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IDENTIFICATION AND CHARACTERISATION OF TEMPERATURE SENSITIVE CYTOPLASMIC-GENETIC MALE STERILES IN RICE (*ORYZA SATIVA* L.)

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ABSTRACT

Eight male sterile lines of rice comprising five from WA and three from MS 577 A sources were studied for temperature influenced male sterility-fertility transformation under natural field and controlled growth chamber conditions. Of them WA-based Pusa 33-16A (JP 24 A) showed the highest tendency for reversible sterility- fertility alteration. Along with its B-line JP 24 A was characterized using parameters like sensitive stage to temperature, critical sterility point and critical fertility point. JP24A and JP24B became resistance 21 and 24 days before heading respectively. Critical sterility point of JP 24 A is 33.8°C as against 35.1°C of JP 24 B. The CMS line possibly genetically linked with temperature sensitive genetic male sterility is of value in commercial hybrid seed production as it requires no maintainer to produce its seed.

Key words : *Oryza sativa*, rice, hybrid, temperature sensitive male sterility

When cytoplasmic genetic male sterility-fertility restoration system is worldwide used for exploitation of hybrid vigour across crops, the discovery of readily usable nuclear gene-governed photoperiod sensitive male sterility system in rice has proved a potential alternative making hybrid seed production relatively less cumbersome and economical, besides several other advantages over the former. Like nuclear genes that determine reversibly male sterility and fertility with change of environmental conditions, there are instances in pepper, pearl millet, sorghum, maize and wheat that temperature or photoperiod change could bring about similar sterility/fertility transformation as well as in the cytoplasmic-genetic male sterility system, wherein interaction of nuclear (*fr*) and cytoplasmic (*ms*) genes results in male sterility [1-7]. Such temperature and photoperiod influenced CMS systems are known by temperature sensitive cytoplasmic male sterility (TCMS) and photoperiod sensitive cytoplasmic male sterility (PCMS) systems respectively. Instability of male sterility as expressed by pollen shedding under certain environments is an indicator to the existence of such systems among CMS lines. Pollen shedding is otherwise a serious problem in

commercial hybrid seed production. Transformation of such a problem into an opportunity depends on identifying appropriate locations/seasons for hybrid seed production and CMS multiplication. Keeping such advantages in view efforts have been made to identify environment sensitive CMS lines in rice and physiologically characterize them for possible use in commercial hybrid seed production.

MATERIAL AND METHODS

Eight cytoplasmic-genetic male sterile lines viz., IR 62828 A, IR 58025 A, JP 24 A, Pusa 33-16 A, Pusa 33-119 A and Pusa 33-123 A of wild abortive (WA) source

Table 1. Environmental influence on sterility behaviour of WA and MS 577 A-based cytoplasmic male sterile lines.

CMS line	Percentage sterility under natural field conditions at Katrain, (Kharif, 1992)		Percentage sterility under natural field conditions at Delhi, (Kharif, 1992)		Percentage sterility in growth chamber (cool regime)*	
	Pollen	Spikelet	Pollen	Spikelet	Pollen	Spikelet
WA-Based						
IR 62829 A	100.00	100.0	100.0	100.0	100.0	100.0
IR 58025 A	43.5	51.0	100.0	100.0	28.9	13.2
Pusa 33-16 A	45.2	49.3	100.0	100.0	19.5	16.7
Pusa 2-33-123 A	39.5	48.5	100.0	100.0	11.1	21.0
Pusa 1.33-119 A	78.7	82.2	100.0	100.0	25.0	19.5
MS 577A-Based						
Pushpa A	100.0	100.0	100.0	100.0	100.0	100.0
Mangala A	45.2	100.0	55.0	100.0	40.0	100.0
Intan Mutant A	68.5	59.2	100.0	100.0	11.1	21.0

Place/(months)	Temperature	Average	Range (°C)
Katrain (June-Oct)	Minimum	16.2	8.9-19.8
	Maximum	28.3	23.6-30.6
Delhi (June-Oct)	Minimum	24.1	15.8-28.2
	Maximum	35.6	33.3-38.9
*Growth chamber	-	20.0	18.0-22.0

Stage : Panicle initiation; Photoperiod: 10h (0700-1700 hr)

and Pushpa A, Mangala A and Intan Mutant A of MS 577 A source formed the experimental material.

Nurseries of the male sterile lines were raised at Delhi in the month of June. One set of the CMS lines each with 20 seedlings was transplanted at Katrain in Himachal Pradesh with two replications following a simple RBD. Pollen sterility was recorded from bagged and unbagged panicles in five plants each of the CMS lines. Those lines, which showed pollen fertility were selected and intensively studied at Delhi.

The second set of the CMS lines each of 20 seedlings planted in pots at Delhi was studied under natural high temperature conditions during August-October as well as under conditions of controlled temperature and photoperiod. One plant each of the CMS lines at the onset of stage IV (between pollen-pistil primordia formation and PMC formation stages) was transferred to a growth chamber maintained at a day temperature of 22°C and night temperature of 18°C under short photoperiod of 10 hrs (0700-1700 hrs) for 10 days. The promising JP 24 A (Pusa 33-16A) with its B line chosen for indepth study were screened for their fertility- sterility alteration behaviour under four different temperature conditions in the growth chambers (Tables 3 to 4). The plants were scored for pollen sterility/fertility and spikelet sterility. Pollen fertility was examined at fortnightly interval by staining anthers at anthesis stage with 1% I-KI solution. Deeply stained round pollen grains were taken as fertile and non-stained and shrivelled ones as sterile. Spikelet sterility was determined as percentage seed set.

Table 2. Determination of critical temperature and sensitive stage for JP 24 A and JP 24 B

C-TGMS line	Date of total sterility (Hyderabad)	Critical temperature		Days before heading
		Max. (°C)	Min. (°C)	
JP 24 A	July 6	33.9	24.2	21
	July 23	**33.8**	**23.5**	
	August 10	35.1	25.1	
JP 24 B	July 5	35.5	24.3	24
	August 13	**35.1**	**25.1**	
	August 14	36.1	23.6	

Figures in bold show the critical sterility point, i.e. the lowest temperature at which a line becomes completely sterile

Table 3. Determination of critical temperature for fertility restoration on the basis of fertility transformation pattern under different temperatures

CMS/B line	Chamber 1											
	panicle 1				panicle 2				panicle 3			
	AS	RS	US	F	AS	RS	US	F	AS	RS	US	F
JP 24 A	78.6	14.3	7.1	0.0	96.2	3.8	0.0	0.0	94.6	5.4	0.0	0.0
JP 24 B	78.4	21.6	0.0	0.0	66.9	33.1	0.0	0.0	45.6	48.8	5.6	0.0
CMS/B line	Chamber 2											
	panicle 1				panicle 2				panicle 3			
	AS	RS	US	F	AS	RS	US	F	AS	RS	US	F
JP 24 A	30.6	20.4	49.0	0.0	31.3	17.3	32.9	18.4	11.7	5.6	16.9	65.7
JP 24 B	4.3	95.7	0.0	0.0	0.0	0.0	17.1	18.5	64.4	43.2	1.2	1.8
CMS/B line	Chamber 3											
	panicle 1				panicle 2				panicle 3			
	AS	RS	US	F	AS	RS	US	F	AS	RS	US	F
JP 24 A	14.4	11.3	18.6	55.7	81.7	1.8	5.2	11.3	80.3	6.6	3.2	10.0
JP 24 B	14.6	5.3	32.1	47.9	7.6	2.1	22.1	68.3	19.2	9.4	31.4	40.0
CMS/B line	Chamber 4											
	panicle 1				panicle 2				panicle 3			
	AS	RS	US	F	AS	RS	US	F	AS	RS	US	F
JP 24 A	13.0	28.0	37.3	20.6	0.0	11.6	10.0	78.3	0.0	11.9	9.9	78.1
JP 24 B	12.5	11.2	43.8	32.5	12.5	11.1	29.6	44.4	12.7	0.0	22.7	64.6

	Chamber			
Regime	1	2	3	4
Maximum temperature	35°C	30°C	24°C	24°C
Minimum temperature	26°C	22°C	20°C	24°C
Mean	30.5°C	26°C	22°C	24°C
Light duration	14.30 h	9 h	9 h	9 h

AS-aborted sterile, RS-round sterile, US-unaborted sterile and F- fertile

The sensitive stage was determined by the integrated use of physical-morphological index and tracking technique [8]. The day, when pollen is completely sterile in a plant kept under natural conditions is taken as the tracking date. The sensitive phase of panicle development (stamen-pistil primordial stage) being between 15-24 days before heading, the days are counted backward from the tracking date upto 24 days and the date, on which maximum temperature was > 30°C is noted for computing the 'sensitive stage' (Table 2). The lowest temperature among the three maximum temperatures recorded is taken as the critical sterility point. [8]. Before the start of the treatment care was taken to label the critical tillers (tillers that were in stage IV) and monitor continuously on their behaviour in and out of the chamber. Pollen and spikelet sterility of the critical tillers were determined at anthesis and harvest respectively.

RESULTS AND DISCUSSION

Instability as expressed by pollen shedding and self-fertility is not uncommon among CMS lines in various hybrid crops including rice. Attributing this phenomenon all along to either outcrossing or impurity of B-line, breeders have been engaged all along in genetic purification of the A and B lines. Strangely none has realized that it could be as a result of the presence of yet another male fertility influencing gene sensitive to environment along with the nuclear sterility gene (*fr*), which in interaction with cytoplasmic gene (*ms*) induces cytoplasmic genetic male sterility. It was Shi [9], who reported first the possibility of nuclear male sterility gene (*fr*) being closely linked with photoperiod sensitive sterility gene. Under such a situation, CMS lines, in all likelihood, would be vulnerable to changes in day length. This has been the basis for undertaking the present study of eight cytoplasmic-genetic male sterile lines derived from two diverse sources of cytoplasmic sterility namely wild abortive (WA) and MS 577 A.

Results on the behaviour of the CMS lines at high temperature under field conditions during *kharif* (wet season) at Delhi and at low temperature under field conditions at Katrain, a high altitude location in Himachal Pradesh during June-October (Av. Max. & Min. temperature 28.3 and 16.2°C respectively) as well as in growth chambers maintained in the temperature range of 23.3-30.6°C (Table 1) reveal all except Mangala A among the MS 577 A based CMS lines to remain completely pollen and spikelet sterile under high temperature conditions during *Kharif* at Delhi. Their behaviour, however, was found to differ with change of temperature. Among the WA-based A-lines, IR 62829 A was not at all influenced by change in temperature regime. It remained completely sterile at Delhi and Katrain as well as in the growth chamber maintained at low temperature. Others showed a tendency to revert back

to fertile phase to different degrees under low temperature conditions at Katrain as well as in the growth chamber maintained at low temperature. Pusa 33-123 A, in particular, was found to be maximum vulnerable, the percentage pollen and spikelet fertility being in the range of 50.5 and 89.0 respectively, followed by IR 58025 A, Pusa 33-16A and Pusa 33-119 A in decreasing order.

Environment-influenced male sterility systems are known to be governed by specific temperature or day length sensitive nuclear gene in recessive state [10-12]. The present findings as well as earlier reports on temperature/photoperiod determined expression of sterility/fertility in some of the cytoplasmic- genetic male sterile lines suggest indirectly the possibility of the environment sensitive gene(s) being either loosely linked or non-linked to the nuclear sterility gene *'fr'* but located on the same chromosome. But for the foregoing inference, there is no direct evidence to prove temperature sensitive genic male sterility gene to be different from the nuclear fertility (*Fr*)/sterility (*fr*) gene and the gene conferring genetic male sterility (*st*), which does not involve cytoplasmic (*c*) gene in its expression of male sterility.

Although six of the eight A-lines showed a tendency for reversible sterility-fertility transformation with changing temperature, Pusa 33-16 A (JP24 A) and its B line (JP 24 B) only were chosen for intensive study and characterization in terms of stage of panicle development sensitive to temperature and critical sterility and fertility points. Sensitive stage of panicle development determined by employing physical/morphological indicator method and tracking technique was 21 days before heading for JP 24 A while it was 24 days for JP 24 B. Critical sterility point of JP 24 A (33.8°C) was lower than that of JP 24 B (35.1°C).

Table 4. Behaviour of the CMS/B line sensitive to temperature for percentage spikelet fertility under high and low temperature regimes

CMS/B line	Chamber 1			Chamber 2			Chamber 3			Chamber 4		
	Spikelet sterility (%) in panicles			Spikelet sterility (%) in panicles			Spikelet sterility (%) in panicles			Spikelet sterility (%) in panicles		
	1	2	3	1	2	3	1	2	3	1	2	3
JP 24	100.0	100.0	100.0	82.1	90.3	92.4	73.0	33.3	27.0	71.7	30.3	28.3

The CMS lines associated with temperature sensitive genic male sterility system would be of considerable value in commercial hybrid seed production. When it remains completely sterile at a specific temperature regime, a wide choice of promising pollinator lines with restorer gene(s) could be used for production of progressively

heterotic hybrids and their tendency to turn fertile at another temperature regime would facilitate maintenance of the same without the aid of its maintainer (B) line, without which maintenance of CMS line is not possible in 3- line breeding. This advantage makes seed production relatively easy and economical. Especially in wheat, wherein outcrossing is still a hurdle in making hybrid technology economically viable, environment-influenced CMS would be of considerable value. Existence of such an effective system has been reported in wheat, maize and sorghum [3-7]. Whereas in bread wheat [7] alloplasmic line of Norin 26 with *Aegilops crassa* cytoplasm has been found to be male sterile under short day (< 14.5 hr) conditions with no influence of temperature, in maize partly day length-influenced CMS line Qun-6-ms

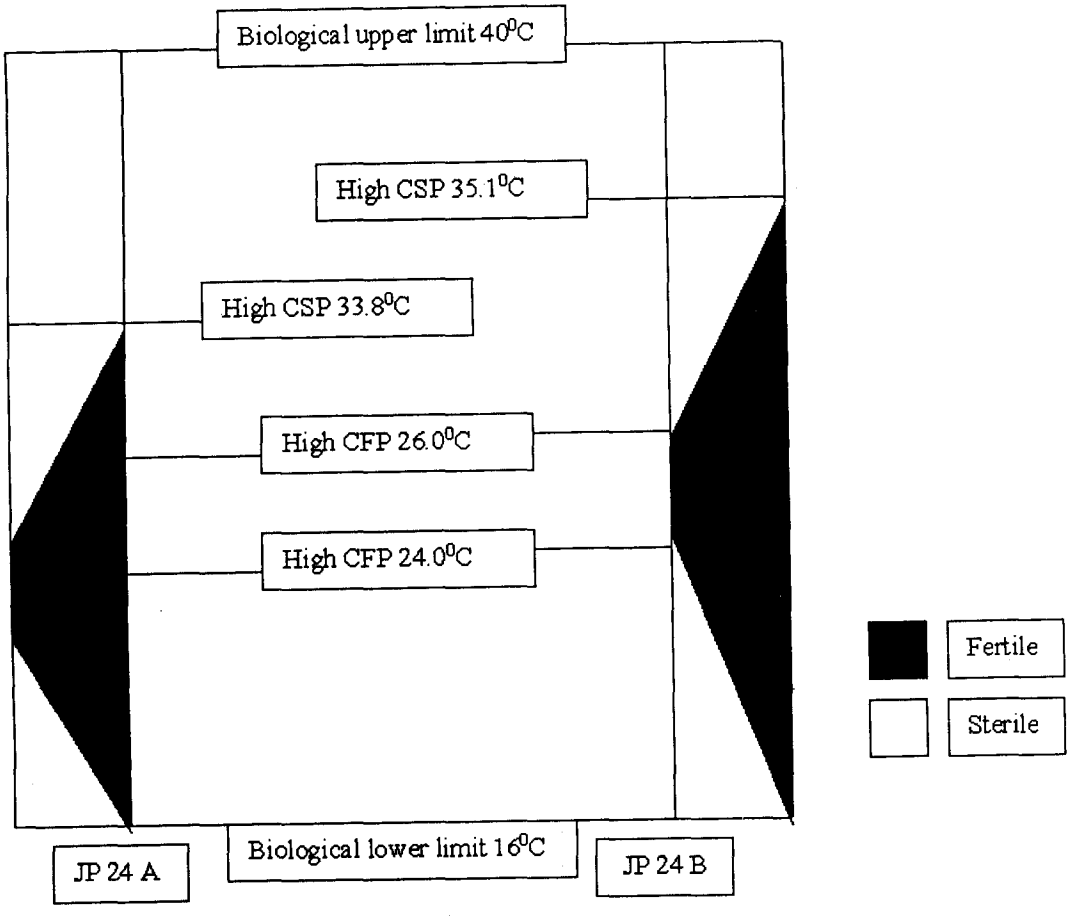

Fig. 1. Critical sterility and fertility points of JP 24A and JP 24B

remains sterile under high temperature and turns fertile under low temperature. In the case of sorghum, where A-2 cytoplasm based CMS lines in India are reported to be sterile under low temperature and fertile under high temperature, Chinese source based CMS lines behave just opposite of the former by remaining sterile at high temperature and fertile under low temperature.

In the present study, influence of temperature has been observed in WA as well as MS 577 A based CMS lines. Of these only one of the WA-based CMS lines JP 24 A (Pusa 33-16 A) along with its B line was studied under different temperature regimes for their fertility-sterility transformation behaviour. The results reveal both of them to be of high CSP-high CFP type (Fig. 1). Yet, they differ from each other in their degree of transformation. As against the CSP of 33.8°C in JP 24 A, it is 35.1°C in JP 24 B. and though the CFP of both fall under the same range, CFP of JP 24 A might be closer to 26°C than that of JP 24 B. From the comparative study of the A and B lines for their temperature- influenced sterility-fertility behaviour (B line showing relatively higher CSP and CFP than the A-line) there is room to believe that the TGMS gene could be linked to the nuclear sterility gene (*fr*) to have some degree of influence on *'fr'* as well.

The knowledge on the behaviour of the CMS lines under study could also be made use of in conceiving and developing stable male sterile lines for the conventional 3-line hybrid breeding. For instance, to have stable CMS line the nuclear genome should have the fertility gene in recessive condition *'fr'* with environment sensitive male sterility gene (*Tg* or *Pg*) in dominant state so that neither temperature nor day length would affect its stability for pollen sterility. Also, depending on the EGMS gene in dominant or recessive state advantages of two-line and three-line breeding can be made use of under high temperature conditions for hybrid seed production. The A-line in such a state will be more stable for sterility because of combined effect of both *'fr'* and *'tg'* genes. Seed multiplication of the CMS line can be done with ease taking advantage of fertility transformation under low temperature conditions. To minimize the risk of temperature fluctuations, the CMS line can be planted with its B-line in appropriate proportion. If high temperature during CMS seed multiplication makes the CMS partly or completely sterile, the B line planted along would act as a maintainer to ensure reasonable seed yield.

REFERENCES

1. J. A. Martin and J. H. Crawford. 1951. Several types of sterility in *Capsicum frutescens*. Proc. Amer. Soc. Hort. Sci., 57: 335-338.
2. M. L. H. Kaul. 1988. Male sterility in higher plants. Springer- Verlag, Berlin, Germany: 1996.

3. Z. Kongtian and F. Hongyi. 1981. Effect of high temperature on fertility of the male sterile lines in sorghum. *In* : Annual Report (1980). Institute of Genetics Academica Sinica., Science Press, Beijing, China : 120.
4. K. T. Zhang and H. Y. Fu. 1982. Effect of high temperature treatment on male sterility in sorghum. Acta Genet., **9**: 71-77.
5. U. R. Murthy. 1997. Two line hybrids in cereals with special reference to sorghum. pp 330-331. *In*: Intl. Symp. The Genetics and Exploitation of Heterosis in crops. D. F., CIMMYT, Mexico.
6. Z, Swngyum and Zong-long. 1997. Two line hybrid breeding in maize p. 218. Ibid.
7. K. Murai, H. Hirohira and K. Tsunewaki. 1992. Photoperiod- sensitive cytoplasmic male sterility in wheat. *In* : Proc. First International Crop Science Congress. 14-22, July, 1992, Iowa State Centre, Ames., Iowa. USA. : 92.
8. J. Ali, E. A. Siddiq, F. U. Zaman, M. J. Abraham and I. Ahmed 1995. Identification and characterization of temperature sensitive genic male sterile sources in rice (*Oryza sativa* L.). Indian J. Genet., **55**: 243-259.
9. M. S. Shi. 1985. The discovery and study of the photosensitive recessive male sterile rice (*Oryza sativa* L. Sub sp. *japonica*). Scientia Agricultura Sinica., **2**: 44-48.
10. Z. Zhang, S. C. Yuan and C. Z. Xu. 1987. The influence of photoperiod on the fertility changes of Hubei Photoperiod Sensitive Genic Male Sterile Rice (HPGMR). Chinese J. Rice Sci., **1**: 137-143.
11. L. P. Yuan. 1992. The strategy of breeding rice PGMS and TGMS lines. Hybrid Rice., **1**: 1-4.
12. Z. G. Zhang, H. L. Zeng, S. C. Yuan, B. X. Wang, Y. Z. Li and D. P. Zhang. 1992. Restudies on the model of photo-thermoreaction of fertility alteration in photosensitive genic male sterile rice. Journal of Huazhong Agricultural University., **11**: **1-6.**