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GENETIC DIVERSITY IN A AND B LINES BASED ON TRAITS INFLUENCING OUTCROSSING IN RICE (*ORYZA SATIVA* L.)

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ABSTRACT

Seventeen pairs of CMS (A) and maintainer (B) lines were evaluated for fifteen traits (both floral and morphological) influencing outcrossing in rice. Considerable variation exhisted in the population for all the traits. B lines had bigger anthers, greater plant height and early flowering whereas A lines were characterized by pronounced stigmatic traits, longer duration of anthesis and poor panicle exsertion. Male sterility followed by source sterile cytoplasm and geographical diversity were the main factors responsible for the diversity. V20 A can be used as potential source of sterile cytoplasm. Some Punjab male sterile line (PMS) were found promising but need improvement in panicle exsertion. Seven clusters were formed and these clusters can be utilised to identify and develop CMS lines with good outcrossing ability.

Key words: CMS lines, floral morphology, outcrossing, genetic diversity, rice.

In the development of rice hybrids utilising A, B and R system, production and maintenance of A lines is a basic and vital component as commercial seed production of hybrids, needs CMS line seed in large quantities. Thus, high degree of outcrossing on A lines is essential.

Rice, being an autogamous plant, does not encourage outcrossing. However, higher outcrossing was reported as a function of floral morphology and flowering behaviour of both A line and its male parent [1]. Limited studies on floral as well as morphological characters that influence outcrossing have been done [2–4]. Therefore, it is necessary to evaluate both A and B lines for those traits that influence outcrossing. With this background, a set of seventeen pairs of A and B lines was evaluated for fifteen traits, i.e., anther length, anther breadth, anther size, stigma length, stigma breadth, stigma size, stigma protrusion,

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style length, duration of anthesis, plant height, flag leaf angle, panicle length, panicle exsertion, days to flowering and pollen fertility.

Genetic diversity studies were carried out to estimate amount of diversity existing in the population based on above traits and factors responsible for diversity were also identified. The genetically diverse parents are likely to produce high heterotic effects and desirable segregates [5]. These studies are useful for development of CMS lines with potential outcrossing ability.

MATERIALS AND METHODS

The seventeen pairs of A and B lines of different origin used in the study are listed in Table 1. The experiment was laid out during dry season in the wet lands of the University of Agricultural Sciences, Bangalore. The anther and stigma characters, style length and pollen sterility were measured under microscope using occular micrometer. Stigma protrusion was measured quantitatively based on frequencies of stigma lobe protruding outside and the genotypes were classified as single lobe protrusion (S), double lobe protrusion (D) no lobe protrusion (zero) and their combinations, i.e. (D+O) (D+S), (S+D), (S+O), (O+S) etc. Based on the relative advantage these observations were converted to score for convenience in statistical analysis. The angle between flag leaf and the panicle was measured with protractor and recorded as flag leaf angle. Duration of anthesis was recorded from the time of opening of the first spikelet on a panicle to the complete closure of all spikelets in that panicle the same day. Five random panicles from each unit of genotype were taken for observation and mean values calculated for each trait. Genetic diversity was calculated using the weighted pair average method as suggested by Sokal and Sneath [6]. By this method a dendrogram was made on the axis of dissimilarity between genotypes and group of genotypes. The nodes, number of genotypes in each node and extent of dissimilarity are given in Table 3 and the dendrogram in Fig. 1.

Table 1. List of CMS lines and maintainers of rice used in the experiments

CMS line/maintainer	Origin	Sterile cytoplasm
V20A & V20 B	China	WA
IR 54752 A & B	IRRI	WA
IR 62829 A & B	IRRI	WA
IR 58025 A & B	IRRI	WA
Mangala A & B	Local	MS 577A
Pushpa A & B	Local	MS 577A
Intan Mutant A & B	Local	MS 577A
PMS 1A & B	Punjab	WA
PMS 2A & B	Punjab	WA
PMS 3A & B	Punjab	WA
PMS 4A & B	Punjab	WA
PMS 5A & B	Punjab	WA
PMS 6A & B	Punjab	WA
PMS 7A & B	Punjab	WA
PMS 8A & B	Punjab	WA
PMS 9A & B	Punjab	WA
PMS 10A & B	Punjab	WA

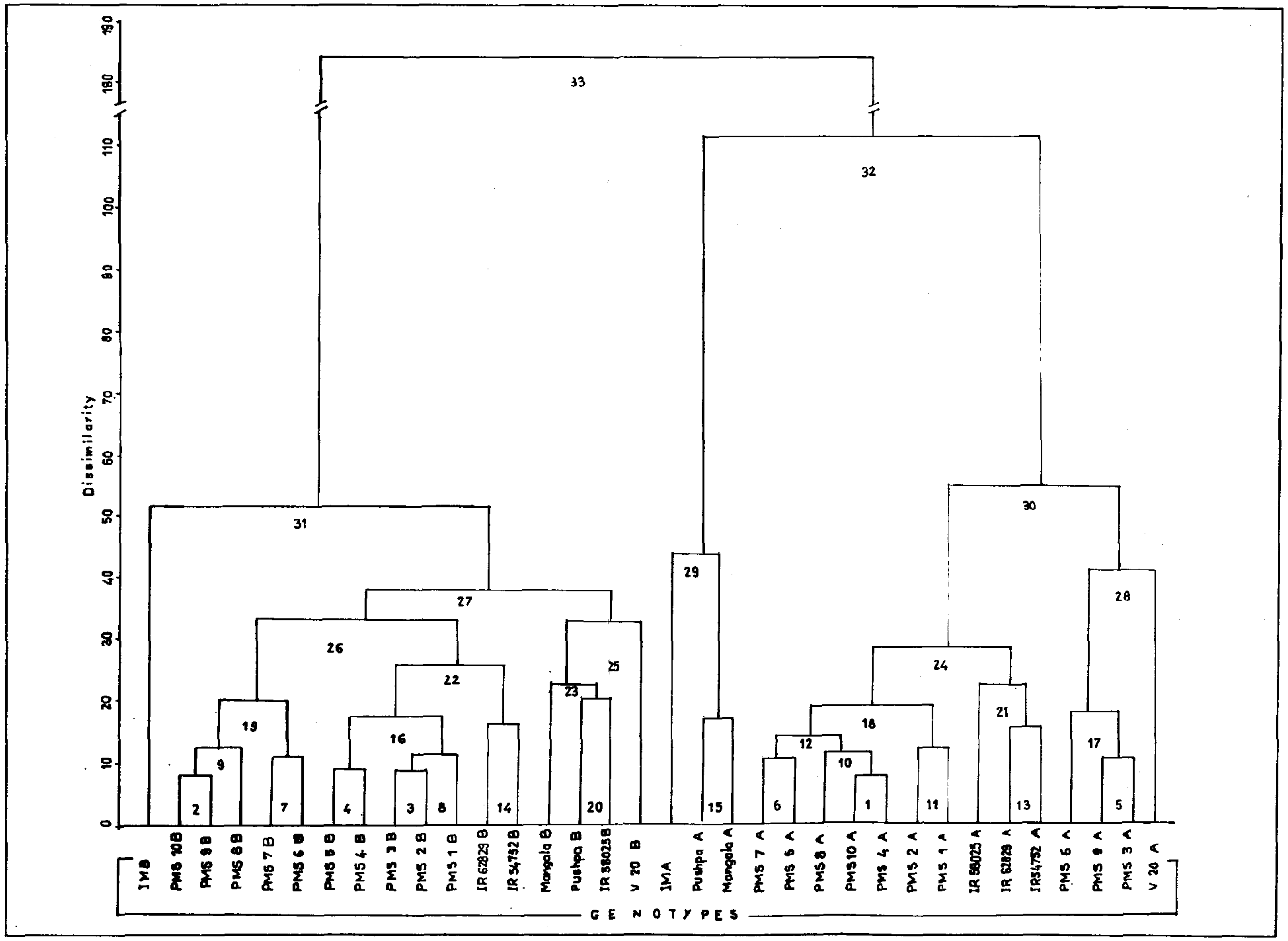

Fig. 1. Dendogram showing genetic diversity in a set of 17 pairs of A & B lines based on traits influencing outcrossing.

RESULTS AND DISCUSSION

A perusal of Table 2 shows that the variability in the population within the group of A lines and group of B lines was considerable for all the traits under study revealing scope of development of CMS lines with good outcrossing ability. Similar results were reported earlier [7] with anther and stigma characters in rice.

In general, the anthers of B lines were bigger than those of A lines. However such difference was not observed in case of Mangala, Pushpa and Intan Mutant, which have a different mechanism of male sterility as they were derived from MS 577A source.

The stigma traits of A lines were more pronounced than those of B lines except in the Intan Mutant. Similar trend was observed by Ramachandra [8]. Stigma protrusion and duration of anthesis were much more in A lines than in B lines as was mentioned in Annual Report of IRRI 1981. Delay and/or failure in pollination prolonged the duration of anthesis and consequently, CMS lines had a longer duration of anthesis [2, 9].

The percentage of panicle exsertion was poor in all A lines, especially in the PMS lines and V20A. Poor panicle exsertion and dwarfness of CMS lines may be due to sterile cytoplasm [10]. The A lines with good panicle exsertion are IMA (89.8%), Pushpa (86.6%) and IR 62829 A (75.3%). Virmani et al. [2] attributed better seed set to better panicle exsertion. Well exserted panicles were also advocated for higher seed set by others [4, 11]. Chinese workers spray GA3 to increase panicle exsertion in A lines to get higher seed set.

The maintainer lines flowered earlier than their corresponding CMS lines. Similar trend was observed earlier [12, 13]. Staggering of sowing dates is necessary to have synchronous flowering. PMS 6A and PMS 7A had about 85% pollen sterility which need purification. V20A and V20B recorded 100 and 0% sterility indicating their stability for male sterility and fertility respectively.

Not much difference was observed between A and B lines with regard to flag leaf angle and panicle length.

V20A has pronounced stigma traits and stable male sterility mechanism. Since it has short stature, poor panicle exsertion and susceptibility to many pests and diseases, it may not be useful for direct utilization in hybrid production. However, it may be used as a source of sterile cytoplasm in the development of male sterile lines. PMS 3A, 6A and 9A appeared promising provided their panicle exsertion is improved.

Table 2. Mean values of floral and morphological characters of CMS and maintainer lines of rice

CMS/maintainer line	Anther length (mm)	Anther breadth (mm)	Anther size (mm^2)	Stigma length (mm)	Stigma breadth (mm)	Stigma size (mm^2)	Style length (mm)	Stigma protrusion (score)	Pollen sterility (%)	Anthesis duration (min)	Plant height (cm)	Flag leaf angle (°)	Panicle exsertion (%)	Panicle length (cm)	Days to 50% flowering
V20 A	2.03	0.45	0.92	1.66	0.56	1.86	0.53	4.5	100.0	273.0	53.3	27.0	69.4	20.6	90
V20 B	2.23	0.76	1.69	1.30	0.39	1.02	0.51	4.5	0.0	98.0	58.2	30.0	82.2	21.2	88
IR 54752 A	2.21	0.65	1.46	1.52	0.60	1.82	0.54	4.5	98.6	238.0	90.0	18.5	64.5	27.1	124
IR 54752 B	2.32	0.71	1.65	1.52	0.60	1.42	0.54	3.5	1.2	98.0	95.0	15.0	78.9	28.6	121
IR 62829 A	2.62	0.52	1.37	1.91	0.47	1.80	0.31	4.5	96.3	237.2	80.0	22.3	75.3	22.5	122
IR 62829 B	2.70	0.57	1.58	1.80	0.42	1.52	0.30	3.5	1.5	107.0	85.0	18.0	82.3	21.0	120
IR 58025 A	2.28	0.55	1.25	1.52	0.60	1.18	0.54	2.0	100.0	236.6	75.3	18.5	64.5	22.0	106
IR 58025 B	2.41	0.64	1.54	1.40	0.31	0.86	0.70	2.0	0.5	101.0	78.6	20.2	79.9	21.6	100
MANGALA A	1.97	0.42	0.83	1.25	0.42	1.06	0.55	2.0	0.0	205.0	62.6	14.0	73.8	21.0	100
MANGALA B	2.01	0.43	0.86	1.26	0.40	1.00	0.59	2.0	0.1	75.0	80.8	16.0	79.2	23.2	97
PUSHPA A	2.40	0.41	0.98	1.61	0.42	1.36	0.55	3.5	1.0	211.8	74.8	15.0	86.6	24.0	105
PUSHPA B	2.38	0.44	1.0	1.44	0.37	1.06	0.48	2.0	1.5	95.0	77.9	18.0	98.7	25.6	102
IMA	2.19	0.64	1.40	1.33	0.43	1.28	0.83	4.5	1.0	250.0	82.4	28.0	89.8	19.6	107
IMB	2.09	0.64	1.33	1.50	0.45	1.30	0.55	4.5	1.5	60.0	90.5	58.0	106.2	20.2	105
PMS 1A	1.90	0.39	0.74	1.36	0.43	1.17	0.63	4.5	100.0	256.0	65.1	17.5	60.0	21.5	128
PMS 1B	1.97	0.68	1.34	1.10	0.31	0.68	0.52	4.5	1.5	105.0	72.1	16.0	64.1	20.6	122
PMS 2A	1.84	0.39	0.72	1.36	0.43	1.54	0.92	4.5	100.0	251.6	75.1	22.0	64.0	22.7	129
PMS 2B	1.97	0.68	1.34	1.10	0.31	0.92	0.69	3.5	0.8	110.4	76.6	20.8	68.3	23.2	122
PMS 3A	1.98	0.37	0.74	1.50	0.55	1.66	0.69	4.5	96.5	284.0	71.6	29.0	58.0	18.9	120
PMS 3B	2.10	0.66	1.39	1.20	0.33	0.80	0.74	3.5	4.5	107.0	75.8	25.0	72.0	19.2	124

(Contd.)

Table 2 (contd.)

CMS/maintainer line	Anther length (mm)	Anther breadth (mm)	Anther size (mm^2)	Stigma length (mm)	Stigma breadth (mm)	Stigma size (mm^2)	Style length (mm)	Stigma protrusion (score)	Pollen sterility (%)	Anthesis duration (min)	Plant height (cm)	Flag leaf angle (°)	Panicle exsertion (%)	Panicle length (cm)	Days to 50% flowering
PMS 4A	1.76	0.35	0.63	1.40	0.44	1.34	0.72	4.5	93.2	265.0	68.1	35.0	57.4	23.1	120
PMS 4B	2.09	0.62	1.30	1.45	0.46	1.32	0.47	3.5	6.8	108.0	68.8	30.0	74.4	24.4	124
PMS 5A	2.02	0.34	0.69	1.36	0.44	1.20	0.76	4.5	90.3	251.0	69.0	31.5	58.6	22.1	122
PMS 5B	2.12	0.64	1.36	1.15	0.31	0.70	0.50	3.5	8.4	109.0	70.2	25.0	78.6	21.8	117
PMS 6A	1.99	0.30	0.60	1.43	0.43	1.24	0.73	4.5	88.6	298.0	68.8	36.5	69.1	22.0	124
PMS 6B	1.90	0.61	1.16	1.10	0.32	0.70	0.69	3.5	2.3	100.0	76.0	30.5	94.1	23.2	120
PMS 7A	2.37	0.43	1.01	1.55	0.54	1.68	0.72	4.5	86.5	256.0	75.2	35.5	54.7	22.5	119
PMS 7B	2.28	0.71	1.62	1.11	0.37	0.82	0.66	3.5	10.3	100.0	74.2	30.0	103.2	19.6	115
PMS 8A	1.96	0.37	1.73	1.58	0.47	1.54	0.74	4.5	98.0	246.0	70.6	38.5	55.9	22.5	121
PMS 8B	2.11	0.64	1.35	1.36	0.41	1.12	0.56	3.5	3.5	83.0	70.5	36.0	87.2	24.5	114
PMS 9A	2.23	0.45	1.00	1.58	0.53	1.68	0.80	4.5	99.0	285.0	76.2	36.5	66.7	21.3	120
PMS 9B	2.52	0.77	1.94	1.13	0.35	0.80	0.70	4.5	2.5	91.0	68.9	32.5	89.6	23.8	117
PMS 10A	1.85	0.36	0.67	1.52	0.53	1.62	0.62	4.5	98.0	257.0	64.4	31.6	56.2	20.1	116
PMS 10B	1.95	0.65	1.45	1.25	0.35	0.86	0.47	4.5	6.4	93.0	70.9	30.8	91.2	20.9	116
Mean	2.14	0.51	1.14	1.40	0.43	1.23	0.61	3.8	41.2	174.4	74.5	25.1	74.8	22.2	114
Mean of A lines	2.09	0.43	0.93	1.50	0.48	1.47	0.65	4.2	79.2	252.3	73.1	26.3	65.6	22.0	116
Mean of B lines	2.16	0.59	1.35	1.30	0.38	0.99	0.57	3.5	53.1	96.6	75.9	23.9	84.1	22.5	113
SE	0.04	0.02	0.06	0.03	0.02	0.06	0.02	0.1	1.1	14.0	1.5	1.3	2.5	0.4	2
CV (%)	10.68	25.77	31.01	14.27	20.35	28.64	22.47	23.3	80.1	46.7	11.5	31.5	19.4	9.6	9
Range	0.94	0.47	1.30	0.81	0.29	1.18	0.62	2.5	100.0	238.0	42.3	24.	41.5	9.7	40

Table 3. Dendogram of genotypes for forming clusters based on dissimilarity index

Node	Group 1	Group 2	Dissimilarity index	Number of genotypes in fused group
1	PMS 4A	PMS 10A	7.9	2
2	PMS 9B	PMS 10B	8.3	2
3	PMS 2B	PMS 3B	8.8	2
4	PMS 4B	PMS 5B	8.9	2
5	PMS 3A	PMS 9A	11.1	2
6	PMS 5A	PMS 7A	11.4	2
7	PMS 6B	PMS 7B	11.4	2
8	PMS 1B	(PMS 2B + PMS 3B) NODE 3	11.7	3
9	PMS 8B NODE 1	PMS 9B + PMS 10B) NODE 2	11.8	3
10	PMS 4A + PMS 10A	PMS 8A	12.2	3
11	PMS 1A NODE 10	PMS 2A NODE 6	12.6	2
12	PMS 4A + PMS 8A	PMS 5A + PMS 7A	14.5	5
13	IR 574752 A	IR 62829 A	16.3	2
14	IR 574752 B	IR 62829 B	16.5	2
15	MANGALA A	PUSHPA A	17.6	2
16	NODE 8	NODE 4	17.9	5
17	NODE 5	PMS 6A	18.7	3
18	NODE 11	NODE 12	19.6	7
19	NODE 7	NODE 9	19.9	5
20	IR 58025 B	PUSHPA B	23.2	3
21	NODE 13	IR 58025 A	26.0	7
22	NODE 14	NODE 16	26.0	7
23	NODE 20	MANGALA B	27.7	3
24	NODE 21	NODE 18	28.9	10
25	V20 B	NODE 23	32.8	4
26	NODE 22	NODE 19	32.9	12
27	NODE 25	NODE 26	32.0	16
28	V20 A	NODE 17	43.5	4
29	NODE 15	IMA	43.9	3
30	NODE 28	NODE 24	49.6	14
31	NODE 27	IMB	51.4	17
32	NODE 30	NODE 29	110.8	17
33	NODE 32	NODE 31	183.7	34

GENETIC DIVERSITY

The genetic diversity among genotypes is shown by dendrogram (Fig. 1). The diversity was based on characters that influence outcrossing. A perusal of the dendrogram and general situation presented in Table 4 reveals that basically the whole population can be divided into two groups, i.e., CMS lines and maintainers, based on sterility factor. The CMS lines depending on the source of male sterility (sterile cytoplasm), were divided into two groups, viz. CMS lines derived from WA type sterile cytoplasm and those derived from MS 577 A cytoplasm. Among the three CMS lines with MS 577A type male sterility, IMA differed with the other two as the source of male sterility in IMA has not been confirmed (Mahadevappa, personal communication). Further, geographical diversity has also played its role in causing diversity. CMS lines from IRRI were clustered together whereas V20A and CMS lines derived from India though they had similar sterile cytoplasm, maintained some distance. Geographical diversity might have been the reason for IMA and IMB to differ from the rest.

Table 4. Variability for floral traits influencing open pollination in A & B* populations in rice

Floral trait	Mean		Range		CV (%)	
	A	B	A	B	A	B
Anther length (mm)	2.09	2.18	1.76–2.62	1.90–2.70	11.00	10.6
Anther breadth (mm)	0.44	0.64	0.30–0.65	0.42–0.77	22.2	14.8
Anther size (mm^2)	0.90	1.37	0.60–1.46	0.86–1.69	28.1	20.00
Stigma length (mm)	1.50	1.30	1.25–1.91	1.10–1.80	10.4	14.6
Stigma breadth (mm)	0.49	0.38	0.42–0.60	0.31–0.60	13.4	14.6
Stigma size (mm^2)	1.47	0.99	1.06–1.86	0.68–1.52	17.5	26.3
Stigma protrusion (score)	4.12	3.53	2.00–4.50	2.00–4.50	20.3	24.2
Style length (mm)	0.66	0.57	0.31–0.92	0.30–0.74	22.1	20.3
Duration of anthesis (min)	252.33	96.50	205–298.6	75.00–110	9.6	13.8
Days to flowering (No.)	116.18	106.20	90–129	88.0–124	9.3	9.5
Pollen sterility	79.20	53.50	0.0–100.0	001–10.3	47.60	98.0
Plant height	73.10	75.90	53.3–90.0	58.2–95.0	11.70	11.5
Flag leaf angle	26.30	23.9	14.0–38.5	15.0–58.0	31.10	39.5
Panicle exsertion	65.60	84.1	54.7–89.8	64.1–106.2	15.7	14.1
Panicle length	22.00	22.7	18.9–27.1	19.2–28.6	8.5	10.7

*A—Cytoplasmic–genic male sterile lines.

B—Maintainers.

CLUSTERING

Based on the extent of relative dissimilarity among genotypes with regards to the traits influencing outcrossing, the population was divided into seven clusters. Cut off point at 40 dissimilarity units was fixed as minimum dissimilarity. The clusters and genotypes included in each cluster are listed in Table 5. Three solitary clusters were formed with V20A, IMA and IMB probably due to their geographical diversity. The maximum number of genotypes 16 were placed in cluster VI (all maintain lines except IMB). This indicates that all the maintainers are almost similar with regards to the characters that influence outcrossing, whereas the CMS lines differed much from each other. Cluster IV contained Mangala A and Pushpa A. Cluster II contained PMS 3A, PMS 6A and PMS 9A, which hold good promise provided their panicle exsertion is improved. Cluster III, had all the CMS lines from IRRI and other PMS lines which also can be used in hybrid development.

Table 5. Clusters of genotypes based on charactes influencing allogamy in rice

Cluster No.	No. of genotypes	Genotypes
1	1	V20A
2	3	PMS 3A, PMS 6A, PMS 9A
3	10	IR 54752 A, IR 62829 A, IR 58025A, PMS 1A, PMS 2A, PMS 4A, PMS 5A, PMS 7A, PMS SA, PMS 10A
4	2	Pushpa A, Mangala A
5	1	IMA
6	16	V20B, PMS 1B, PMS 2B, PMS 3B, PMS 4B, PMS 5B, PMS 6B, PMS 7B, PMS 8B, PMS 9B, PMS 10B, IR 58025B, IR 54752 B, IR 62829 B, Pushpa B, Mangala B
7	1	Intan Mutant B

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