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ISOLATION AND CHARACTERIZATION OF A REVERSE TEMPERATURE SENSITIVE GENIC MALE STERILE MUTANT IN RICE

A. JAUHAR ALI AND E. A. SIDDIQ*

Department of Crop Improvement,
Agricultural College and Research Institute, Tiruchirapalli 620 009

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ABSTRACT

A spontaneous male sterile mutant isolated from a farmer's field at Katrain, Himachal Pradesh during early *kharif* (< 24°C) when stubble-planted at Delhi under high temperature (> 30°C) condition set seeds indicating thus its temperature-influenced behaviour of sterility-fertility alteration. Physiological characterization of the mutant designated as JP 38 S under natural and controlled growth chamber conditions has revealed its critical sterility and fertility points to be 24°C and 30.5°C respectively. Critical stage of sensitivity to temperature is 16 days before heading. The mutant source, representing a reverse form of the widely known normal temperature sensitive genic male sterility (TGMS), that remains sterile at high temperature and turns fertile at low temperature will be as much valuable as that of the latter in commercial hybrid seed production in the hills with low temperature and seed multiplication of JP 38 S in the plains with high temperature.

Key Words : Rice, hybrid, male sterility, reverse temperature sensitive male sterility, characterization, critical sterility point, critical fertility point, seed production

Two-line hybrid breeding in rice started with the chance discovery of a photoperiod sensitive genic male sterile (PGMS) plant named later as Nongken 58 S in the *japonica* variety Nongken 58 [1-3] closely followed by temperature sensitive genic male sterile (TGMS) sources like Annong 1s in China and Norin PL 12 in Japan [4, 6]. The TGMS sources remain male sterile under high temperature and fertile under low temperature conditions [4]. Subsequently, Chinese have identified 'IV A' followed by 'Dian Xin 1A', as TGMS sources of different kinds. Their sterility-fertility behaviour is just opposite of earlier reported TGMS lines. Unlike Annong 1s and Norin PL 12, the typical TGMS sources, the newly found one remains sterile under low temperature (<24°C) and reverts to fertile phase under high temperature (> 27°C) [7, 8]. Such reverse TGMS types can also be utilized in commercial hybrid seed production in the tropics. Keeping in view, the potential of

*National Professor, Directorate of Rice Research, Hyderabad 500 030

this reverse TGMS type the present study was undertaken with the specific objective of physiologically characterizing the same.

MATERIALS AND METHODS

A spontaneous male sterile mutant isolated from a farmer's field at Katrain, (Himachal Pradesh) during early *kharif* when cooler regime (< 24°C) prevails was tagged and stubble-planted under high temperature regime (> 30°C) at the Indian Agricultural Research Institute, Delhi. The stubble-raised mutant was studied for pollen sterility by staining with I-KI 1% and spikelet sterility as number of filled fertile to unfilled sterile grains. Seed harvested from the stubble-planted material was used for sowing at Hyderabad during early *rabi*, 1992-93. Pollen and spikelet sterility was recorded. Exclusively constructed growth chambers were used to physiologically characterize it in terms of critical sterility point (CSP), the temperature at which the plant becomes completely male sterile and critical fertility point (CFP), the temperature at which the plant shows maximum fertility. The responsive stage (pistil- stamen primordia formation phase) was determined by split-opening primary tillers at different intervals and correlating the size of the developing panicle with flag leaf length. When the flag leaf was about 1/5th of the length of preceding leaf the length of the developing panicle inside was 0.2 cm, an indication of pistil-stamen primordia formation phase. The date of emergence of panicles exhibiting fertility transformation suggested that the date of their placement under low temperature regime in growth chamber was the responsive stage i.e., pistil-stamen primordia phase [9, 10]. Ten seedlings uniformly of 30 day old were transplanted in buckets at one seedling per bucket. The growing conditions provided in the various growth chambers were as under:

Chamber - 1: Light duration of 14 hours and 30 minutes;
Maximum temperature of 35°C and minimum temperature of 26°C.

Chamber - 2: Light duration of 9 hours;
Maximum temperature of 30°C and minimum temperature of 22°C.

Chamber - 3 : Light duration of 9 hours;
Maximum temperature of 24°C and minimum temperature of 20°C

Chamber - 4 : Light duration of 9 hours;
Constant temperature of 24°C

Chamber - 5 : Natural condition (Hyderabad)

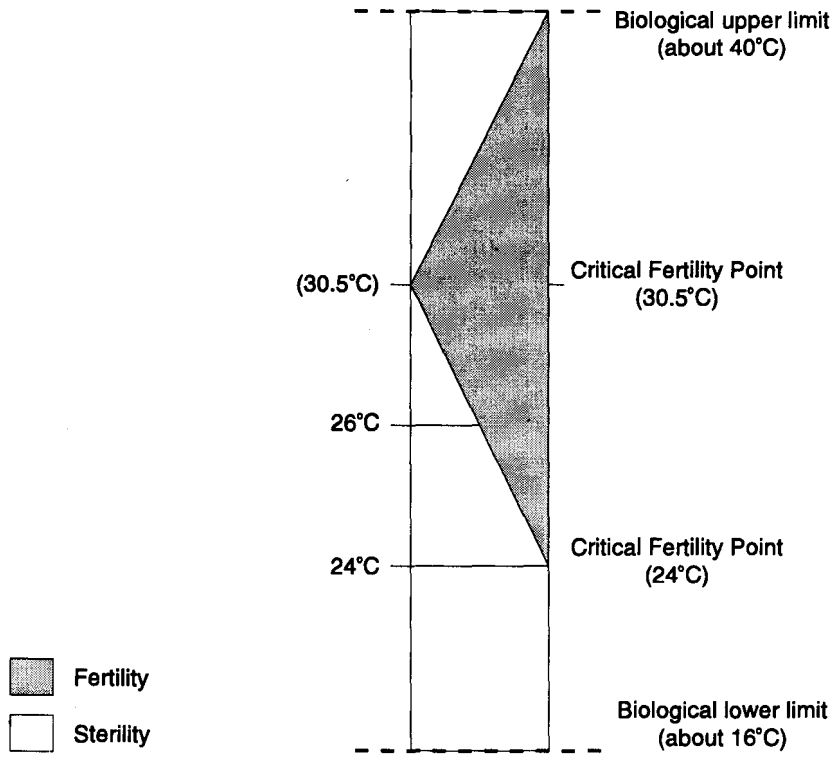

Fig. 1. Critical sterility and fertility points of JP. 38 S

Light intensity measured at the top of the plant in the growth chambers was about 220 μ mol $m^{-2}S^{-1}$ PAR (17800 Lux).

Data on pollen and spikelet fertility/sterility were recorded for three critical panicles that emerged during the period of above listed temperature treatments. Pollen fertility was determined by smearing and staining with I-KI (1%) solution of anthers from five spikelets. Deeply stained and round pollen grains were taken as fertile (F), while lightly stained round pollen and one-half stained as partially fertile (PF). Deshaped and non- stained as aborted sterile (AS) and round nonstained as round sterile (RS). In order to understand the pattern of fertility-sterility transformation in the test line, these pollen categories were recognized. The proportion of the various categories under different temperature regimes (chambers) was determined by fixing emerging panicles at different dates and studying the shape and stainality of their pollen under microscope. The critical panicles were numbered according to the order of their emergence as critical panicle number 1 (CP 1) critical panicle number 2 (CP 2) and critical panicle number 3 (CP 3).

RESULTS AND DISCUSSION

Chinese were the first to report that the spontaneous CMS mutant Dian Xin 1A, of *japonica* background to be just opposite of the typical TGMS in its temperature-influenced fertility-sterility transformation behaviour [8]. Closely following this report breeders at Yunnan, China have been successful in identifying another similarly behaving TGMS 'IVA' of spontaneous origin [7]. These reverse TGMS lines remain sterile under low temperature (< 22-24°C) and turn fertile under high temperature (> 27°C) [7, 8]. JP 38 S is a spontaneous male sterile variant accidentally isolated in a farmer's field at Katrain, (Himachal Pradesh) in the month of September, when the entire crop was fertile there. The stubble-planted variant was found to set seeds during the first week of October at Delhi. Subsequent study of the plant raised at Hyderabad as transplanted *rabi* crop revealed it to show a very high degree of pollen and spikelet fertility. The stubbles of it planted there itself in the last week of December turned completely pollen and spikelet sterile during the first week of March (Table 1).

Table 1. Behaviour of the spontaneous mutant JP 38 S under different temperature regimes

Low temperature regime (Jul-Aug. 1992) (Katrain, H.P.) Seed sown		High temperature regime (Sep-Oct. 1992) (New Delhi) Stubble-planted		Low temperature regime (Feb-Mar 1993) (Hyderabad) Stubble-planted		High temperature regime (Apr-Mar 1993) (Hyderabad) seed sown	
Sterility(%)		Sterility(%)		Sterility(%)		Sterility(%)	
Pollen	Spikelet	Pollen	Spikelet	Pollen	Spikelet	Pollen	Spikelet
100.0	100.0	1.6	2.0	100.0	100.0	0.0	0.0

Study of the variant under controlled temperature conditions confirmed it to remain totally fertile with as high as 92.8 per cent pollen fertility under high temperature (chamber 1) regime. With progressively decreasing mean temperature conditioned in chambers 2, 3 and 4, the line reverted gradually to sterile state. Under moderate temperature partially fertile and round sterile types of pollen increased to 51 and 41 per cent respectively with fertile pollen reduced to less than 10 per cent. The sterility was nearly complete in chambers 3 and 4 maintained at low temperature. Round sterile type of pollen rose to 58-85 per cent and abortive pollen type between 8 and 42 per cent (Table 2). The trend of increase or decrease of spikelet sterility was also similar to that of pollen sterility. It was completely spikelet fertile (96%)

at high temperature (chamber 1), while it reverted to high degree of sterility at moderate temperature (chamber 2) and total sterility under low temperature (chambers

Table 2. Determination of critical temperature for fertility restoration on the basis of pollen and spikelet fertility transformation under different temperatures for JP 38S

Temperature regime	Critical panicle 1				Critical panicle 2				Critical panicle 3			
	AS	RS	PF	F	AS	RS	PF	F	AS	RS	PF	F
Chamber 1	16.2	17.3	19.9	46.6	0.0	3.0	4.2	92.8	0.0	11.0	36.5	52.5
		(93.8)				(96.2)				(94.7)		
Chamber 2	22.8	19.5	54.4	3.3	11.4	35.4	51.0	2.1	21.2	41.2	27.3	10.2
		(8.7)				(6.7)				(7.1)		
Chamber 3	10.6	85.8	14.2	0.0	41.7	58.3	0.0	0.0	19.0	59.8	11.7	9.5
		(0.0)				(0.0)				(1.8)		
Chamber 4	8.2	75.8	16.0	0.0	0.0	19.7	42.7	37.5	0.0	14.4	17.9	67.6
		(0.0)				(0.0)				(0.0)		

AS: Aborted sterile RS: Round sterile PF: Partial fertile F: Fertile

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

Figures within parenthesis: percentage spikelet fertility and pertains to the same panicle chosen for pollen study

3 and 4) conditions (Table 2). The sensitive stage to fertility-sterility alteration was 16 days before heading as determined by using morpho-physical techniques [10]. The findings on the temperature response of JP 38 S thus suggest its critical fertility point (CFP) and critical sterility point (CSP) to be 24° and 30° respectively (Fig. 1). The reverse TGMS sources are as good as normal TGMS. But their exploitation in hybrid seed production and the reverse TGMS multiplication would depend on identifying appropriate environment. Whereas hybrid seed production can be done at high altitude low temperature regions, their seed multiplication in the plains characterized by high temperature. To know whether the reverse TGMS is governed

by an allele at the same locus of the normal TGMS gene or altogether by a different gene there is need for indepth genetic investigations.

REFERENCES

1. M. S. Shi. 1981. Preliminary report of breeding and utilization of late *japonica* natural double-purpose line. J. Hubei Agricultural Sciences., **7**: 1-3.
2. M. S. Shi. 1985. The discovery and study of the photosensitive recessive male-sterile rice (*Oryza sativa* L. sub. sp. *japonica*). Scientia Agricultural Sinica., **2**: 44-48.
3. M. S. Shi and J. Y. Deng. 1986. The discovery, determination and utilization of the Hubei photosensitive genic male-sterile rice (*Oryza sativa* sub sp *Japonica*). Acta Genetica. Sinica., **13**: 107-112.
4. Z. C. Tan, Y. Y. Li, L. B. Chen, G. Q. Zhou. 1990. Studies on ecological adaptability of dual purpose line Annong s-1. Hybrid Rice., **3**: 35-38.
5. K. Maruyama, H. Araki and E. Amano. 1990a. Enhancement of outcrossing habits of rice plant by mutation breeding. Gamma Field Symposia., **29**: 11-25.
6. K. Maruyama, H. Araki and H. Kato. 1990b. Thermosensitive genetic male sterility induced by irradiation 1999b. *In*: Rice Genetics II. P.O. Box. 933, Manila, the Philippines. 227-235.
7. Z. G. Zhang, S. C. Yuan, H. L. Zeng, Y. Z. Li, Z. C. Li and C. L. Wei. 1991b. Preliminary observation of fertility changes in the new type temperature sensitive male sterile rice IVA. Hybrid Rice, **1**: 31-34.
8. Y. M. Jiang. 1988. Studies on the effect of high temperature on fertility of the male sterile lines in Dian type hybrid rice (in China) J. Juinnan Agric. Univ., **3**(2): 99-107.
9. J. Ali. 1993. Studies on temperature sensitive genic male sterility and chemical induced sterility towards development of two line hybrids in rice (*Oryza Sativa L.*) Unpubl. Ph.D. Thesis, Indian Agricultural Research Institute, New Delhi.
10. 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*). Indian J. Genet., **55**: 243-259.