R	BSMRAU	23	Acc-7407/ CHINA-2R	BSMRAU
09	Acc-7393/ MATAL-R	BSMRAU	24	Acc-7408/ CHINA-1R	BSMRAU
10	Acc-7394/ ACI-1R	BSMRAU	25	Acc-7409/ 1R-509R	BSMRAU
11	Acc-7395/ LP-70R	BSMRAU	26	Acc-7410/ BU-521R	BSMRAU
12	Acc-7396/ LP-106R	BSMRAU	27	Acc-7411/ BU-507R	BSMRAU
13	Acc-7397/ LP-108R	BSMRAU	28	Acc-7412/ BU-329R	BSMRAU
14	Acc-7398/ GOLD-R	BSMRAU	29	Bu dhan-2	BSMRAU
15	Acc-7399/ DOYEL-R	BSMRAU			

BSMRAU= Bangabandhu Sheikh Mujibur Rahman Agricultural University

III. Results and Discussion

Significant differences among genotypes in respect of reproductive traits are prerequisites of multivariate analysis. The replicated information of the present study of 29 restorer rice genotypes on analysis of variance revealed significant differences among all the genotypes for all the characters studied. A considerable amount of genetic diversity was observed and therefore diversity analysis was carried out through multivariate analysis. The value of 'V' statistics was showing significant difference among 29 genotypes.

Principal component analysis

The principal component analysis yielded Eigen values of each principal component axes of ordination of genotypes with the first axes totally accounted for the variation among the genotypes, while five of these with Eigen values above unity accounted for 71.2%. The first two principal axes accounted for 37.6 % of the total variation among the 21 characters describing 29 CMS restorer lines of rice (Table

02). Quantification of variability from genetic point of view is very scanty that the first axis largely accounted for the variation among the genotype (22.57) followed by second axis (15.04).

Table 02. Eigen values and percentage of variation for corresponding 21 component characters in 29 CMS restorer lines of rice

Identifier/Characters	Eigen values	% of total variation accounted for	Cumulative percent
Days to 10% Flowering	4.739	22.57	22.6
Days to 50% Flowering	3.159	15.04	37.6
Plant height	2.894	13.78	51.4
Effective tillers per plants	2.490	11.86	63.3
Total tillers per plants	1.676	7.98	71.2
Panicle exertion rate (%)	1.359	6.47	77.7
Days to maturity	1.202	5.73	83.4
Anther length	0.855	4.07	87.5
Anther breadth	0.614	2.92	90.4
Filament length	0.440	2.10	92.5
Pollen fertility (%)	0.385	1.83	94.4
Pollen sterility (%)	0.320	1.53	95.9
Panicle length	0.261	1.24	97.1
Primary branches per panicle	0.205	0.97	98.1
Secondary branches per panicle	0.151	0.72	98.8
Filled grains per panicle	0.119	0.57	99.4
Unfilled grains per panicle	0.082	0.39	99.8
Grain length-breadth ratio	0.031	0.15	99.9
1000 grain weight	0.015	0.07	100.0
Grain yield per plant	0.001	0.00	100.0
Yield (ton/ha)	0.000	0.00	100.0

Construction of scatter diagram

Based on the values of principal component scores 1 and 2 obtained from the principal component analysis, a two dimensional scatter diagram ($Z_1 - Z_2$) using component score 1 as X- axis and component score 2 as Y- axis was constructed, which has been presented in (Figure 01). The position of the genotypes in the scatter diagram was apparently distributed into five groups, which indicated that there exists considerable diversity among genotypes. The scattered diagram for the rice genotype revealed that the genotype number Acc-7386/ SHAKTI-1R, Acc-7394/ ACI-1R, Acc-7407/ CHINA-2R, Acc-7404/ HB-8R and Acc-7409/ 1R-509R were distantly located which suggested that the genotype were more diverged from rest of the genotypes.

Principal coordinate analysis

Principal coordinate analysis (PCO) was performed on auxiliary of Principal component analysis. This analysis helps in estimating distances (D^2) for all 630 combinations between pairs of genotypes. The highest inter genotype distance (2.422) was observed between the genotypes Acc-7386/ SHAKTI-1R and Acc-7394/ ACI-1R.

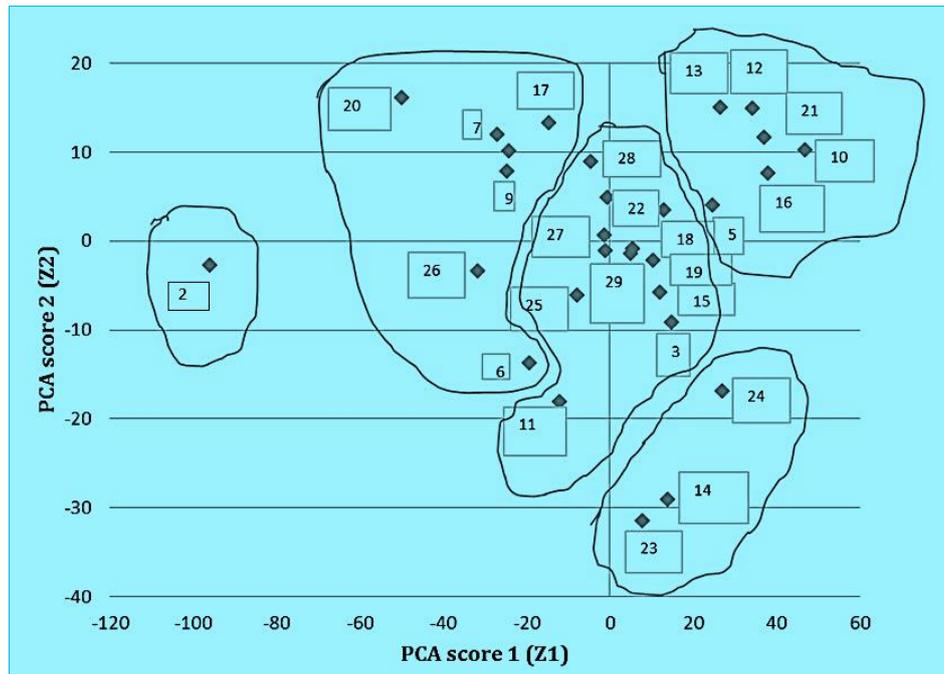


Figure 01. Scatter distribution of 29 CMS restorer lines of rice based on their principal component scores super imposed with clusters.

Table 03. Five of each higher and lower inter genotypic distance (D^2) between pairs of CMS restorer lines of rice

5 highest inter genotypic distances		
Sl. Number	Genotypes combination	Distances
01	(Acc-7386/ SHAKTI-1R)/ (Acc-7394/ ACI-1R)	2.422
02	(Acc-7394/ ACI-1R) / (Acc-7409/ 1R-509R)	2.19
03	(Acc-7394/ ACI-1R) / (Acc-7395/ LP-70R)	2.046
04	(Acc-7388/ BHD-2R) / (Acc-7404/ HB-8R)	1.894
05	(Acc-7385/ HB-9R) / (Acc-7386/ SHAKTI-1R)	1.863
5 lowest inter genotypic distances		
Sl. Number	Genotypes combination	Distances
01	(Acc-7396/ LP-106R) / (Acc-7397/ LP-108R)	0.458
02	(Acc-7396/ LP-106R) / (Acc-7405/ BU-3R)	0.498
03	(Acc-7397/ LP-108R)/ (Acc-7405/ BU-3R)	0.503
04	(Acc-7390/ HERA-2R) / (Acc-7399/ DOYEL-R)	0.537
05	(Acc-7390/ HERA-2R) / (Acc-7395/ LP-70R)	0.567

The fifth highest pair distance (1.863) was observed between the genotypes Acc-7385/ HB-9R and Acc-7386/ SHAKTI-1R. The lowest distance (0.458) was observed between the genotypes Acc-7396/ LP-106R and Acc-7397/ LP-108R. The fifth lowest pair distance (0.567) was observed between the genotypes Acc-7390/ HERA-2R and Acc-7395/ LP-70R. The difference between the highest and the lowest inter-genotypes distance indicated the prevalence of variability among the 29 genotypes of CMS restorer line of rice (Table 03).

There were marked variations in intra-cluster distances, which ranged from 0.000 to 1.066 (Table 04). The magnitudes of the intra-cluster distances were not always proportional to the number of genotypes in the clusters. In the present study it was found that although both cluster III & IV

composed of the largest number of genotypes (12) & (7) respectively and their intra-cluster distances were first (1.066) and fourth (0.927) among the five clusters (Table 04).

The intra-cluster distances in all the 5 clusters were more or less low indicated the genotypes within the same cluster were closely related. The highest intra-cluster distances was computed for cluster III (1.066) composed of 12 genotypes followed by the cluster V (0.978) composed of 3 genotypes. However the lowest value (0.000) of intra-cluster distance in cluster II indicated only one genotype (Acc-7386/ SHAKTI-1R) constituted this cluster might have diverged characters, which contributed to the formation of this cluster (Table 04).

Table 04. Average inter cluster distance (D^2) and intra-cluster distance (bold) for 29 CMS Restorer lines of rice

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
Cluster I	0.959				
Cluster II	47.86	0.000			
Cluster III	8.49	40.28	1.066		
Cluster IV	18.25	29.63	10.86	0.927	
Cluster V	15.91	46.62	12.54	20.53	0.978

Canonical variate analysis

Statistical distances represent the index of genetic diversity among the clusters. The inter-cluster distances were bigger than the intra-cluster distances suggesting wider genetic diversity among the genotypes of different groups. The inter-cluster distance was maximum between cluster I and II (47.86) while the distance was minimum between cluster I and III (8.49). The maximum values of inter-cluster distance indicated that the genotypes belonging to cluster I was far diverged from those of cluster II. These relations were also reflected in the scatter diagram (Figure02). Basher et al., (2007) reported that inter-cluster distances were larger than intra-cluster distances in a multivariate analysis in rice. The genotypes belonging to clusters III and V having greater intra-cluster distance are recommended for inclusion in a hybridization program as they are expected to produce good segregants.

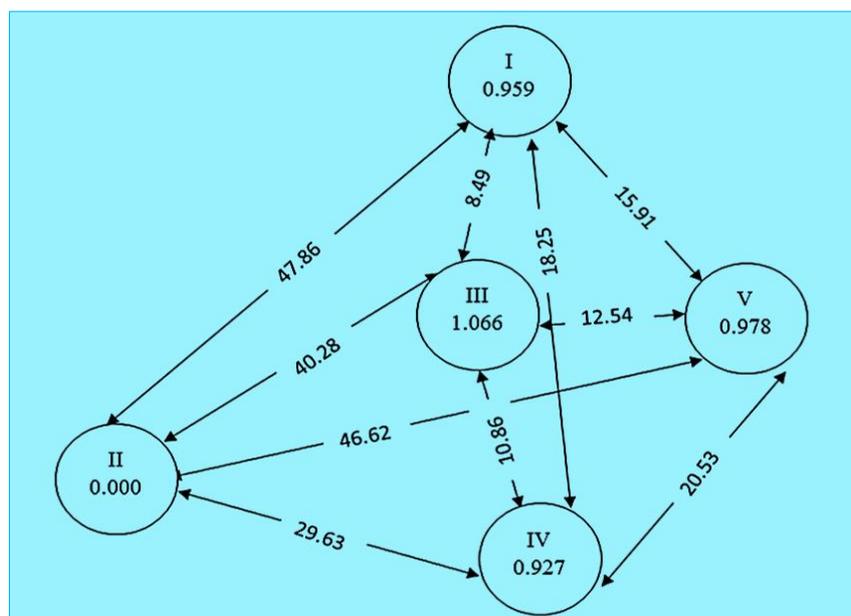


Figure 02. Diagram showing intra and inter cluster distances of twenty nine CMS restorer line of rice.

Table 05. Distribution of twenty nine CMS restorer lines of rice in five clusters

Cluster	Member	Genotypes no.	Local Name
I	6	5, 10, 12, 13, 16 & 21	Acc-7389/ BHD-3R, Acc-7394/ ACI-1R, Acc-7396/ LP-106R, Acc-7397/ LP-108R, Acc-7400/ MOYNA-R, Acc-7405/ BU-3R
II	1	2	Acc-7386/ SHAKTI-1R
III	12	1, 3, 4, 11, 15, 18, 19, 22, 25, 27, 28 & 29	Acc-7385/ HB-9R, Acc-7387/ BHD-1R, Acc-7388/ BHD-2R, Acc-7395/ LP-70R, Acc-7399/ DOYEL-R, Acc-7402/ BU-1R, Acc-7403/ BU-7R, Acc-7406/ BU-2R, Acc-7409/ 1R-509R, Acc-7411/ BU-507R, Acc-7412/ BU-329R & Basmati Indian
IV	7	6, 7, 8, 9, 17, 20 & 26	Acc-7390/ HERA-2R, Acc-7391/ HERA-5R, Acc-7392/ HERA-10R, Acc-7393/ MATAL-R, Acc-7401/ SL-8R, Acc-7404/ HB-8R & Acc-7410/ BU-521R
V	3	14, 23 & 24	Acc-7398/ GOLD-R, Acc-7407/ CHINA-2R & Acc-7408/ CHINA-1R

Table 06. Cluster mean for twenty one characters in CMS restorer lines of rice

Characters	Clusters				
	I	II	III	IV	V
Days to 10% Flowering	117.83	86.00	109.42	112.57	90.67
Days to 50% Flowering	122.50	120.00	116.58	117.29	112.33
Plant height	96.83	105.00	99.25	103.00	102.67
Effective tillers per plant	11.17	10.00	11.58	9.29	14.33
Total tillers per plant	13.83	11.00	13.58	11.14	16.67
Panicle exertion rate (%)	1.67	2.00	1.42	1.43	1.67
Days to maturity	141.00	139.00	138.50	137.71	138.33
Anther length	78.79	78.59	76.97	76.49	81.53
Anther breadth	11.82	11.44	11.18	11.13	12.24
Filament length	40.52	41.22	38.52	37.78	40.52
Pollen fertility (%)	82.00	87.00	85.83	83.57	87.33
Pollen sterility (%)	18.00	13.00	14.17	16.43	12.67
Panicle length	25.47	26.90	25.73	26.67	25.97
Primary branches per panicle	8.50	13.00	9.08	10.14	9.00
Secondary branches per panicle	28.33	50.00	33.17	37.86	30.33
Filled grains per panicle	73.00	191.00	99.75	130.00	81.00
Unfilled grains per panicle	30.00	53.00	23.17	23.57	26.67
Grain length-breadth ratio	3.53	4.08	3.45	3.69	3.43
1000 grain weight	14.83	15.00	21.58	21.00	17.67
Grain yield per plant	26.83	65.40	43.41	47.39	51.47
Yield (ton/ha)	7.17	17.00	11.67	12.57	13.67

Non-hierarchical clustering

Non-hierarchical clustering using co-variance matrix grouped 29 CMS restorer line of rice into five different clusters. These results confirmed the clustering pattern of the genotypes obtained through principal component analysis. So the results obtained through PCA were confirmed by non-hierarchical clustering. Number of genotypes in five clusters ranged from 1 to 12 in different clusters (Table 05). The distribution pattern indicated that the maximum number of genotypes (12) was included in cluster III followed by cluster IV (7), cluster I (6), cluster V (3) and minimum genotype constituted of the cluster II (Acc-7386/ SHAKTI-1R).

Intra-cluster mean

The genotypes in cluster I showed the highest desirable mean value for pollen sterility (%) (Table 06). Similarly the genotypes in cluster II showed the highest desirable mean value for plant height, panicle exertion rate (%), filament length. It also showed highest mean value for panicle length, primary branches per panicle, secondary branches per panicle, filled grains per panicle, unfilled grains per panicle, grain length-breadth ratio, grain yield per plant and yield (ton/ha) and lowest mean value for days to 10% flowering followed by the genotypes in cluster III which showed the highest desirable mean value for 1000 grain weight. The genotypes in cluster IV showed lowest desirable mean value for days to maturity. Finally the genotypes in cluster V showed the highest desirable mean value for effective tillers per plant, total tillers per plant, anther length, anther breadth, pollen fertility (%) and grain yield per plant. From the overall discussion it was revealed that in most of the cases genotypes of cluster II showed the maximum values where as in most of the cases the lowest values were found in cluster I & IV. These results indicated that these characters had the highest contribution towards the divergence among the 29 CMS restorer line of rice. Bidhan et al. (2002) found that days to 50% flowering, grain length and grain yield per plant were major yield contributing characters to rice genetic diversity.

IV. Conclusion

Considering genetic diversity for reproductive traits considerable variability was observed among the 29 CMS restorer lines of rice. As per cluster analysis based on 21 characters the 29 CMS restorer lines of rice were grouped into 5 clusters. The genotypes of cluster II exhibited the highest mean value for 11 out of 21 characters studied. The genotypes of cluster I exhibited the lowest mean value for 8 out of 21 characters studied. The scattered diagram for the rice genotype revealed that the genotype number Acc-7386/ SHAKTI-1R, Acc-7394/ ACI-1R, Acc-7407/ CHINA-2R, Acc-7404/ HB-8R and Acc-7409/ 1R-509R were distantly located which suggested that the genotype were more diverged from rest of the genotypes. The highest intra-cluster distances was computed for cluster III (1.066) composed of 12 genotypes. However the lowest value (0.000) of intra-cluster distance in cluster II indicated only one genotype (Acc-7386/ SHAKTI-1R) constituted this cluster might have diverged characters, which contributed to the formation of this cluster. Genotypically distant parents are usually able to produce higher heterosis. Considering magnitude of genetic distance, contribution of different characters towards the total divergence, magnitude of cluster means for different characters and performance of the genotypes Acc-7386/ SHAKTI-1R, Acc-7394/ ACI-1R, Acc-7407/ CHINA-2R, Acc-7404/ HB-8R and Acc-7409/ 1R-509R may be considered to perform better if used in hybrid breeding program. For getting precise idea about diversity molecular approach is recommended.

Acknowledgement

Authors are very much grateful to the Ministry of Science and Technology, Government of Bangladesh for financial support to complete this research.

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