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STUDIES ON STABILITY OF CYTOPLASMIC MALE STERILE LINES AND THEIR FLORAL TRAITS INFLUENCING OUTCROSSING IN RICE (*ORYZA SATIVA* L)

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ABSTRACT

One of the problems encountered in the hybrid breeding of crop plants is the pollen shedding in male sterile lines under certain environments; hence, studies on cytoplasmic male sterility (CMS) breakdown and relative stability of CMS lines for their sterility was undertaken. Twelve CMS lines were evaluated over two seasons at Delhi. The CMS lines PMS2A, PMS3A and PMS 10A were completely pollen sterile, had zero spikelet fertility and hence highly stable while PMS 5A and IR 58025A were stable. Remaining CMS lines were unstable. The CMS lines PMS 3A, PMS 10A and IR 58025A had comparatively longer stigma, style and anthers length favourable for outcrossing during seed production of A × B and A × R combinations. In general, all CMS lines were semi- dwarf in height, had medium to high tillering and medium duration except IR 62829A (early). The differential response of CMS lines possessing one kind of cytosterile source 'wild abortive (WA)' observed was due primarily to differences in nuclear background of CMS lines and interactions of cytoplasm with nuclear genes.

Key words : Cytoplasmic male sterility, breakdown, stability, hybrid vigour, *Oryza sativa* L

The importance of stable male sterility in hybrid rice technology needs no emphasis. The first stable cytoplasmic male sterile line in rice was developed by Shinjyo and Omura [1] and later on various workers [2-6] reported development of CMS lines. Identification of cytoplasmic male sterile lines based on their relative stability for sterility, wide adaptability and good combining ability is of paramount importance in exploitation of hybrid vigour. However, one of the problems encountered in the hybrid breeding of crop plants is the pollen shedding in male sterile lines under certain environments. The breakdown of male sterility is largely attributed to temperature variations. The stage at which temperature affects a pollen mother cell (PMC) is not precisely known in CMS lines of various crops including rice. Pre-meiotic stage is, however, considered to be the most vulnerable stage to temperature. High temperature at 35-41°C during anthesis induces pollen sterility [7] while low temperature sensitivity is maximum at microsporo release stage [8]. Satake [9] observed that low temperature between 15-17°C at booting stage was critical for inducing sterility in cold tolerant and between 17-19° C in cold sensitive varieties.

Stability of sterility in CMS lines depends on the stage at which pollen abortion takes place. Male sterility of sporophytic system is more stable than gametophytic system. In sporophytic system pollen grains abort at uninucleate stage and in gametophytic system abortion occurs at bi or trinucleate stage [10, 11]. Stage at which pollen abortion occurs depends upon genetic distance between cytoplasmic donor and nuclear parents [11]. In the present study 12 CMS lines developed from wild abortive cytosterile source in different nuclear backgrounds were evaluated for their sterility behaviour and floral attributes across two seasons in the rice farm of Indian Agricultural Research Institute, New Delhi with the objective to identify stable CMS lines possessing floral structure favourable for outcrossing for utilisation in hybrid breeding programme.

MATERIALS AND METHODS

A group of CMS lines developed at Regional Research Station, Punjab Agricultural University, Kapurthala (1-10) and International Rice Research Institute (IRRI), Philippines (11-12) listed below constituted the materials for this experiment.

Sl. no.	CMS line	Parentage/pedigree
1	PMS 1A	Phulpattes 72/Jaya
2	PMS 2A	Phulpattes 72/Mutant-15
3	PMS 3A	Basmati mutant
4	PMS 4A	IR 8/Sigadis/ / NS 200
5	PMS 5A	PB 134/PB 133
6	PMS 6A	PB 134/PB 133
7	PMS 7A	IR 305-3-17-13/IR 661-1-140-3
8	PMS 8A	IR 747-82-6-3/IR 665-40-6-3
9	MS 9A	PAU 169-49-3-1-1/PAU 29-295-3-28
10	PMS 10A	RP 2B-849
11	IR 58025A	IR 48483 A/8 Pusa 167-120-3-2
12	IR 62829A	IR 46828/IR 29744-94-3-2-2-2-3-3

Experiment I (Kharif 1992)

The seeds of CMS lines were sown in raised nursery bed. Thirty day old seedlings were transplanted in unreplicated manner in the main field with a row × plant spacing of 20 × 15 cm. Plot size consisted of 3 rows 5 m long with row to row spacing of 20 cm, recommended agronomic practices were followed.

Experiment II (Kharif 1993)

In this experiment the thirty day old seedlings of CMS lines were transplanted in randomised complete block design (RBD) with 3 replications. Plot size was $3m^2$ consisting of 4 rows with inter and intra row spacings of 20 cm and 15 cm respectively. CMS lines plots were isolated by erecting 3 m high polyethlene sheets and growing Sesbania plants (Experiment I) all around the experimental field to avoid cross contamination from foreign pollens. Further to avoid outcrossing from pollen dehisced from the instable CMS lines panicles were bagged with butter paper bag at inflorescence initiation. To estimate pollen sterility spikelets from lower, middle and top portion of five panicles per plant from randomly selected 25 and 5 (Experiment I) plants were collected at the time of flowering and fixed in 1:3 acetoalcohal. Anthers of each spikelet were smeared in 1% acetocarmine and pollen grains were observed under binocular microscope. Count of completely unstained shrivelled pollen grains indicating sterility was taken and expressed in percentage. Spikelet sterility estimates were taken from number of fertile and total spikelets from bagged and equal number of unbagged panicles per plant. Spikelet sterility bagged and unbagged in per cent was calculated as

$$\text{Spikelet sterility \%} = \frac{\text{Number of sterile spikelets}}{\text{Total number of spikelets}} \times 100$$

Data on 5 randomly selected plants per CMS line were recorded for morphological and floral characteristics viz; plant height (cm), number of effective tillers per plant, panicle length (cm), number of primary branches per panicle, number of spikelets per panicle, days to 75% flowering, stigma length (mm), style length (mm) and anther length (mm). Statistical analysis was done using RBD model.

RESULTS AND DISCUSSION

Analysis of variance (Table 1) exhibited highly significant differences among CMS lines for all characteristics studied except stigma length, number of effective tillers per plant and panicle length indicating that CMS lines differed significantly among themselves for these characteristics. Data on percentage of pollen sterility and spikelet fertility of bagged and unbagged panicles over two seasons and various floral and agronomic traits have been presented in Table 2.

Table 1. Analysis of variance for sterility and other agronomic traits of CMS lines, Kharif 1993

Source	d.f.	Pollen sterility	Spikelet fertility (U)	Spikelet fertility (B)	Stigma length	Style length	Anther length	Days to 75% flowering	Plant height	No.of effective tillers/ plant	Panicle length	No. of primaries /panicle	No. of spikelets panicle
Replications	2	116.9	0.539	0.27	0.004	0.153	0.005	5.36*	5.02	35.0	0.85	0.26	779.2
Treatment	11	407.6	4.108*	1.93*	0.014	0.161*	0.063**	167.99**	49.47*	59.1	2.78	1.44*	1856.0
Error	22	813.8	1.378	0.79	0.008	0.051	0.016	1.30	18.24	86.8	1.60	0.57	399.2

*Significant at 5% level, **significant at 1% level, (U) = Unbagged panicles, (B) = Bagged panicles

Table 2. Mean performance of CMS lines for sterility and other agronomic traits over two seasons

CMS lines	Pollen sterility (%) (B)		Spikelet fertility (%) (U)		Spikelet fertility (%) (B)		Style length (mm)		Stigma length mm	
	Kh 92	Kh 93	Kh 92	Kh 93	Kh 92	Kh 93	Kh 92	Kh 93	Kh 92	Kh 93
PMS 1A	98.5	80.9*	0.82	1.30	10.91	9.56	0.79	0.92	1.68	2.52
PMS 2A	100.0	100.0	0.33	0.21	0.4	2.42	0.83	1.00	1.62	2.32
PMS 3A	100.0	100.0	0.00	0.18	0.03	6.62	1.04	0.99	2.05	2.47
PMS 4A	100.0	84.6	0.00	6.33	0.49	12.64	0.95	0.95	1.01	1.62
PMS 5A	99.1	100.0	0.60	0.30	0.98	1.10	0.97	1.12	1.90	2.57
PMS 6A	94.7	97.3	6.43	0.54	7.12	2.09	0.78	0.95	1.45	2.53
PMS 7A	99.4	89.1	0.02	3.43	0.60	5.93	0.88	0.99	2.27	2.74
PMS 8A	73.4	67.2**	0.50	9.46	2.44	28.82	0.95	1.08	2.00	2.53
PMS 9A	87.3	97.3	4.16	2.94	3.50	5.78	0.93	1.08	1.92	2.93
PMS 10A	100.0	100.0	0.00	0.35	2.96	1.78	1.00	1.01	2.46	2.56
IR 58025A	99.4	99.0	0.00	0.89	1.38	3.03	1.24	1.14	2.23	2.26
IR 62829A	94.9	83.6	3.37	5.21	5.08	13.35	1.01	1.07	2.39	2.01
SE (d)		± 7.47		± 3.86		± 7.25		± 0.11		± 0.18

Table 2 (Contd.)

CMS lines	Plant height (cm)		No. of effective tillers/plant		No. of spiklets per panicle		Anther length (mm)		Days to 75% flowering	Mean panicle length (mm)	No. of primary branches/ panicle
	Kh 92	Kh 93	Kh 92	Kh 93	Kh 92	Kh 93	Kh 92	Kh 93	Kh 93	Kh 93	Kh 93
PMSA 1A	56.8	65.87	8.4	2.33	121.2	190.0	1.35	1.77	108.3	22.48	9.60
PMS 2A	57.0	65.07	8.4	33.27	156.3	199.5	1.26	1.91	109.3	22.65	10.13
PMS 3A	61.4	71.26	11.8	26.81	188.3	187.5	1.46	1.87	109.3	24.75	10.16
PMS 4A	64.6	70.27	12.6	31.27	172.7	183.3	1.30	1.91	106.7	22.75	9.60
PMS 5A	64.2	65.20	12.2	28.93	186.3	193.3	1.19	1.95	107.7	22.15	9.17
PMS 6A	78.4	64.30	13.8	26.73	152.5	178.0	1.08	1.89	108.3	21.94	10.53
PMS 7A	65.20	66.23	12.0	33.73	152.4	143.8	1.69	2.21	107.0	24.72	10.13
PMS 8A	68.80	74.87	13.0	30.07	141.3	169.3	1.54	2.03	103.0	24.12	9.93
PMS 9A	68.40	72.33	11.0	26.07	109.2	128.7	1.31	2.01	103.0	23.10	8.07
PMS 10A	67.40	65.82	10.6	27.68	159.0	176.3	1.64	1.75	106.3	23.49	10.07
IR 58025A	56.40	68.40	15.8	18.87	177.0	171.7	1.80	1.66	100.0	23.51	10.07
IR 62829A	55.80	60.20	22.20	34.93	125.5	125.7	1.80	1.86	82.3	22.31	9.20
S.E. (d)		± 3.49		± 7.61		± 26.72		± 0.12	± 0.93	± 1.68	± 1.29

Kh = Kharif; Kh 92 = Data unreplicated; B = Bagged panicles; U = unbagged panicles.

Relative stability of cytoplasmic male sterile lines

Of the 12 CMS lines studied possessing WA cytoplasm PMS 2A, PMS 3A and PMS 10A were completely pollen sterile, had 100 per cent pollen sterility in bagged panicles and very low seed set in unbagged panicles over both the seasons, hence found to be highly stable. The CMS lines PMS 5A, and IR 58025A recorded > 99 per cent pollen and spikelet sterility in bagged panicles and < 3 per cent seed set in unbagged panicles during kharif 1992 and 1993 seasons and were found to be stable. Spikelet fertility of bagged and unbagged panicles was as low as 0.00 and 0.21 per cent and as high as 9.46 and 28.82 per cent, respectively. The variation in seed set in unbagged panicles may be due to pollens from unstable CMS lines. The CMS lines PMS 1A, PMS 4A, PMS 6A, PMS 7A, PMS 8A, PMS 9A and IR 62829A showed low pollen and spikelet sterility, hence were found to be unstable. The CMS line IR 58025A [6, 12, 13], PMS 5A and PMS 10A [12, 14], PMS 1A and PMS 2A[12], PMS 3A [14, 15] are reported to be stable. In some cases inconsistent behaviour of CMS lines was observed over seasons/environments. For example, PMS 4A was completely pollen sterile during kharif 1992 and partial during kharif 1993. The differential behaviour of CMS lines over seasons indicated the presence of genotype × environment interaction for male sterility. These findings were in conformity to those of earlier workers [14-17]. It is, therefore, essential to evaluate the CMS lines for their stability over environments before exploitation for heterosis.

Breakdown of sterility in CMS lines may also be due to occasional outcrossing. Virmani and Wan [18] were of the opinion that some maintainers in addition to major sterility maintaining genes might carry minor genes for fertility which get expressed under certain environmental conditions resulting into instability. Hassan and Siddiq [19] observed differences in pollen sterility due to reciprocal cross, while [20] cited genetic imbalance as the cause of sterility has also been reported [20]. According to Nishiyama [21] all stages of reproductive phase ranging from pollen formation to fertilization are vulnerable to climatic fluctuations, therefore, occurrence of some fertile plants in perfect male sterile lines could be due to fluctuations in temperature and day length. The differential response of CMS lines possessing one kind of cytosterile source i.e. 'WA' was due primarily to differences in nuclear background of lines and MCS interaction of cytoplasm with nuclear genes. Choudhary *et al.* [11] also emphasised the role of genetic diversity between cytoplasmic donors and nuclear parents.

Floral traits

Differences for floral traits viz., style length, stigma length and anther length over environments were observed for all CMS lines. The range for anther length varied from 1.08 to 1.80 mm in Kharif 1992 and from 1,66 to 2.21 mm in Kharif 1993. CMS line PMS 10A and IR58025A in 1992 and PMS 7A in 1993 produced the longest anther. All CMS lines except PMS 10A showed increase in anther length in Kharif 1993. Style length ranged between 0.78 to 1.24 mm and 0.92 to 1.14 mm in 1992 and 1993 season, respectively. PMS 10A had the longest style in both the seasons, while PMS 6A and PMS 1A showed shortest in Kharif 1992 and 1993, respectively. Other CMS lines with long style were PMS 3A, PMS 5A and IR 58025A. The range for stigma length was from 1.62 to 2.46 mm in Kharif 1992 and from 2.01 to 2.93 mm in Kharif 1993. PMS 10A in Kharif 1992 and PMS 9A in 1993 produced the longest stigma followed by PMS 7A over both the seasons. In general, the CMS lines PMS 3A, PMS 10A, IR 58025A had comparatively long stigma, style and anthers favourable for outcrossing during maintenance of 'A' lines and seed production of A × R hybrids.

Agronomic traits

All CMS lines were dwarf in height, had very high tillering (> 25 per plant) during Kharif 1993 except IR 58025A and medium tillering during kharif 1992 except PMS 1A and PMS 2A. The mean plant height varied from 55.80 to 78.40 cm in Kharif 1992 and from 60.20 to 74.87 cm in Kharif 1993. IR 62829A was found to be the shortest while PMS 6A and PMS 8A the tallest in 1992 and 1993 season, respectively. The number of effective tillers per plant ranged from 8.4 to 22.2 and from 18.87 to 34.93 in 1992 and 1993 seasons respectively. IR 62829 A recorded the maximum number of effective tillers in both the seasons while IR58025A in 1993 and PMS 1A and PMS 2A in 1992 had minimum number of effective tillers. In general, panicle bearing tillers were observed 2-3 times more in Kharif 1993 than Kharif 1992 had minimum number of effective tillers. This was due to the favourable climatic conditions of kharif '93 resulting into healthy crop stand. The number of spikelets per panicle ranged from 109.2 to 188.3 in 1992 and from 125.17 to 199.5 in 1993 season. PMS 3A and PMS 2A had highest spikelets in Kharif 1992 and 1993; respectively followed by PMS 5A. PMS 9A in 1992 and IR62829A in 1993 produced lowest number of spikelets per panicle. The CMS lines PMS 3A, PMS 5A and IR 58025A didnot exhibit variation for spikelet number over two seasons.

The data for 75% flowering, panicle length and number of primary branches per panicle were recorded for Kharif 1993 experiment. The range for mean number of days to 75% flowering ranged between 82.3 to 109.3. IR 62829A was the earliest to flower while all other CMS lines exhibited medium flowering. PMS 2A and PMS 3A took maximum number of days to flower. The mean panicle length ranged from 21.94 to 24.74 cm. PMS 3A produced the longest panicles followed by PMS 7A, PMS 8A, IR 58025A and PMS 10A while PMS 6A had the shortest. The range for number of primary branches per panicle varied between 8.07 to 10.53. PMS 6A had the maximum number of primary branches followed by PMS 2A and PMS 3A while PMS 9A recorded the minimum. To conclude the CMS line PMS 3A, PMS 10A and IR58025A with complete pollen sterility, semi dwarf plant type, medium duration, possessing longer stigma, style and anther length favouring outcrossing during maintenance and hybrid seed production could be utilized in hybrid breeding programme.

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