

Genetic variability in cytoplasmic male sterile lines in rice (*Oryza sativa* L.)

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Abstract: Diversification of cytoplasmic male sterile (CMS) lines in hybrid rice technology contribute a pivotal role towards the development of superior hybrids. A study was conducted on fifteen elite CMS lines of diverse source of origin to estimate nature and magnitude of genetic divergence and correlation analysis of different agro-morphic traits. Out of fifteen CMS lines, six (6) CMS lines namely IR 58025A, IR 79156A, IR 80559A, IR 68897A, DRR 5A and Pusa 6A exhibited complete pollen sterility (100 %). Based on eight characters, the genotypes were grouped into five (5) clusters at 74% similarities coefficient. The genetic distances between the few CMS lines showed some correlation with geographic origin. In some case, the correlation between the genotypes not suggested the same trend which might be due to genetic drift. The CMS lines having the characters in the genetically distant cluster could, therefore, offer an important role in the development of hybrid rice varieties.

Key Words: Cytoplasmic male sterile lines, Genetic diversity, Hybrid rice.

I. Introduction

Rice (*Oryza sativa* L.) is a universal food crop, feeding more than half of the world's population every day. In India, it occupies about one-quarter of cropped area and contributes 40% to 43% of total food grain production and 46% of total cereal production (Krishnaiah and Shoba Rani, 2000). In concern over the growing population in India, it needs to increase the productivity of rice (Sidharthan *et al.* 2007). The venture of second green revolution in India specially in Eastern India is basically focused on improved rice productivity. Hybrid rice offers a wide opportunity to improve rice productivity in India. Hybrid rice has the potential to increase yields by 15% to 20% over those of conventionally bred varieties (Virmani, 1994). Chinese rice scientists developed rice hybrids utilizing cytoplasmic male sterile (CMS) system which boosted up the yield by about 20 percent over semi dwarf rice varieties (Yuan, 1977 and Yuan *et al.* 1989). The CMS lines play a pivotal role in success and sustenance of hybrid rice technology. In fact, the belated success of hybrid rice technology in India was basically due to the nonavailability of CMS lines suited to tropics (Ahmed *et al.* 1998). Knowledge of genetic diversity among prospective parental lines is important for the success of a hybrid rice breeding program as it determines the magnitude of heterosis in F1 hybrids to a large extent (Kaladhar *et al.* 2004).

II. Materials and Methods

The experimental materials for the present study consist of fifteen cytoplasmic male sterile (CMS) lines of rice (Table - 1) namely IR 58025A, IR 68897A, IR 80555A, IR 79156A, IR 80559A, PMS 3A, PMS 10A, Satabdi A, DRR 4A, DRR 5A, DRR 6A, COMS 15A, COMS 18A, APMS 6A and PUSA 6A. The investigation was carried out at Rice Research Station (Govt. of West Bengal), Chinsurah during the boro season 2011-12. The lines were planted in randomized block design with three replications. Each CMS line was sown in single row with plant to plant spacing 15 cm and row to row spacing 30 cm. Normal recommended cultural practices and plant protection measured were followed. Observations were recorded for plant height (in cm), days to 50% flowering, panicles per plant, spikelets per panicle, panicle length (in cm), 1000 seed-weight (in gm), pollen sterility percentage (%) and spikelet fertility percentage (%). The pollen testing was done by using 2% iodine – potassium iodide stain (Rosamma *et al.* 2005) by keeping 10 plant of each CMS in isolation. Spikelet fertility was observed on ten spikelets of different plant by bagging the panicles with butter paper prior to anthesis. The data were statistically analyzed by using software SPAR 2 version. The UPGMA based dendrogram of fifteen cytoplasmic male sterile (CMS) lines of rice was computed with the help of Software Darwin 5.0 version.

III. Results and Discussion

The CMS lines are highly sensitive to environmental conditions resulting in fertile pollen production (Pardhe *et al.* 2011). The result evinced that out of fifteen (15) cytoplasmic male sterile (CMS) lines, six (6) CMS lines namely IR 58025A, IR 79156A, IR 80559A, IR 68897A, DRR 5A and Pusa 6A performed complete

pollen sterility (100 %), Seven (7) CMS lines had pollen sterility ranging from 97.2% to 99.5% and others two (2) had 92.33% and 96% pollen sterility with the mean of 98.45% in the existing agroclimatic situation (Table-2).

In hybrid breeding program, utilization of parental lines with considerable variability is of primary concern for exploitation of maximum level of heterosis or hybrid vigor in the F₁ seed production (Rajendra *et al.* 2012). In order to have good parental lines for hybrid rice technology, it is necessary to assess the genetic diversity among parental lines (Bar-Hen *et al.* 1995). Analysis of variance revealed high and significant variation for all the characters under the study indicating considerable variation present in the materials. The estimation of genetic parameters like genotypic coefficient of variation, heritability and genetic advance are presented in table - 4. The maximum range of genotypic variation was observed for spikelet fertility percentage. The phenotypic coefficient of variation (PCV) was greater than genotypic coefficient of variation (GCV) in all included characters. After keen observations, it was indicated close resemble between the corresponding estimation of PCV and GCV in almost all characters except spikelet fertility, concluded that the environment had little role to play in the expression of this character.

High heritability percentage was observed in almost all characters except for spikelet fertility percentage (55.69 %). Highest heritability percentage was recorded 99.64 % for the character 1000 seed-weight, followed by days to 50% flowering (99.37 %), Plant height (97.13 %) and spikelets per panicle (91.06 %) respectively. The characters panicle per plant, 1000 Seed weight, spikelets per panicle, panicle length, days to 50% flowering and plant height exhibited high heritability coupled with moderate genetic advance which conclude due to the interaction between additive and non-additive gene action.

The phenotypic and genotypic correlations among the traits are presented in table- 5. The phenotypic and genotypic correlations were closely agreed for the most of characters, where as in some cases the difference was higher that signified the role of environmental effects in estimating these characters. Highest strong negative significant correlation was observed in between Spikelet fertility percentage and pollen sterility percentage at both genotypic and phenotypic level [$r = -0.892$ (at genotypic level), -0.843 (at phenotypic level)] respectively followed by in between plant height and days to 50% flowering [$r = -0.699$ (at genotypic level), -0.693 (at phenotypic level)], 1000 seed weight and days to 50% flowering [$r = -0.405$ (at genotypic level), -0.403 (at phenotypic level)] respectively. Panicle length and spikelet per panicle were correlated positively and significantly at both genotypic and phenotypic level [$r = 0.616$ (at genotypic level), 0.669 (at phenotypic level)] respectively followed by spikelet per panicle and 1000 Seed weight [$r = 0.580$ (at genotypic level), 0.548 (at phenotypic level)] respectively. Non significant and negligible associations were also present in between some characters like days to 50% flowering and spikelet per panicle, days to 50% flowering and panicle per plant etc.

Analysis of variance revealed the CMS lines differed significantly for all the characters under the study. This signified that it would be judicious to classify the population on the basis of degree of divergence. The fifteen CMS lines based on eight morphological characters were grouped into five (5) clusters at 74% similarities coefficient. The distribution pattern indicated that maximum nine (9) number of genotypes were included in cluster V, while cluster I and cluster II consisted of single genotype in each. The cluster III and IV comprised of two genotypes in each (Fig 1).

Again the fifteen CMS lines were grouped into twelve (12) clusters at 90% similarities coefficient. The clusters I, II, IV, V, VI, VII, VIII, IX and X consisted of single genotype in each. The clusters III, XI and XII consisted of two genotypes in each. The genetic distances between the CMS lines showed some correlation with geographic origin (Table - 6). Both the lines IR 79156A and IR 80555A occupied same cluster I above 90% similarities coefficient. The correlation also suggested they were closest ($D = 0.452$). The others three IRR1 originated CMS lines namely IR 80559A, IR 68897A and IR 58025A were also belonged to cluster I at 74% similarities coefficient. But in case of IR 80559A and IR 68897A (both from IRR1) not suggested the same trend ($D = 2.187$) which might be due to genetic drift.

IV. Tables And Figure

Table 1: Origin of Cytoplasmic Male Sterile (CMS) Line or A line and their respective maintainer or B line

Sr. No.	CMS Line (A line) and Maintainer line (B line)	Origin
1	IR 58025A & B	IRRI [*] , Philippines
2	IR 68897A & B	IRRI [*] , Philippines
3	IR 80555A & B	IRRI [*] , Philippines
4	IR 79156 A & B	IRRI [*] , Philippines
5	IR 80559A & B	IRRI [*] , Philippines
6	PMS 3A & B	PAU [*] , Punjab
7	PMS 10A & B	PAU [*] , Punjab
8	Satabdi A & B	RRS [*] , Chinsurah, W.B
9	DRR 4A & B	DRR [*] , Hyderabad
10	DRR 5A & B	DRR [*] , Hyderabad
11	DRR 6A & B	DRR [*] , Hyderabad
12	COMS 15A & B	TANU [*] , Coimbatore
13	COMS 18A & B	TANU [*] , Coimbatore
14	APMS 6A & B	APRRS [*] , Andhra Pradesh
15	PUSA 6A & B	IARI [*] , New Delhi

* APRRS - Andhra Pradesh Rice Research Institute, Maruteru, India

DRR - Directorate of Rice Research, Hyderabad, India

IARI - Indian Agricultural Research Institute, New Delhi, India

IRRI - International Rice Research Institute, Philippines

PAU - Punjab Agricultural University, Ludhiana, India

RRS - Rice Research Station, Chinsurah, West bengal, India

TANU – Tamilnadu Agricultural University, Coimbatore, India

Table 2: Mean values of CMS lines for various traits.

Genotypes	Plant Height (cm)	Days to 50% flowering	Panicle / Plant	Spikelet /Panicle	Panicle Length (cm)	1000 S-W(gms)	Pollen Sterility %	Spikelet Fertility %
IR 58025A	87.33	97.33	16.00	122.00	17.83	18.40	100.00	0
IR 68897A	104.33	87.00	14.67	132.00	18.77	19.57	100.00	0
IR 80555A	89.00	71.33	11.67	131.00	16.00	21.43	97.90	8.97
IR 79156 A	93.67	91.67	12.00	127.67	20.00	18.37	100.00	0
IR 80559A	78.67	105.00	16.00	156.67	23.33	17.93	100.00	0
PMS 3A	62.00	98.33	9.33	133.33	18.27	16.40	92.33	4.93
PMS 10A	64.00	101.00	8.67	125.00	22.53	16.73	97.20	1.43
Satabdi A	85.67	84.67	14.00	162.67	20.00	19.90	97.67	1.23
DRR 4A	63.00	93.67	10.33	170.33	21.67	19.70	99.60	0
DRR 5A	79.00	94.67	12.00	119.33	17.97	17.70	100.00	0
DRR 6A	91.67	90.33	13.33	131.00	19.67	15.80	99.27	0
COMS 15A	95.00	74.33	8.67	148.67	19.33	22.37	97.27	6.1
COMS 18A	90.67	80.67	8.67	179.33	18.83	25.07	99.57	1.33
APMS 6A	89.33	85.33	8.67	113.00	15.47	16.87	96.00	3.3
PUSA 6A	82.00	103.00	14.33	122.33	14.20	14.13	100.00	0
Mean	83.69	90.56	12.07	138.29	18.92	18.69	98.45	1.82
Range	62.00 - 104.33	71.33 - 105.00	8.67 - 16.00	113.00 - 179.33	14.20 - 23.33	14.13 - 25.07	92.33 - 100	0 - 8.97
SE (1%)	1.19	0.53	0.70	3.60	0.70	0.09	0.80	1.09

Table 3 : Analysis of Variance (ANOVA) table of various traits.

SL. NO.	Characters	Source of Variance M.S (df)		
		Replication (2)	Variety(14)	Error (28)
1	Plant Height	66.42	440.93**	4.30
2	Days to 50% flowering	0.80	401.78**	0.85
3	Panicle / Plant	26.69	22.41**	1.47
4	Spikelet /Panicle	607.29	1227.80**	38.90
5	Panicle Length	30.4	18.9**	1.50
6	1000 Seed-Weight	0.04	23.19**	0.03
7	Pollen Sterility %	3.83	11.59**	1.93
8	Spikelet Fertility %	28.19	17.03**	3.57

*significant at 5% probability level, **significant at 1% probability level,

Table 4 : Comparative study of Critical Difference (CD), Coefficient of Variance (CV), Genetic Coefficient of Variance (GCV), Phenotypic Coefficient of Variance (PCV), Heritability % (H%) and Genetic Advance (GA), Genotypic Variance (σ^2_g), Phenotypic Variance (σ^2_p) and Environmental Variance (σ^2_e) of different characters

Characters	CD		CV	GCV	PCV	H %	GA	σ^2_g	σ^2_p	σ^2_e
	at 1%	at 5%								
Plant Height	4.68	3.47	2.40	13.97	14.18	97.13	25.23	145.54	149.84	4.30
Days to 50% flowering	2.08	1.54	1.07	13.39	13.43	99.37	27.49	133.65	134.49	0.85
Panicle / Plant	2.74	2.03	10.21	22.22	24.46	82.56	41.50	6.98	8.45	1.47
Spikelet /Panicle	14.07	10.43	4.51	14.39	15.09	91.06	28.29	396.30	435.21	38.91
Panicle Length	2.76	2.04	6.47	12.74	14.29	79.50	23.46	5.82	7.32	1.50
1000 Seed-Weight	0.38	0.28	0.89	14.87	14.89	99.64	30.52	7.72	7.75	0.03
Pollen Sterility %	3.14	0.32	1.42	1.83	2.32	62.51	3.79	3.22	5.15	1.93
Spikelet Fertility %	4.26	3.16	67.48	75.66	100.38	55.69	116.39	4.49	8.06	3.57

Table 5: Correlation Analysis among pairs of the characters

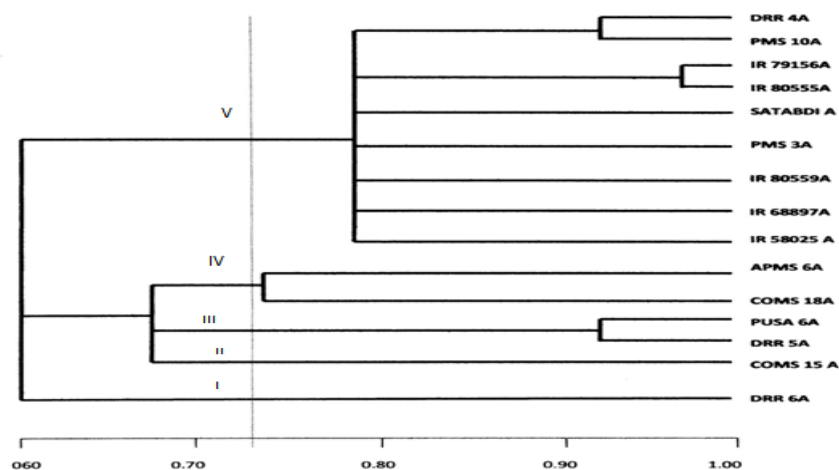
Characters	Plant Height	Days to 50% flowering	Panicle / Plant	Spikelet /Panicle	Panicle Length (cm)	1000 S-W	Pollen Sterility %	Spikelet Fertility %
Plant Height	1							
Days to 50% flowering	P-0.693** G-0.699**	1						
Panicle / Plant	P 0.105 G 0.135	P -0.086 G -0.102	1					
Spikelet /Panicle	P 0.197 G 0.213	P 0.045 G 0.049	P -0.114 G -0.112	1				
Panicle Length	P -0.111 G -0.144	P 0.543* G 0.618*	P -0.004 G 0.027	P 0.669** G 0.616**	1			
1000 Seed-Weight	P 0.490 G 0.499	P -0.403 G -0.405	P -0.277 G -0.305	P 0.548* G 0.580*	P 0.138 G 0.155	1		
Pollen Sterility %	P 0.404 G 0.519*	P -0.164 G -0.213	P 0.319 G 0.321	P 0.101 G 0.168	P -0.021 G 0.047	P 0.141 G 0.194	1	
Spikelet Fertility %	P 0.187 G 0.221	P -0.389 G -0.527*	P -0.199 G -0.207	P -0.075 G -0.129	P -0.195 G -0.345	P 0.229 G 0.299	P-0.892** G-0.843**	1

*significant at 5% probability level, **significant at 1% probability level, P=Phenotypic correlation coefficient, G= Genotypic correlation coefficient

Table 6: Genetic distance (D) between the cytoplasmic male sterile (CMS) lines

	IR 58025A	IR 68897A	IR 80555A	IR 79156A	IR 80559A	PMS 3A	PMS 10A	Satabdi A	DRR 4A	DRR 5A	DRR 6A	COMS 15A	COMS 18A	APMS 6A	PUSA 6A
IR 58025A		0.693	0.500	1.792	0.982	0.375	0.417	0.083	1.037	0.067	0.196	0.096	0.305	0.348	0.083
IR 68897A			0.250	0.501	2.187	0.167	1.386	0.693	0.522	0.174	0.131	0.333	0.691	0.174	0.942
IR 80555A				0.452	1.15	1.525	0.218	0.348	0.174	1.115	0.087	0.167	1.009	1.485	0.096
IR 79156A					0.131	0.265	1.556	0.283	0.559	1.307	0.056	2.485	0.022	0.386	1.997
IR 80559A						1.189	0.256	0.183	0.225	1.559	1.268	0.007	0.097	0.226	0.256
PMS 3A							0.986	1.422	0.008	0.169	0.556	0.246	0.516	0.287	0.213
PMS 10A								0.158	0.101	1.021	2.487	0.126	0.316	0.589	1.010
Satabdi A									1.089	1.026	1.087	0.246	0.586	0.387	0.589
DRR 4A										0.222	1.792	1.525	2.072	1.021	1.086
DRR 5A											1.056	1.089	0.014	0.018	0.041
DRR 6A												0.286	1.056	0.120	0.786
COMS 15A													1.084	1.014	0.008
COMS 18A														0.281	0.052
APMS 6A															0.579
PUSA 6A															
PUSA 6A															

Fig -1 : Dendrogram of fifteen cytoplasmic male sterile (CMS) lines in rice based on eight morphological characters



V. Conclusion

Analysis of variance revealed high and significant variability, therefore the CMS lines may be used for further manipulation to develop superior hybrid rice varieties. Perusal of data were indicated close resemble between the corresponding estimation of PCV and GCV in almost all characters except spikelet fertility, evinced that the environment had little role to play in the expression of this character. Out of fifteen CMS lines, six (6) CMS lines namely IR 58025A, IR 79156A, IR 80559A, IR 68897A, DRR 5A and Pusa 6A exhibited complete pollen sterility (100 %) at this agroclimatic condition. The genotype IR 80555A performed most early flowering (71.33days) as well as maturing CMS line followed by COMS 15A (74.33 days), both can be used as female parents in developing early hybrid varieties. The range of days to 50% flowering of CMS lines namely COMS 18A, Satabdi A, APMS 6A, IR 68897A, IR 79156A, DRR 4A, DRR 5A and DRR 6A was varied from 80 days to 95 days, so these CMS lines were suitable as female parents for mid-early hybrid varieties. The phenotypic and genotypic correlations were closely agreed for the most of characters, where as in some cases like in between panicle per plant and panicle length, panicle length and spikelet fertility percentage the difference was higher that signified the role of environmental effects in estimating these characters. The CMS lines having the

characters in the genetically distant cluster could offer a pivotal role in for the development of hybrid rice varieties.

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