Indian J. Genet., 55 (3): 243–259 (1995)

IDENTIFICATION AND CHARACTERIZATION OF TEMPERATURE SENSITIVE GENIC MALE STERILE SOURCES IN RICE (*ORYZA SATIVA* L.)

J. ALI, E. A. SIDDIQ,* F. U. ZAMAN, M. J. ABRAHAM AND ILYAS AHMED

Division of Genetics, Indian Agricultural Research Institute, New Delhi 110 012 and Directorate of Rice Research, Hyderabad

(Received: January 31, 1995; accepted: June 5, 1995)

ABSTRACT

Identification and isolation of the usable sources of temperature sensitive genic male sterility–fertility system for developing two-line hybrid technology in rice were the objectives of the study. Among the several male sterile plants isolated from stagger/late planted germplasm, breeding lines at different stages of development, and mutagen-treated populations, six strains namely, SM 3, SM 5, F 61, JP 2, JP 8-1A-12 and SA 2 (F 43), remained sterile at high temperature and turned fertile at low temperature. The six lines were studied in growth chambers maintained at specific temperature ranges, decided on the basis of sensitive stage which was determined by a newly developed 'tracking technique' to identify the critical sterility point (CSP), i.e. the temperature at which a plant is completely male sterile, and the critical fertility point (CFP), i.e. the temperature at which the plant turns completely male fertile. The lines were categorized into high CSP–low CFP, high CSP–high CFP, and low CSP–low CFP types. Among them, JP- 8-1-A-12, F-61 and SA 2 (F 43) belonging to the low CSP-low CFP group were found to be the most promising sources of stable sterility. Transfer of temperature sensitivity from them to other productive agronomic backgrounds and identification of appropriate locations for optimization of seed production would make the two-line approach a commercial reality.

Key words: *Oryza sativa*, rice, temperature-sensitive male sterility, hybrid, two-line breeding.

The cytoplasmic-genetic male sterility–fertility restoration-based hybrid technology has become a commercial reality in China since the late seventies and is also gaining ground in several major rice growing countries like India and Vietnam. However, excessive dependence on a single source of cytoplasmic male sterility (cms), viz. WA and the cumbersome process of seed production and parental line development warrant the development of alternate approaches to exploit hybrid vigour in rice. Two-line breeding is

*Present address of corresponding author: Deputy Director General (CS), ICAR, Krishi Bhavan, New Delhi 110001.

one such possibility that emerged following the chance discovery of a photoperiod-sensitive plant called Nongken 58 S, in the *japonica* variety Nongken 58 by Prof. Shi Min Shong of China [1, 2]. The day-length dependent fertility–sterility behaviour of this spontaneous mutation aroused a lot of interest among rice breeders, leading to isolation of new sources of photoperiod- as well as temperature-sensitive genic male sterility (PGMS/TGMS) [3–7]. While China has already succeeded in developing two-line hybrids using Nongken 58 S based PGMS lines, the possibilities of exploiting the mutagen-induced temperature-sensitive mutations Norin Pl 12 and H 89-1 are being explored in Japan and IRRI [8, 9]. The present study has been undertaken with the objective of isolating new sources of TGMS and characterizing them to identify the most appropriate ones for tropical countries like India.

MATERIALS AND METHODS

ISOLATION OF TEMPERATURE-SENSITIVE MALE STERILES

Germplasm, breeding lines and mutagen-treated populations formed the base material.

About 2000 accessions, mostly belonging to the *indica* group, maintained at the Directorate of Rice Research (DRR), Hyderabad, were raised in four staggered plantings at 7-day intervals from December 14, 1991 onward at Hyderabad. The test entries were examined at flowering/maturity for pollen/spikelet fertility in the plants suspected to be sensitive to different dates of planting.

The breeding lines derived from several crosses at different stages of their development were screened at the Indian Agricultural Research Institute (IARI), Delhi, as well as DRR, Hyderabad, for the plants remaining totally sterile during the normal flowering month of September in the kharif seasons of 1991 and 1992.

M_2 populations of four varieties (K 39-2, IET 11988, Himdhan and IET 11990) treated with gamma rays (25 kR), ethyl methane sulphonate (0.1%, pH 7, 6 h) and sodium azide (002 M, pH 3, 6 h) were screened for male sterile mutations.

Completely male sterile plants isolated from the above materials were tagged and observed for their sterility–fertility behaviour in the later formed tillers during the cooler months of November–December, and in the stubbles (ratoons) of the same plants during rabi season at Hyderabad. The test plants were examined for pollen fertility by staining with iodine potassium iodide (I-KI).

CHARACTERIZATION OF STABLE TEMPERATURE-SENSITIVE MALE STERILES

Six male sterile lines that were consistently sensitive to the changing temperature regimes were taken for intensive study and characterization. They comprised SM 3 and SM5

from the germplasm, JP 2 and JP 8-1A-12 from breeding lines of unknown crosses, and SA 2 (F 43) and F 61—sodium azide-induced mutations in the variety IET 11990 (Table 1). Growth stage of panicles sensitive to change of temperature was determined by the physical and "tracking" methods developed as described below.

Physical-cum-morphological index method. The stages of panicle development from the day of its initiation were determined by periodically split-opening the main tillers of the test lines. Using the observations on the main tiller, the corresponding developmental stages of the secondary and tertiary tillers were determined. For instance, when the main tiller is at stage V, the pollen mother cell (PMC) formation stage (panicle length 2.5 cm), the secondary tillers which are younger by 6–8 days are at stage IV, i.e. stamen–pistil primordia stage (panicle length 2 mm).

Also, the flag leaf length was used as a morphological index to determine the stage of panicle development. When the flag leaf is about 4–5 cm long, or 20% of the length of the preceeding leaf, the panicle developing inside is at stage V.

Tracking method. The date of panicle emergence and pollen fertility status of each test line raised in adequate population at one-day intervals in nethouse were recorded daily. The date when the first spikelet protruded out was taken as the day of panicle emergence. Five spikelets were examined on the same day for pollen fertility. The observations were continued till the pollen was found to be totally sterile in the newly emerging panicles and 3–4 such days were chosen as tracking dates. On the basis of earlier reports suggesting that the most sensitive phase of panicle development, i.e. the stamen–pistil primordia stage, lies between 24–15 days before heading, the day of critical stage was determined with the help of weather chart showing maximum–minimum temperatures. Days were counted backward from the tracking date between 15 and 24 days and the dates, when the maximum temperature in this period was above 30°C were noted. The temperature record for the 24–15-day preheading period of each test line was examined for three tracking dates as presented in Table 5, and the dates on which the maximum temperature was above 30°C were regarded as critical temperature days coinciding with the sensitive stamen–pistil primordia phase. In the event of more than one date qualifying as critical temperature days, the day with maximum mean temperature was taken as critical. If that too was not discriminative enough, high minimum temperature was used as the criterion for judgement. In the case of SM 3, for instance, 24th, 23rd and 19th days before heading had similar maximum temperatures (> 30°C) and, therefore, the maximum mean temperature for the day was used as the next alternative. Among the prospective days, the 22nd day had the highest mean temperature, and was taken as the day of critical temperature during the sensitive stage. If several days were identical in respect of all the foregoing parameters, then two consecutive critical days with the highest mean temperatures were taken together as the sensitive period.

Determination of critical fertility and sterility points. Four sets of the six temperature-sensitive lines were raised keeping one seedling or stubble per bucket. Four growth chambers each maintained at specific temperature range and light hours were set up and compared with the natural conditions of nethouse (chamber 5) as follows:

Chamber No.	Light duration (h)	Temperature range °C
1	14 h 30 min	26–35
2	9 h	22–30
3	9 h	20–24
4	9 h	24
5	Natural nethouse conditions	

When the test plants reached growth stage IV, a complete set of six lines was transferred to each chamber for 20 days.

Data on pollen and spikelet fertility were recorded on three critical panicles (the panicles in which the critical stage coincided with critical temperature) that emerged during the period of temperature treatment in the growth chambers. Anthers from the top five spikelets of the three panicles were smeared and stained with 1% I-KI solution. Deeply stained round pollen grains were categorised as fertile, the lightly and partially stained round pollen grains were counted as sterile (transformation stage prior to abortion) and the round or deformed but unstained pollen grains as aborted sterile. The change in the sterility–fertility pattern (ratio) was determined from the proportion of pollen grains of these two categories in each sample. The sterility–fertility pattern of spikelets was also recorded on the same set of critical panicles at maturity. The critical panicle (i.e panicles under observation) were numbered in order of their emergence. The temperature range, at which the plants produced higher proportion of fertile and unaborted sterile (partially stained) pollen was taken as the critical fertility point (CFP), while the lowest among the maximum temperatures of the three tracking dates coinciding with the sensitive stage of the three panicles causing complete pollen sterility was recognized as the critical sterility point (CSP) (Table 6).

RESULTS

Isolation of temperature sensitive male steriles. Screening of the germplasm under the 3rd and 4th staggered plantings and a set of late planted high yielding varieties so as to ensure flower production during the high temperature period of the season led to the isolation of 23 sterile plants, 10 plants from 9 germplasm accessions and 13 in the varieties

Pusa Basmati 1, Pranava and Pathara. Study of all the 23 sterile plants raised from the stubbles during February–March, 1993 revealed that 11 plants reverted back to fertile phase gradually and degree of transformation varied among them. Pranava SM 3 and GP SM 5 showed the highest percentage (70–80) of fertility closely followed by Pusa Basmati SM 2 and Pusa Basmati SM 7 with the average fertility of 50%, and the remaining seven plants were in the range of 1.6–20.0% fertility (Table 2).

Table 1. Temperature-sensitive male sterile lines of rice

TGMS line	Source	Plant type	Origin
SM 3	Variety Pranava	Semitall	DRR, Hyderabad
SM 5	Germplasm	Semitall	DRR, Hyderabad
JP 2	Breeding line of unknown crosses	Semidwarf	IARI, Delhi
JP 8-1A-12	—Do—	Semidwarf	IARI, Delhi
SA 2 (43)	Sodium azide induced mutant of IET 11990	Semidwarf	IARI, Delhi
F 61	—Do—	Semidwarf	IARI, Delhi

Out of the several spontaneous plants obtained in the breeding populations of unknown crosses during kharif (wet) season at Delhi, 8 plants had fertility restored when plants from their stubbles were raised during rabi at Hyderabad. Five of them, viz. JP 8-8, JP 8-1A-12, JP 1, JP 2 and JP 27, had high pollen and spikelet fertility (Table 3).

Table 2. Sterility–fertility transformation behaviour of male-sterile plants identified under staggered planting of rice germplasm

Male sterile plant	Parent variety	Rabi, 1992		Plants from stubbles*	
		pollen fertility (%)	spikelet fertility (%)	pollen fertility (%)	spikelet fertility (%)
SM 5	Not known	0.0	0.0	79.9	48.7
SM 3	Pranava	0.0	0.0	70.8	47.2
SM 2	Pusa Bas-1	0.0	0.0	51.7	35.6
SM 7	Pusa Bas-1	0.0	0.0	50.8	31.9
SM 6	Not known	0.0	0.0	20.0	16.0
SM 19	Rasi	0.0	0.0	18.4	21.5
SM 15	Pathara	0.0	0.0	17.5	22.9
SM 27	Ajaya	0.0	0.0	14.4	21.4
SM 17	Pathara	0.0	0.0	3.5	7.5
SM 16	Pathara	0.0	0.0	1.6	5.4

*Plants raised from stubbles were screened in October–November, 1992 and February–March, 1993.

In the M_2 generation of the varieties IET 11990, Himdhan and IET 11988 treated with sodium azide, ethyl methane sulphonate and gamma rays, 68 completely male sterile mutants were isolated during kharif at Delhi. Among them, 16 plants produced seeds in the late formed tillers which emerged towards the end of kharif season when temperature was low. Fourteen such mutations were isolated from sodium azide treated IET 11990. Their pollen fertility ranged from less than 2% in F 40-2 to 76% in F 43

(Table 4). The mutants F 1-3, F 3-2 and F 61 with pollen fertility 71.6, 59.5 and 64.7%, respectively, were considered promising.

Characterization of stable temperature sensitive lines. Six TGMS lines were characterized using the indices of temperature-sensitive stage of panicle development and the critical sterility and fertility points.

Sensitive stage. As determined by the integrated use of the physical-cum-morphological method and the tracking technique, the sensitive stage was found to vary in different lines. It was 17 d (days prior to heading) in SA 2 (F 43), 19 d in JP 2 and F 61, 22 d in SM 3, 23 d in JP 8-1A-12, and 24 d in SM 5 (Table 6).

Table 3. Sterility–fertility transformation behaviour of male sterile segregates/spontaneous mutants isolated from breeding populations of rice

Mutant	Source	Kharif 1992 (Delhi)		Rabi 1992–93 (Hyderabad)	
		pollen fertility (%)	spikelet fertility (%)	pollen fertility (%)	spikelet fertility (%)
JP 8-8	Unknown	0.0	0.0	64.4	82.4
JP 8-1A-12	—Do—	0.0	0.0	61.5	84.1
JP 1	—Do—	0.0	0.0	70.7	85.9
JP 2	—Do—	0.0	0.0	58.7	79.5
JP 27	—Do—	0.0	0.0	60.8	81.2

Note. Stubbles of completely sterile plants isolated during kharif 1992 at IARI, Delhi were grown as ratoon plants in rabi 1992–93 at DRR, Hyderabad.

Table 4. Sterility–fertility transformation behaviour of male sterile mutants of rice in M_2 generation

Parent variety	Mutant	Mutagen	Kharif 1992 (Delhi)		Rabi 1992–93 (Hyderabad)	
			pollen fertility (%)	spikelet fertility (%)	pollen fertility (%)	spikelet fertility (%)
IET 11990	F 1-3	Sodium azide	0.0	0.0	71.6	62.5
	F 3-2	—Do—	0.0	0.0	59.5	35.8
	F 13-3	—Do—	0.0	0.0	12.7	19.2
	F 27-1	—Do—	0.0	0.0	44.2	40.5
	F 35-1	—Do—	0.0	0.0	4.7	7.8
	F 35-3	—Do—	0.0	0.0	45.9	30.8
	F 37	—Do—	0.0	0.0	19.3	31.5
	F 40-2	—Do—	0.0	0.0	1.3	11.0
	F 43	—Do—	0.0	0.0	76.0	58.2
	F 45-1	—Do—	0.0	0.0	20.0	8.5
	F 45-2	—Do—	0.0	0.0	4.1	10.5
	F 61	—Do—	0.0	0.0	64.7	59.1
	F 64	—Do—	0.0	0.0	46.2	30.8
Himdhan	G 17	—Do—	0.0	0.0	10.8	2.8
	B 93-1	EMS	0.0	0.0	5.9	4.7
IET 11988	I4	Gamma rays	0.0	0.0	22.0	35.0

Table 5. Determination of critical stage of panicle development in rice sensitive to temperature

TGMS line	Dates of total sterility in 1993	Maximum and minimum temperatures (°C) on different days prior to heading									Critical temperatures (°C)	
		15	16	17	18	19	20	21	22	23	24	max. min.
SM 3	9.8	32.0/22.4	32.3/22.2	35.6/23.1	36.1/23.6	35.1/25.1	35.1/25.1	34.1/23.1	33.9/23.3	30.9/23.3	30.0/22.6	**33.9** **23.3**
	13.8	26.4/21.7	30.3/22.0	26.8/23.3	31.8/21.9	32.0/22.1	32.3/22.2	35.6/23.1	36.1/23.6	35.1/25.1	35.1/25.1	36.1 23.6
	14.8	28.1/22.4	26.4/21.7	30.3/22.0	26.8/23.8	31.8/21.9	32.0/22.4	32.3/22.2	35.6/23.1	36.1/23.6	35.1/25.1	35.6 23.1
SM 5	5.8	35.1/25.1	35.1/25.1	34.1/23.1	33.9/23.3	30.9/22.5	30.0/22.6	29.0/22.2	28.4/22.6	31.7/23.1	32.3/23.0	**32.3** **23.0**
	11.8	26.8/23.3	31.8/21.9	32.0/22.4	32.3/22.2	35.6/23.1	36.1/23.6	35.1/25.1	35.1/25.1	34.1/23.1	33.9/23.3	33.9 23.3
	13.8	26.4/21.7	30.3/22.0	26.8/23.3	31.8/21.9	32.0/22.1	32.3/22.2	35.6/23.1	36.1/23.6	35.1/25.1	35.1/25.1	35.1 25.1
JP 2	26.7	32.8/24.2	32.5/24.4	33.8/24.9	30.3/24.0	35.0/24.4	34.3/23.7	33.2/23.6	33.5/23.2	33.8/23.5	32.4/23.6	**35.0** **24.4**
	6.8	36.1/23.6	35.1/25.1	35.1/25.1	34.1/23.1	33.9/23.3	30.9/22.5	30.0/22.6	29.0/22.2	28.4/22.6	31.7/23.1	33.9 23.3
	10.8	31.8/21.9	32.0/22.4	32.3/22.2	35.6/23.6	36.1/23.6	35.1/25.1	35.1/25.1	34.1/23.1	33.9/23.3	30.9/22.5	36.1 23.6

Critical fertility and sterility points. The critical temperature for fertility induction as determined by pollen and spikelet fertility-sterility under high (26–35°C), moderately (22–30°C), low (20–24°C) and constant low (24°C) temperature regimes also varied among the test lines (Tables 7, 8).

All the lines except SA 2 (F 43) showed complete pollen sterility only at high temperature. SA 2 (F 43) became sterile at high as well as moderate temperatures. Sterility was characterized only on the basis

Table 6. Critical temperatures for different TGMS lines of rice

TGMS Line	Dates of total sterility in 1993	Critical temperature (°C)		Days before heading
		max.	min.	
SM 3	9.8	**33.9**	**23.3**	22
	13.8	36.1	23.6	
	14.8	35.6	23.1	
SM 5	5.8	**32.3**	**23.0**	24
	11.8	33.9	23.3	
	13.8	35.1	25.1	
JP 2	26.7	35.0	24.4	19
	6.8	**33.9**	**23.3**	
	10.8	36.1	23.6	
JP 8-1A-12	9.8	**30.9**	**23.3**	23
	10.8	33.9	23.3	
	17.8	32.0	22.4	
SA 2	30.7	**31.7**	**23.1**	17
	6.8	35.1	25.1	
	9.8	35.1	23.1	
F 61	30.7	32.8	24.2	19
	5.8	**30.9**	**22.5**	
	6.8	33.9	23.3	

Figures in bold show their critical sterility points (CSP), i.e. the lowest temperature at which a line becomes completely sterile.

Table 7. Determination of critical temperature for fertility restoration on

TGMS Line	Chamber 1											
	Panicle 1				Panicle 2				Panicle 3			
	AS	RS	US	F	AS	RS	US	F	AS	RS	US	F
SM 3	97.1	2.9	0.0	0.0	100.0	0.0	0.0	0.0	92.9	7.1	0.0	0.0
SM 5	92.6	7.4	0.0	0.0	96.9	3.1	0.0	0.0	89.4	10.6	0.0	0.0
JP 2	80.0	10.9	9.1	0.0	88.1	6.8	5.1	0.0	94.1	5.9	0.0	0.0
JP 8-1A-12	78.2	21.2	0.6	0.0	100.0	0.0	0.0	0.0	89.1	10.9	0.0	0.0
SA 2	100.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	75.7	24.3	0.0	0.0
F 61	84.8	15.2	0.0	0.0	100.0	0.0	0.0	0.0	96.8	3.2	0.0	0.0

TGMS Line	Chamber 3											
	Panicle 1				Panicle 2				Panicle 3			
	AS	RS	US	F	AS	RS	US	F	AS	RS	US	F
SM 3	91.8	3.6	4.1	0.4	72.7	4.5	8.4	14.4	82.7	3.6	5.2	8.5
SM 5	43.8	0.0	25.7	30.5	16.7	0.0	37.9	45.4	66.7	16.6	8.7	7.7
JP 2	7.9	7.5	20.5	64.1	10.2	6.9	4.1	78.8	2.9	7.8	32.9	57.1
JP 8-1A-12	69.9	28.9	1.2	0.0	16.3	28.0	32.2	23.5	5.2	0.0	14.4	80.4
SA 2	61.2	29.1	9.7	0.0	8.4	19.6	29.4	42.1	10.2	11.0	18.2	60.5
F 61	53.4	35.8	10.7	0.0	11.3	19.0	51.0	18.6	0.0	13.1	21.6	65.3

Regime	Chamber 1	Chamber 2	Chamber 3	Chamber 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.

of aborted pollen. The round poorly stained pollen grains were ignored. A tendency for transformation to fertile phase was evident from the increasingly higher proportion of fertile and semistained pollen with decreasing temperature. With the exception of SA 2 (F 43), all other lines tended to improve fertility even at moderate temperature, and fertility restoration was more striking at low temperature. The lines, however, differed from each other in the degree of fertility restoration. The range of pollen fertility varied in different

the basis of fertility transformation pattern under different temperatures

Chamber 2											
Panicle 1				Panicle 2				Panicle 3			
AS	RS	US	F	AS	RS	US	F	AS	RS	US	F
80.2	17.9	1.9	0.0	56.6	30.1	11.5	1.8	66.5	23.0	10.5	0.0
65.0	28.1	6.8	0.0	55.0	23.8	18.3	2.7	50.5	40.5	9.0	0.0
49.4	15.4	35.3	0.0	10.7	4.4	36.5	46.4	11.8	14.0	29.9	44.9
9.5	70.0	18.6	1.9	0.0	27.9	46.1	26.1	18.0	51.3	27.9	2.8
100.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	65.0	35.0	0.0	0.0
56.6	25.4	18.0	0.0	0.0	33.1	40.2	26.9	0.0	14.2	62.3	23.4

Chamber 4											
Panicle 1				Panicle 2				Panicle 3			
AS	RS	US	F	AS	RS	US	F	AS	RS	US	F
72.7	1.0	12.1	15.2	97.8	0.0	1.1	1.1	96.8	0.6	2.6	0.0
43.6	6.0	26.5	23.9	11.8	0.0	20.4	45.8	46.0	1.6	18.5	33.9
8.4	9.3	28.4	53.8	2.0	4.4	24.1	67.5	10.3	17.9	56.4	15.4
22.2	47.5	30.3	0.0	0.0	19.7	42.7	37.5	0.0	14.4	17.9	67.6
59.0	10.0	19.1	11.9	29.1	14.2	38.1	18.7	48.0	15.0	37.0	0.0
21.0	38.8	40.1	0.0	0.0	11.1	20.4	68.4	0.0	10.6	23.6	65.9

chambers for the same genotype as well as different genotypes in the same chamber. While in chamber 1 all the genotypes were sterile, in chambers 2, 3 and 4, fertility restoration was 1.9 (JP 8-1A-12) to 46.4% (JP 2); 0.4 (SM 3) to 80.4% (JP 8-1A-12); and 1.1 (SM 3) to 68.4% (F 61), respectively.

Spikelet fertility was also restored following the trend broadly similar to that of pollen fertility. All the lines were completely sterile at high temperature and, except SM 3, all of them started reverting back to fertile state with the lowering of temperature. As in the case of pollen sterility, here also the lines differed in the degree of fertility restoration.

Barring JP 2, which had the highest spikelet fertility of 79% at moderate temperature, maximum fertility restoration occurred at low temperature in all other lines: from 25% in

Table 8. Spikelet fertility of selected lines sensitive to temperature under different temperature regimes

TGMS line	Chamber 1			Chamber 2			Chamber 3			Chamber 4		
	spikelet fertility (%)			spikelet fertility (%)			spikelet fertility (%)			spikelet fertility (%)		
	panicle No. 1	panicle No. 2	panicle No. 3	panicle No. 1	panicle No. 2	panicle No. 3	panicle No. 1	panicle No. 2	panicle No. 3	panicle No. 1	panicle No. 2	panicle No. 3
SM 3	0.0	0.0	0.0	0.0	0.0	0.0	14.7	24.6	16.4	25.0	7.6	5.6
SM 5	0.0	0.0	0.0	0.0	3.1	0.0	70.6	65.0	68.4	71.6	68.2	66.7
JP 2	0.0	0.0	0.0	66.7	78.4	77.2	34.0	43.6	62.2	29.6	57.1	64.7
JP 8-1A-12	0.0	0.0	0.0	2.0	6.2	3.8	67.8	83.8	85.3	77.3	89.1	84.6
SA 2 (F 43)	0.0	0.0	0.0	0.0	0.0	0.0	12.8	35.8	38.6	11.1	16.7	21.9
F 61	0.0	0.0	0.0	0.0	18.1	26.2	76.4	74.0	78.6	77.2	73.2	41.0

SM 3 to 85% in JP 8-1A-12. The lines also differed in their response to constant low temperature. The percentage of fertile pollen was similar in SM 3, SM 5 and JP 8-1A-12, which was lower than SA 2 (F 43) and higher than the fertility level recorded in F 61 at low temperature.

DISCUSSION

The advantages of the environment sensitive genic male sterility-based two-line hybrids over the presently popular cytoplasmic male sterility-based three-line hybrids prompted the present study with the specific objective of isolating and characterizing temperature-sensitive genic male sterile sources suitable for tropical environments of India.

Isolation of temperature sensitive male steriles. Germplasm, breeding lines and mutagen-treated populations were used as the material for isolation of TGMS sources assuming that inherent variability, if any in the former two groups of genetic materials and induced variability in the mutagen-treated, which is not detectable in the range of normal temperatures, could express only under appropriate temperature. Isolation of photoperiod-sensitive male sterile Nongken 58 S under long-day conditions by Chinese workers [10] and Norin PL-12 under high temperature conditions in the gamma ray-treated populations by Japanese workers [5] supports this assumption. In the case of mutational approach, the choice of parental varieties was restricted to those adapted to high altitudes in the hope that the probability of recovering TGMS mutants with low CFP–high CSP in them would be high and they are likely to be prone to physiological imbalances associated with the change of temperature. Among the mutagens, sodium azide appeared most promising. Its efficiency however, was genotype dependent, with the highest frequency of TGMS mutations isolated in IET 11990. Among the several male sterile lines recovered in

the present study, 11 in germplasm, 7 in the breeding lines and 17 in the mutagenized populations appear to be sensitive to changing temperature regimes. Further study of the lines under controlled temperature suggests that they differ widely in the degree of transformation to fertile phase; the level of fertility restoration being as high as 80 to less than 25%. Although the environment-sensitive male sterility–fertility system has been reported to be a simple recessive trait [11–13], genetic basis of the wide differences observed in fertility restoration require more intensive study. From utility point of view, SM 3, SM 5, JP 8-1A-12, JP 2, SA 2 (F 43) and F 61, which showed high degree of fertility restoration at low temperature are more interesting.

Characterization of promising temperature-sensitive male sterile lines. The temperature-sensitive stage and critical temperature range for sterility–fertility transformation are the two important parameters that help characterize TGMS sources. Precise information on these two indices is important for choosing the appropriate source for development of two-line hybrids.

The temperature responsive stage of EGMS sources is determined by physically examining the developing panicles and correlating the observations with easily measurable morphological indicators, such as, flag leaf (nth leaf) length [14, 15]. By relating flag leaf length periodically with the size of the developing panicle, the following stages have been identified:

Flag leaf length		Panicle	
cm	relative	size (cm)	stage
4.0	1/5 of (n-1)th leaf	0.2	IV (pistil–stamen primordia formation)
6–8	3/5 of (n-1)th leaf	2.5–3.5	V (pollen mother cell formation)
—	4/5 of (n-1)th leaf	—	VI (beginning of meiosis)
Collar—auricle of flag leaf and (n-1)th leaf at same level		—	VII (meiosis in middle of the panicle)

Based on the stage of panicle development on the main tiller, the corresponding stages in the secondary and tertiary tillers can also be judged. If the panicle length of the main tiller is 5.0 cm (between stages V and VI) for instance, panicle in the secondary tiller will be in

stage-II (initiation of primary and secondary rachis branch primordia). Although the secondary tillers emerge 10–14 days after the main tiller, the difference in flowering time between them is only 7–8 days. This knowledge enabled us to study the test lines at the desired temperature and stage of panicle development.

In addition to the physical-cum-morphological indicators, the tracking technique developed in the present investigation was useful in determining precisely the sensitive stage for sterility/fertility induction. This technique requires daily recording of pollen fertility/sterility of the test lines in an environment similar to that of Hyderabad, where temperature is always fluctuating and flowering occurs almost throughout the year. As described in the Materials and Methods, flowering was recorded till the day the pollen was found completely sterile. Taking this day as the 'tracking date' the days are counted backward up to 15–24 days to determine the temperature coinciding with the 'sensitive stage' with the help of a weather chart. The earlier reports show that the most sensitive stage of panicles is the stamen–pistil primordia stage which lies between 24–15 days before heading depending on the maturity duration of a variety [14–16]. The sensitive stage so determined by the tracking technique varied from 24 days before heading in SM 5 to 17 days in SA 2 (F 43). The day of sensitive stage of different TGMS lines was in good agreement with the respective dates of their fertility transformation under controlled temperature. The slight variation observed in some cases may be attributed to several factors that influence the growth pattern. Among them, the fluctuating weather which causes 1–2 day difference [16] and differential growth rates of the primary–secondary tillers are important. In general, the present tracking technique has following advantages over earlier used techniques:

1. It is an easier technique.

2. It enables more precise determination of the sensitive stage without injury to the plant (as in the physical split-opening technique) and leaves no scope for arbitrariness (which is possible in the flag leaf index method).

3. Abrupt fluctuations in temperature affecting growth and development are not a limitation as the sensitive stage is determined by counting back from the sterility induction day.

4. It enables simultaneous determination of temperatures of the critical fertility and sterility points.

The technique in combination with flag leaf index becomes even more efficient in precise determination of the sensitive stage.

Characterization of promising TGMS sources. On the basis of their CSP and CFP, determined by the tracking technique and exposure to controlled temperature, the six TGMS lines were found to be high temperature sterile–low temperature fertile type, and could be divided in the following groups:

1. High CSP (> 32°C) — low CFP (20–24°C)
2. High CSP (> 32°C) — high CFP (24–30°C)
3. Low CSP (30–32°C) — low CFP (20–24°C)

The fourth possible group of low CSP–high CFP was not found. Essentially, the grouping is based on the relative difference between the CSP and CFP designated by Zhang et al. [7] in case of photoperiod sensitive genetic male sterility (PGMS) as the temperature related photoperiod sensitivity (TRPS). During this photoperiod, according to these authors, the pace of transformation to sterility or fertility is determined by daylength. Unlike the PGMS system, the phenomenon of sterility–fertility transformation is least affected by photoperiod in TGMS system, making it convenient and ideally suitable for tropical situations. It is the CSP and CFP level that largely determines the stability and commercial usefulness of the TGMS lines. If the CFP of a TGMS source is not low enough, even slight drop in temperature during seed production period would result in selfing where 100% male sterility and only hybrid seed set are desired. On the other hand, if CSP is not high enough, multiplication of TGMS lines would be a problem, especially under short-day and high temperature conditions. Thus, an ideal CFP–CSP system is highly environment specific. In China, where moderate temperature is combined with distinct short or long photoperiods, the ideal TGMS line for commercial exploitation should be high CSP–low CFP [12, 17]. For tropical conditions, on the other hand, low CSP–low CFP type appears to be desirable. This is evident from the present findings, where the lines with CSP > 30°C but < 32 °C were completely sterile (85% abortive and 15% round sterile pollen grains) and CFP < 24°C caused up to 80% spikelet fertility restoration.

The fertility–sterility transformation behaviour of the six lines is grouped into three different types, and their usefulness in commercial hybrid breeding is described below.

Type 1: *high CSP–low CFP.* This type is recognised by the Chinese workers as the ideal for China, as it would be safe for both hybrid seed production and maintenance of the TGMS lines. However, such a type is yet to be identified [18]. Although it is not yet clear where to draw the line for high CSP and low CFP, based on the available temperature and photoperiod regimes in a given region, a narrow range can be selected. The lines SM 3 and SM 5 fall under this category. The former with a CSP 33.9°C and its degree of fertility

transformation, has been far below the minimum required even under the low temperatures (20–24°C), indicating that still lower temperature can increase spikelet fertility, further (Fig. 1). Realization of as high as 70% fertility under the natural cool weather conditions

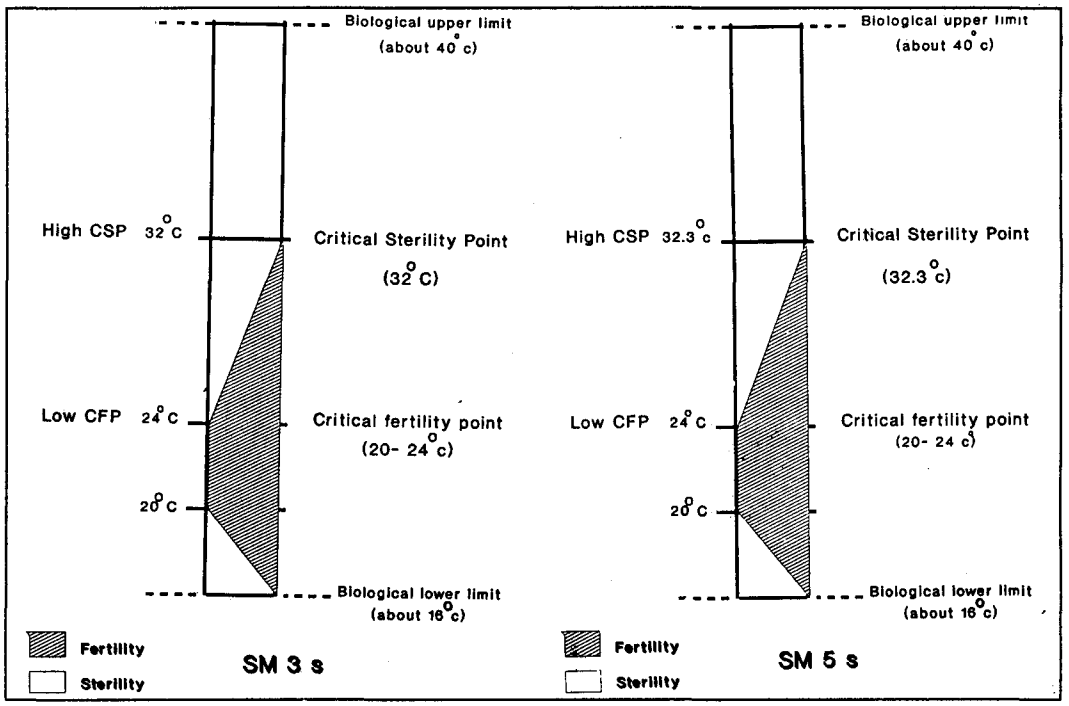

Fig. 1. Critical sterility and fertility points of SM 3s and SM 5s.

(min. 16–20°C; max. 28–32°C) supports the above conclusion. The line SM 5, on the other hand, shows moderately high pollen fertility (45%) at low controlled temperature as well as in field conditions. Therefore, from utility point of view SM 5 has an advantage over SM 3.

Type 2: *high CSP–high CFP.* Majority of the EGMS lines identified by the Chinese workers belong to this type. Zhang et al. [12] classified this type as 7001 S. TGMS lines 8902S and W 7415 S, although belong to this category are not suited to the Chinese conditions. However, they may prove useful under tropical situations. In the present study, JP 2 alone falls under this type. With high CSP (34°C), its fertility transformation behaviour with 45% fertility even at moderate temperature is adequate for commercial exploitation. Its CFP could be close to 26°C (Fig. 2).

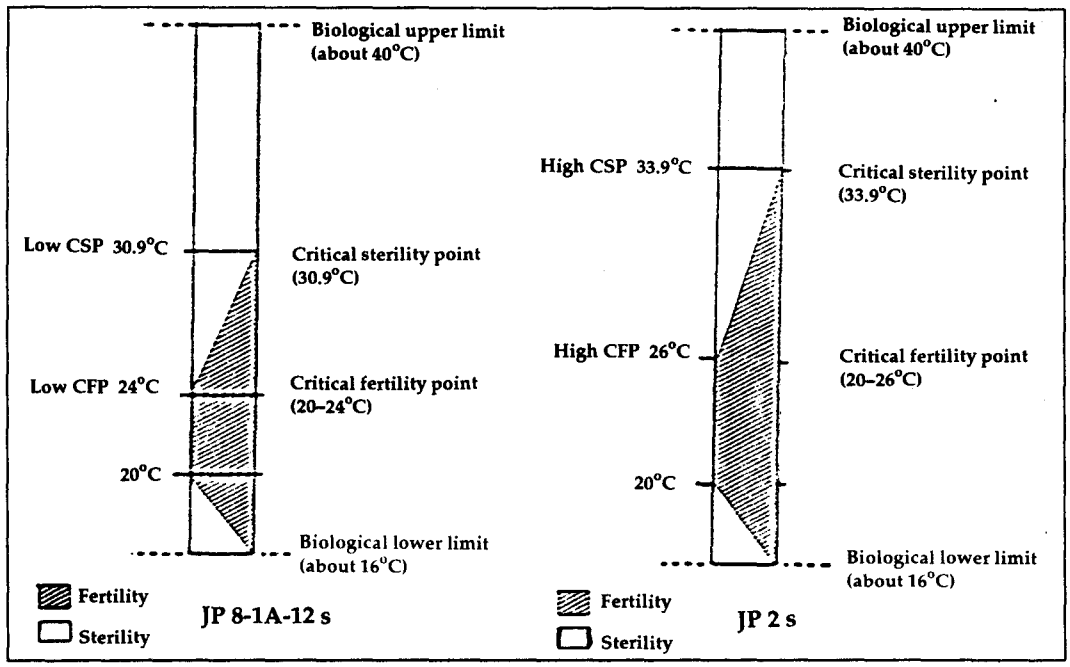

Fig. 2. Critical sterility and fertility points of JP 8-1A-12s and JP 2s.

Type 3: *low CSP–low CFP.* The stable sterility phase over a large region prompted the Chinese workers to use the TGMS lines of this type (Peiai 645 type) commercially in the initial years. However, difficulties experienced in the maintenance of such TGMS lines ultimately limited their utilization [12, 17]. In tropical countries like India, where the sterile phase is required to be more stable, this TGMS type would be useful. Three lines in our collection, JP 8-1A-12, SA 2 (F 43) and F 61, belong to this type (Figs. 2, 3). Among them, JP 8-1A-12 manifesting total pollen sterility even at high temperatures in the lower range (30.9°C) and high (> 80%) pollen/spikelet fertility at low temperature appears to be most promising. Fertility decline even at 24°C suggests its CFP to be around 20°C. The fertility transformation behaviour of SA 2 (F 43) and F 61 suggests the CFP of the former to be the same as of JP 8-1A-12 (20°C) and CFP of the latter 24°C.

In the absence of the required day-length differences to make use of PGMS system, the day-neutral TGMS system, is most appropriate for two-line hybrid breeding in many tropical countries of Asia. Successful use of TGMS, however, depends on choosing highly region-specific sources. An ideal TGMS for tropical situation is the one which combines low CSP (30–32°C) and low CFP (20–24°C). Stable sterility and high degree of fertility restoration with seed set exceeding 40% are important considerations while selecting the right source. Out of the six lines studied by us, JP 8-1A-12 and F 61 belong to the low CSP–low CFP

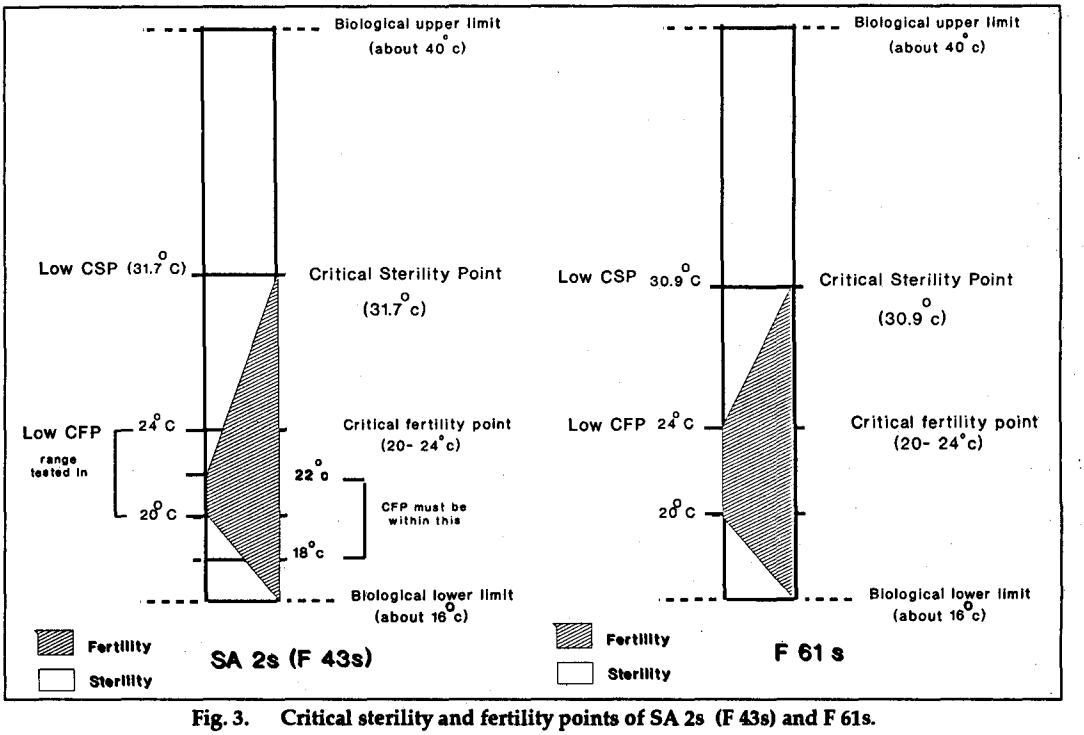

Fig. 3. Critical sterility and fertility points of SA 2s (F 43s) and F 61s.

category, and therefore appear to be most promising. These lines are also productive semidwarfs. It would be worthwhile to study their suitability in hybrid seed production on field scale at place(s), where stable and desired temperature regimes are available.

REFERENCES

1. M. S. Shi. 1981. Preliminary report of breeding and utilization of late *japonica* natural double purpose line. J. Hubei Agric. Sci., 7: 1–3.

2. M. S. Shi and J. Y. Deng. 1986. The discovery, determination and utilization of the Hubei photosensitivity genic male sterile rice (*Oryza sativa* ssp. *japonica*). Acta Genet. Sinica, 13 (2): 107–112.

3. Y. G. Lu and J. Wang. 1986. Fertility transformation and genetic behaviour of Hubei photoperiod sensitive genic male sterile rice. *In*: Hybrid Rice. IRRI Philippines: 129–138.

4. Z. C. Tan, Y. Y. Li, L. B. Chen and G. Q. Zhou. 1990. Studies on ecological adaptability of dual purpose line An-Nong S-1. Hybrid Rice, 3: 35–38.

5. K. Maruyama, H. Araki and H. Kato. 1991. Thermo-insensitive genetic male sterility induced by irradiation. *In*: Rice Genetics II. IRRI, Philippines: 227–235.

6. Z. G. Zhang, S. C. Yuan, H. L. Zen, Y. Z. Li, Z. C. Li and C. L. Wei. 1991. Preliminary observation of fertility changes in the new type temperature sensitive male sterile IV A. Hybrid Rice, **13**: 31–34.

7. Z. G. Zhang, S. C. Yuan, H. L. Zen, Y. Z. Li and B. X. Wang. 1991. Studies on the fertility changes of photoperiod temperature sensitive genic male sterile W 6154 S in response to temperature. J. Huazhong Agric. Univ., **10**(1): 21–25.

8. L. P. Yuan. 1993. Progress in two-line system in hybrid rice breeding. *In:* New Frontiers in Rice Research (eds. K. Muralidharan and E. A. Siddiq). Directorate of Rice Research, Hyderabad: 86–90.

9. S. S. Virmani. 1993. Global status of hybrid rice research and development in hybrid rice. *In*: Food Security in India (ed. B. R. Barwale). Mc Millan India Limited, Madras: 3–21.

10. M. S. Shi. 1985. The discovery and study of photosensitive recessive male sterile rice (*Oryza sativa* L. subsp. *japonica*). Sci. Agric. Sinica, **2**: 44–48.

11. X. Zhang and Y. Zhu. 1990. A genetic study on sterility of Hubei photoperiod sensitive genic male sterile rice. J. Huazhong Agric. Univ., **9**(4): 481.

12. Z. G. Zhang, S. C. Yuan, H. L. Zen and Y. Z. Li. 1992. Studied on the inheritance of two photoperiodic reactions in photoperiod sensitive genic male sterile rice. J. Huazhong Agric. Univ., **1**(1): 7–14.

13. S. C. Yuan, Z. G. Zhang, H. H. He, H. L. Zen, J. H. Lian and W. X. Wang. 1993. Two photoperiod sensitive genic male sterile rices. Crop Sci., **33**(4): 651–660.

14. S. C. Yuan, Z. G. Zhang and C. Z. Xu. 1988. Studies on the critical stage of pollen fertility changes induced by photoperiod and its development phases in HPGMR. Acta Agron. Sinica, **14**(1): 7–13.

15. M. Wang and G. Mei. 1990. Study on the fertility change sensitized to Nangken 58 S. J. Hauzhong Agric. Univ., **9**(4): 363–368.

16. S. Matsushima. 1970. Crop Science in Rice. Fiji Publ. Co. Tokyo: 379.

17. L. P. Yuan. 1992. The strategy of breeding rice PGMS and TGMS lines. Hybrid Rice, **1**: 1–4.

18. L. P. Yuan. 1992. Increasing yield potential in rice by exploitation of heterosis. *In*: International Rice Research Conference. April 21–25, 1992, IRRI, Philippines.