Australian Journal of Crop Science AJCS

AJCS 6(6):957-962 (2012) ISSN:1835-2707

Characterization of thermo-sensitive genic male sterility (TGMS) rice genotypes (*Oryza sativa* L.) at different altitudes

R. K. Salgotra[1 *], B. B. Gupta[1] and M. I. Ahmed[2]

[1]Division of Plant Breeding and Genetics, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Chatha, Jammu-180 009 (J & K), India
[2]Hybrid Rice Section, Directorate of Rice Research (ICAR), Rajendera Nagar, Hyderabad (A.P.) India

***Corresponding author: rks_2959@rediffmail.com**

Abstract

Thermo-sensitive genic male sterility (TGMS) is the genic sterile system in plants that affects the fertility/sterility response to temperature in hybrid rice breeding. Eight TGMS lines, DDR 1S, DDR 18S, DDR 19S, DDR 20S, DDR 23S, DDR 27S, DRR 28S and DDR 29, showed satisfactory seed-set percentage at high altitude, but complete sterility at low altitude. Characterization of sterility-sensitive stage and floral traits were determined by the tracking method. At low altitude, with an average air temperature of 35.4 °C, TGMS lines DRR 19S, DRR 20S and DRR 29S displayed a sterility-sensitive stage at 21 days prior to normal heading. The TGMS line DRR 1S required a temperature of 36.6 °C for complete sterility at 17 days prior to normal heading. In the remaining seven lines, the temperature for complete sterility ranged from 33.9 °C to 35.8 °C at low altitude. Angle of opened lemma and palea showed a significantly positive correlation with opening duration of lemma and palea and with size of stigma.

Keywords: Characterization; TGMS; sterility fertility behaviour; altitude; hybrid rice.
Abbreviations: CMS-cytoplasmic genetic male sterility; DRR-Directorate of Rice Research; EGMS-environmental sensitive genic male sterility; PGMS-photosensitive sensitive genic male sterility; TGMS-thermo-sensitive genic male sterility; WA-wild abortive.

Introduction

The green revolution in many rice producing countries has enabled global rice production to meet the demands of the world's increasing population. However, by 2050, the world must increase rice production to 880 million tonnes from the present 580 million tonnes in order to keep pace with consumption. Recent progress in plant-breeding research indicates that a significant shift forward in the yield frontiers could be possible through hybrid rice technology. Present hybrid rice technology, with a yield advantage of 2-3 tonnes ha^{-1} over the best inbred varieties, is a major landmark in the history of rice breeding. Initial success by China in the utilization of hybrid rice is increasing grain yields in temperate regions, which in turn is generating considerable interest in the adoption of this technology in tropical and subtropical regions of the country (Virmani, 2006). Although India produces more than enough rice for their needs and is the major exporter of rice in the world, the government sector started hybrid rice research in 1989 to improve grain yield per unit area of land. For heterosis exploitation in rice, the two-line hybrid rice system shows many advantages over the three-line system of hybrid rice, including greater ease in determining the pollinator line in the TGMS male sterility system and a 5 to 15 percent higher heterosis. Several countries such as India, Indonesia, Bangladesh, Malaysia, Myanmar, Philippines, South Korea, Sri Lanka, Vietnam and Thailand have begun their own hybrid rice research and development programs (Virmani, 1998). Results of these programs so far have shown that the degree of success in the use of hybrid rice depends on the extent of heterosis and the efficiency of the seed production techniques (Virmani, 2003). The use of male sterility is a prerequisite for commercial exploitation of heterosis, as rice is a self-pollinating crop. One of the possible alternatives is the two-line breeding system, which is achieved using environmental sensitive genic male sterility (EGMS) and chemical induction of male sterility (Ali et al., 1995). The EGMS is composed of two types: photo-sensitive genic male sterility (PGMS), which is responsive to variations in day length, and thermo-sensitive genic male sterility (TGMS), which is caused by high temperature. India is tropical country with significant temperature variation at different altitudes and in different seasons, making sterility difficult to control. Successful exploitation of this novel male sterility system relies on the knowledge of fertility behaviour of TGMS, since the nuclear sterile gene reacts differently to temperature based on genetic factors (Viraktamath and Virmani, 2001). In the tropics, the cytoplasmic genetic male sterility (CMS) and the thermo-sensitive genic male sterility (TGMS) are the two male sterility systems that can be used (Virmani, 2006). The CMS with wild abortive (WA) cytoplasm is the method used most extensively, although studies have shown that the TGMS system was more effective in increasing seed production efficiency and grain yield (Virmani and Kumar, 2004). Two-line hybrids derived from TGMS lines showed a higher frequency of heterotic combinations than the three-line hybrids derived from CMS lines (Lopez and Virmani, 2000). Keeping in view the importance of TGMS in hybrid rice, the present study was conducted to characterize a set of promising TGMS lines for their sterility/fertility sensitive stages and floral traits and determine their value for use in a two-line hybrid rice breeding program. The Jammu region of Jammu and Kashmir State was chosen as the location for developing hybrid rice seed production of TGMS lines due to varied agro-climatic regions, as high and low temperatures prevail at different altitudes.

Results

Performance of TGMS lines at high altitude

TGMS lines were planted at Bhaderwah, a high altitude location, during kharif 2008. All strains displayed different pollen and spikelet fertility (Table 1). Out of 17 TGMS lines, eight showed good seed-set percentage, which ranged from 33.4% in DRR 23S to 50.0% in DRR 19S. The other nine TGMS lines either could not give satisfactory seed-set percentage or did not flower at all. The eight promising TGMS lines were re-planted during kharif 2009 for confirmation of the initial results, which indeed repeated with 34.1% to 51.2% spikelet fertility (Fig. 1). Maximum, minimum, and mean temperature significantly influenced the pollen and spikelet fertility in all eight TGMS lines at high altitude.

Performance of TGMS lines at low altitude

Most of the TGMS lines planted at the low-altitude Chatha station were found to be sterile, although a few showed various levels of pollen as well as spikelet fertility (Table 1). The eight TGMS lines DDR 1S, DDR 18S, DDR 19S, DDR 20S, DDR 23S, DDR 27S, DRR 28S and DDR 29, which showed satisfactory seed-set percentage at high altitude (Bhaderwah), were completely sterile at low altitude. These eight lines showed complete pollen and spikelet sterility at the prevailing temperature for this low altitude. The few lines shown to be viable may become sterile at higher temperature (low altitude), which might be useful in a different breeding program.

Characterization of TGMS lines

The eight TGMS lines which displayed good self-fertile seed-set percentage at high altitude and complete sterility at low altitude were further characterized at low altitude during kharif 2009. Characterization was based on i) the indices of temperature sterility sensitive stage of panicle development and 2) floral traits. Sterility sensitive stage was determined by the tracking method (Ali et al., 1995) and found to be different in the various TGMS lines (Table 3). The TGMS line DRR 1S required a maximum temperature of 36.6 °C for complete sterility induction, while the other seven lines required a maximum temperature ranging from 35.8 °C (DRR 28S) to 33.9 °C (DRR 29S) for complete sterility. A wide range of variability in various plant characteristics including number of effective tillers per plant, flagleaf area, panicle exertion, panicle length, number of spikelets per panicle, angle of opened lemma and palea, duration of lemma and palea opening, and size of stigma was observed among the different TGMS lines (Table 4). Phenotypic, genotypic and environmental coefficients of variation were low for most of the traits. Coefficients of heritability were medium to low for all the characteristics except for number of spikelets per panicle, which had a coefficient of 83.9%.

Morphological and floral traits

The following is a summary of the mean values of morphological and floral traits. DRR 19S had the highest effective tillers per plant, followed by DRR 28S and DRR 29S (Table 5). DRR 20S and DRR 18S had the maximum flagleaf area of 38.7 cm^2. Most of the TGMS lines had good panicle exertion, which ranged from 91.7 in DRR 28S to 98.9% in DRR 27S. DRR 23S had the maximum stigma size of 1.5 mm^2. A good opening of lemma and palea was observed in DRR 1S, while the maximum duration of opening of lemma and palea was found in DRR 19S.

Correlation among different traits of TGMS lines

The correlation between seven particular characteristics was calculated for all possible combinations of phenotype as well as genotype for promising TGMS lines. Significant phenotypic and genotypic coefficients of correlation were observed between different floral traits and spikelet fertility (Table 2). Panicle exertion, number of spikelets, angle of opened lemma and palea, opening duration of lemma and palea and size of stigma showed significantly positive correlation with size of stigma and spikelet fertility. Effective tillers per plant and flagleaf area did not show any significant correlation with spikelet fertility or other floral traits. Angle of opened lemma and palea showed a significantly positive correlation with opening duration of lemma and palea and with size of stigma. Opening duration of lemma and palea also had a significantly positive correlation with size of stigma.

Discussion

The preliminary step in exploitation of the two-line system of hybrid rice breeding on a large scale is the identification of TGMS lines with stable fertility-transformation behaviour. The lines with complete pollen sterility at high temperature and more than 30% self-seed set at low temperature are considered as promising TGMS lines for commercial exploitation (Lu et al., 1994). The propagation of TGMS lines and seed production of two-line hybrids is not as difficult as that of three-line hybrids, since three-line hybrids require a maintainer line to multiply sterile lines whereas TGMS lines are fertile under certain conditions and can be reproduced by self-pollination. The sterile- and fertile-sensitive phases of TGMS lines need to be determined for different ecological areas, so that the proper locations for sterile line multiplication and hybrid seed production can be established. Out of 17 TGMS lines, eight showed good seed-set percentage ranging from 33.4% in DRR 23S to 50.0% in DRR 19S. Remaining TGMS lines either could not give satisfactory seed set or did not flower at that location, which may be due to having different male sterile genes and/or other genetic factors (Viraktamath and Virmani, 2001). At high altitude, maximum, mean and minimum temperature significantly influenced the pollen and spikelet fertility in all eight promising TGMS lines. Sanchez and Virmani, (2005) and Ramakrishna et al. (2006) also reported that the maximum, mean and minimum temperature played a significant role in the fertility of TGMS lines. The eight TGMS lines DDR 1S, DDR 18S, DDR 19S, DDR 20S, DDR 23S, DDR 27S, DRR 28S and DDR 29 which showed satisfactory seed-set percentage at high altitude (Bhaderwah) were completely sterile at low altitude (Chatha). These lines were further characterized based on the indices of temperature sterility sensitive stage of panicle development

Table 1. Evaluation of TGMS lines of rice at Bhederawah and Chatha during kharif 2008.

TGMS line	Bhaderwah		Chatha	
	Pollen fertility (%)	Spikelet fertility (%)	Pollen fertility (%)	Spikelet fertility (%)
DRR 1S	70.2	48.5	0	0
DRR 4S	0	0	17.6	8.4
DRR 6S	Dnf	Dnf	0	0
DRR 7S	3.6	2.3	28.5	11.0
DRR 17S	Dnf	Dnf	12.0	2.0
DRR 18S	72.6	41.7	1.5	0
DRR 19S	86.6	50.0	0	0
DRR 20S	69.8	44.3	1.0	0
DRR 21S	Dnf	Dnf	14.2	0
DRR 22S	18.5	2.6	22.4	7.4
DRR 23S	61.4	33.4	0	0
DRR 24S	Dnf	Dnf	0	0
DRR 25S	10.5	0	0	0
DRR 26S	0	0	0	0
DRR 27S	70.4	35.6	0	0
DRR 28S	66.4	36.8	0	0
DRR 29S	66.3	40.3	0	0

Dnf = Did not flower.

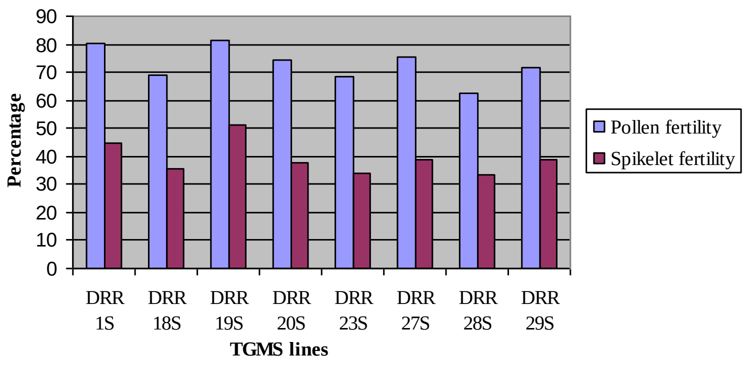

Fig. 1 Pollen and spikelet fertility of promising TGMS lines at high altitude

and floral traits at low altitude by tracking method. Field screening studies were also used by Rangaswamy and Jayamani, (1997) for initial screening and selection of TGMS lines for further characterization. The sterility sensitive stage was found to be different in various TGMS lines. One TGMS line namely, DDR 1S required a maximum temperature of 36.6 °C for complete sterility induction and in the remaining seven lines the maximum temperature ranged from 35.8 °C to 33.9 °C. Santhosh Paikarao et al. (2009) also characterized different TGMS lines for determination of the sterility-sensitive stage in TGMS lines at low altitude. Outcrossing has been reported to be a function of floral morphology and flowering behaviour for the male sterile parent line (Oka and Morishima, 1967). The present study also aimed to evaluate the extent of variation in morphological and floral characters of some promising TGMS lines. Most of the TGMS lines grown during kharif 2009 had good panicle exertion, which ranged from 91.7 to 98.9%. A good opening of lemma and palea, and maximum opening duration of lemma and palea were observed in TGMS lines DRR 1S and DRR 19S, respectively. Greater angle of lemma and palea correlated with greater exertion and surface of the stigma which leads to higher seed-set percentage. These results indicate that visual selection can be used efficiently to identify seed-set potential. Ramakrishna et al. (2006) reported similar findings in their study.

Materials and methods

Plant materials

A set of 17 putative TGMS lines possessing stable sterility, DDR 1S, DDR 4S, DDR 6S, DDR 7S, DDR 17S, DDR 18S, DDR 19S, DDR 20S, DDR 21S, DDR 22S, DDR 23S, DDR 24S, DDR 25S, DDR 26S, DDR 27S, DDR 28S and DDR 29 were used in the present study. They were developed at the Directorate of Rice Research, Hyderabad, India by repeated backcrossing with IRRI TGMS lines. These TGMS lines were evaluated for pollen and spikelet fertility at two different locations. One was Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Chatha. It is located at low altitude with an elevation of 300 m above mean sea level (32° 40’ N, 74° 50’ E). The second was Regional Horticultural Research Sub-Station, Bhaderwah. It

Table 2. Estimates of phenotypic (P) and genotypic (G) correlation between different characters of promising TGMS lines of rice.

Character		Effective tillers/plant (No.)	Flagleaf area (cm^2)	Panicle exsertion (%)	Number of spikelets (No.)	Angle of opened lemma and palea (^{0})	Opening duration of lemma and palea (min)	Size of stigma (mm^2)	Spikelet fertility (%)
Effective tillers/plant	P	1.000	-0.014	0.301	0.401	-0.342	0.124	-0.087	-0.231
(No.)	G		0.121	0.144	0.431	-0.435	0.236	-0.122	-0.332
Flagleaf area (cm^2)	P		1.000	-0.323	0.221	-0.125	-0.111	0.226	0.233
	G			-0.430	0.311	-0.243	-0.172	0.345	0.287
Panicle exsertion (%)	P			1.000	0.098	0.367	-0.204	0.207	0.598*
	G				0.113	0.478	-0.246	0.267	0.653*
Number of spikelets	P				1.000	0.398	0.426	0.358	0.643*
(No.)	G					0.479	0.503	0.485	0.765*
Angle of opened	P					1.000	0.589*	0.594	0.587*
lemma and palea (^{0})	G						0.678*	0.673	0.655*
Opening duration of	P						1.000	0.654*	0.689*
lemma and palea (min)	G							0.699*	0.788*
Size of stigma (mm^2)	P							1.000	0.534*
	G								0.671*
Spikelet fertility	P								1.000
(%)	G								

*Significant at 5% level

is at high altitude with an elevation of 1500 m above mean sea level (34° 20' N, 74° 50' E). Weekly temperature data of these two locations were provided by the Division of Meteorology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu.

Pollen sterility and fertility

Samples to determine pollen sterility/fertility were taken from the first and second panicles. Pollen sterility/fertility was determined by staining pollen grains with 1% IKI solution (1% iodine in 2% potassium iodide) followed by examination by microscope. Pollen grains were classified based on their shape and extent of staining. The unstained withered or spherical pollen grains and the lightly stained round pollen grains were classified as sterile (Govind and Virmani, 1988). The fertile pollen grains were darkly-stained and round. Panicles were harvested at maturity and spikelet fertility was taken from two primary panicles. Spikelet fertility was monitored by counting the number of filled grains and total spikelets per panicle and converted into a percentage value. TGMS lines DDR 1S, DDR 18S, DDR 19S, DDR 20S, DDR 23S, DDR 27S, DRR 28S and DDR 29 showed 30 to 50% spikelet fertility at Bhaderwah and none of them showed any pollen and spikelet fertility at Chatha, Jammu (Table 1) during kharif 2008. To confirm these results, these eight TGMS lines were again planted at Bhaderwah during kharif 2009. The promising TGMS lines that produced self-fertile seed at Bhaderwah were further characterized with regard to sterility sensitive stage indices and morphological and floral traits at Chatha station during kharif 2009. A sterility sensitive stage index is determined by tracking method (Ali et al., 1995).

Sterility sensitive stage of TGMS lines

To characterize the TGMS lines for sterility-sensitive stage under high temperature conditions at Chatha, experimental material was sown in the first week of May during kharif 2009. Transplanting was done when seedlings were 21 days old. Each TGMS line was planted in triplicate in two rows five meters long in a randomized block design, with an area of 20 × 25cm for each plant. Plants were grown based on standard practices. Ten plants were selected randomly from rows of each TGMS plot in each set for recording the observations on angle of opened lemma, average period of time (minutes) from the opening of lemma and palea of individual florets to closing of the florets, length of main panicle, number of panicles per hill, flagleaf area, panicle length, panicle exertion, and size of stigma. The angle of lemma and palea at peak anthesis was measured in degrees when the floret was fully opened. The size of the stigma (mm^2) was likewise determined.Pollen sterility status of each TGMS line was recorded daily from the date of first panicle emergence by staining pollen grains with 1% IKI solution (Govind and Virmani, 1988). The dates when pollen was found completely sterile in the new emerging panicles were considered to be the tracking day/dates. On the basis of earlier reports (Ramakrishna et al., 2006), the most sensitive phase of panicle development, the stamen-pistil primordial stage, lies between 24 and 15 days before heading. The day/date of critical stage was determined with the help of weather data collected by the Division of Meteorology. Days were counted backward from the tracking date between 15 and 24 days, and the date when the maximum temperature during this period was above 30 °C was noted as the critical temperature coinciding with the sensitive stamen-pistil primordial phase.

Statistical analysis

Pollen and spikelet fertility percentage was determined on the basis of the classification developed by Govind and Virmani, (1988). Data collected on morphological and floral traits were analysed by using the standard methods of Panse and Sukhatme, (1984), and broad sense heritability was calculated using the method of Hanson et al. (1956).

Conclusion

The results indicate that TGMS method of hybrid rice production can be used in developing two-line hybrids in north India where there is a distinct regime of temperature at low and high altitude. A temperature range from 20 to 30 ^oC is enough to induce TGMS lines to become fertile at high altitude area. Likewise, seed production of two-line hybrids can be done in low altitude area. Before using the promising CMS lines in two-line hybrid breeding programme it is

Table 3. Determination of sterility sensitive stages of panicle development in TGMS lines sensitive to temperature at Chatha during kharif 2009.

TGMS lines	Date of complete sterility	Maximum and minimum temperature (^{o}C) on different days prior to heading											
		15	16	17	18	19	20	21	22	23	24	Critical temperature	
												Maximum	Minimum
DRR 1S	12-09-2009	35.7/24.9	33.4/22.5	36.6/25.1	35.3/24.5	34.8/25.7	33.1/22.3	31.4/20.6	32.6/21.7	34.3/22.2	32.1/21.0	36.6	25.1
DRR 18S	21-09-2009	33.2/24.1	34.7/24.4	31.9/25.8	32.6/24.9	34.6/25.7	33.2/25.2	32.2/23.5	32.7/23.0	33.1/21.4	33.7/24.2	34.7	24.4
DRR 19S	14-09-2009	32.6/22.5	32.8/23.3	31.6/26.2	32.8/24.9	34.2/25.7	32.6/32.5	35.5/22.3	33.9/24.3	32.9/23.9	33.8/24.8	35.5	22.3
DRR 20S	20-09-2009	33.7/23.6	34.5/24.7	33.6/25.0	32.5/24.2	33.6/23.9	34.0/23.3	35.7/23.3	34.8/23.0	33.5/21.8	32.9/22.0	35.7	23.3
DRR 23S	15-09-2009	33.7/24.9	32.4/24.2	33.8/23.7	33.7/23.5	32.7/21.2	32.7/23.0	34.0/23.5	34.1/24.5	33.7/23.4	33.0/22.9	34.1	24.5
DRR 27S	18-09-2009	33.4/24.5	33.9/23.1	34.0/22.5	33.7/21.8	31.6/20.9	32.0/22.5	34.8/23.8	35.4/25.0	35.3/24.8	34.7/23.8	35.4	25.0
DRR 28S	24-09-2009	33.2/21.4	32.9/23.0	35.8/22.0	34.8/23.0	34.2/21.9	33.7/23.0	33.0/22.7	34.0/23.1	32.4/21.1	33.8/22.8	35.8	22.0
DRR 29S	09-09-2009	31.6/20.4	32.7/22.4	32.7/21.3	33.6/23.0	32.6/20.7	33.7/23.5	33.9/24.2	33.1/23.1	33.2/21.0	31.9/20.4	33.9	24.2

Table 4. Analysis of variance for different characters of TGMS lines at Chatha during kharif 2009.

Source of variation	d f	Effective tillers/plant(No.)	Flagleaf area (cm^2)	Panicle exsertion (%)	Panicle length (cm)	Number of spikelets (No.)	Angle of opened lemma and palea(^{o})	Opening duration of lemma and palea (min)	Size of stigma (mm^2)
Replication	2	2.9	5.6	3.6	2.9	29.7	1.5	16.3	0.01
Treatments	7	8.9*	30.2*	15.0*	14.3*	386.8*	9.4*	142.4*	0.1*
Error	14	3.0	6.8	4.1	4.8	23.2	2.6	27.3	0.03
CD (5%)		3.1	4.7	3.6	2.2	8.6	2.0	9.7	0.3
CV (%)		4.2	7.4	5.1	3.8	6.3	4.1	10.8	16.8
Mean (x±SEm)		21.4±1.2	34.9±1.5	95.2±1.8	16.6±2.0	77.7±2.7	22.4±0.9	47.9±3.0	1.1±0.1
Maximum		15.0	38.7	98.3	19.3	96.3	23.7	56.0	1.5
Minimum		10.3	29.6	91.7	14.0	66.0	21.3	39.0	0.8
PCV (%)		10.2	10.9	2.9	16.9	15.4	9.7	16.9	20.3
GCV (%)		6.9	8.0	1.9	10.6	14.1	6.2	12.9	12.8
ECV (%)		8.0	7.4	2.1	13.1	6.1	7.1	10.9	15.7
Broad sense heritability (%)		45.8	53.7	46.7	39.2	83.9	45.8	58.3	40.0

*Significant at 5% level

Table 5. Mean performance of TGMS lines for different characters of rice at Chatha during kharif 2009.

TGMS lines	Effective tillers/plant (No.)	Flagleaf area (cm^2)	Panicle exsertion (%)	Panicle length (cm)	Number of spikelets (No.)	Angle of opened lemma and palea (^{o})	Opening duration of lemma and palea (min)	Size of stigma (mm^2)
DRR 1S	11.3	29.6	95.0	19.3	69.3	23.7	39.0	1.0
DRR 18S	10.3	36.7	93.3	15.3	67.7	22.3	47.3	1.0
DRR 19S	15.0	34.7	95.0	14.0	71.7	21.3	55.0	0.8
DRR 20S	12.7	38.7	96.7	15.7	66.0	22.7	56.0	1.1
DRR 23S	12.7	35.3	93.3	16.7	76.7	22.3	46.7	1.5
DRR 27S	14.7	33.7	91.7	16.3	87.3	22.7	40.7	1.1
DRR 28S	13.5	32.5	98.9	15.9	77.3	21.5	45.0	1.1
DRR 29S	14.0	38.7	98.3	16.3	96.3	23.0	54.0	1.1
CD (5%)	3.1	4.7	3.6	2.2	8.6	2.0	9.7	0.3

necessary to study their stability in sterility/fertility and suitability in hybrid seed production at large scale.

Acknowledgements

The project was conducted and financially supported by All India Co-ordinated Rice Improvement Project (AICRIP), Directorate of Rice Research, Hyderabad, India. The authors would like to thank Dr. Sukhpal Singh, Senior Technical Assistant (Hybrid Rice), Directorate of Rice Research, Hyderabad for his kind visit to Regional Horticultural Research Sub-Station, Bhaderwah and Sher-e-Kashmir University of Agricultural Sciences & Technology, Chatha, Jammu (J&K), India during the crop season of the TGMS lines.

References

Ali J, Siddiq EA, Zaman FU, Abraham MJ, Ahmed Ilyas (1995) Identification and characterization of temperature sensitive genic male sterility sources in rice (*Oryza sativa* L.). Ind J of Genet 55(3): 243-259

Govind RK, Virmani SS (1988) Genetics of fertility restoration of WA cytoplasmic male sterility in rice. Crop Sci 28: 787-792

Hanson FH, Robinson HF, Comstock RE (1956) Biometrical studies of yield in segregating populations of Korean Lespedeza. Agron J 48: 267-282

Lopez MT, Virmani SS (2000) Development of TGMS lines for developing two-line hybrids for the tropics. Euphytica 114: 211-215

Lu XG, Zhang ZU, Maruyama K, Virmani SS (1994) Current status of two-line method of hybrid rice breeding. In: 'Hybrid rice technology-New Development and future prospects' (Ed SS Virmani), IRRI, Manila, Philippines, p 37-49

Oka HT, Morishima H (1967) In: The ancestors of cultivated rice and their evolution. Japan: Department of Applied Genetics, National Institute of Genetics, p 145-145

Panse VG, Sukhatme PV (1984) Statistical methods for agricultural research workers. ICAR, New Delhi, p 145-152

Ramakrishna S, Mallikarjuna BPS, Mishra B, Virakthamath BC, Ahmed Ilyas (2006) Characterization of thermo-sensitive genetic male sterile lines for temperature sensitivity, morphology and floral biology in rice (*Oryza sativa* L.). Asian J of Plant Sci 5: 421-428

Rangaswamy J, Jayamani P (1997) Field screening of thermo-sensitive genetic male sterile lines in rice. In: Proceeding of International symposium on two line system of heterosis breeding in crops. Chengsha, China CNHRR & DC, Hunon, p 198-203

Santhosh P, Sudheer KS, Ahmed MI (2009) Characterization of temperature sensitive genic male sterile (TGMS) lines in rice (*Oryza sativa* L.). Oryza 44 1-6

Sanchez DL, Virmani SS (2005) Identification of thermo-sensitive genic male sterile lines with low critical sterility point for hybrid rice breeding. Philippine J of Crop Sci 30(1): 19-28

Viraktamath BC, Virmani SS (2001) Expression of thermo-sensitive genic male sterility in rice under varying temperature situations. Euphytica 122: 137-143

Virmani SS (1998) Hybrid rice research and development in the tropics. In: Advances in Hybrid Rice Technology. Virmani SS, Siddiq EA, Muralidharan K (eds), IRRI, Manila, Philippines, p 35-49

Virmani SS (2003) Advances in hybrid rice research and development in the tropics. In: Hybrid Rice for Food Security, Poverty Alleviation and Environmental Protection. Proceedings of the 4th International Symposium on Hybrid Rice Virmani SS, Mao CX, Hardy B (eds.). May, 14-17 2002, Hanoi, Vietnam Los Banos (Philippines): International Rice Research Institute. p 2-20

Virmani SS (2006) Hybrid rice in the tropics: where do we go from here? (Presented in 2nd International Rice Congress, October 9-13, 2006, New Dehli, India.

Virmani SS, Kumar I (2004) Development and use of hybrid rice technology to increase rice productivity in the tropics. Int Rice Res Newl 29(1):10-19.