Indian Journal of Agricultural Sciences **83** (8): 809–14, August 2013/Article

Characterization and genetic inheritance of temperature sensitive genetic male sterility in *desi* cotton (*Gossypium arboreum*)

V SANTHY[1], S M PALVE[2], SONALI VIRKHEDE[3], PRATIBHA MOHAN[4] and B M KHADI[5]

Central Institute for Cotton Research, Nagpur, Maharashtra 440 010

Received: 22 June 2012; Revised accepted: 4 April 2013

ABSTRACT

The two line method of temperature sensitive genetic male sterility (TGMS) is a useful method for production of hybrid seed in crop plants. TGMS 1-1 line has been identified, characterized and stabilized in *desi* cotton (*Gossypium arboreum* L.). It was completely male sterile under high minimum temperature of summer months and exhibited complete pollen fertility under low temperature condition. The Critical Sterility Point was determined as 24°C minimum temperature and 40°C maximum temperature with continuous sunshine and Critical Fertility Point was determined as 18°C minimum and 33°C maximum temperature. The inheritance studies revealed that the trait is governed by single recessive gene. The TGMS 1-1 line developed and characterized was found useful for hybrid seed production in May (100% male sterile) when the minimum temperature was above 24°C and for maintenance when sown in the normal *kharif* by selfing.

Key words: Arboreum, Inheritance, Single recessive gene, Temperature sensitive genetic male sterility (TGMS)

Development and commercial cultivation of hybrid cotton cultivars enabled India to increase productivity during the 1970s and 1980s. Government approval in 2002 for commercial cultivation of transgenic Bt cotton gave further impetus to cotton productivity by securing crop protection from bollworm. As a result, India today occupies the second position in the world with respect to cotton production. With the popularity of Bt cotton, more than 90 % of 11.00 million ha cotton cultivated area is under hybrids. The current technology of hybrid seed production is based on hand emasculation and pollination and hence hybrid seeds are expensive. Therefore, alternative pollination control systems are urgently needed to lower the cost of hybrid seed. In cotton, both conventional as well as nonconventional (using male sterility system) hybrids have been developed and popularized inspite of major limitations in both the systems.

The limitations include narrow genetic base for female/sterile and male/restorer parents, non-availability of suitable sources for male sterile and restorer genes and difficulty in seed multiplication especially of GMS parent, where half plant population in the maintainer test crossed progeny need to be rogued at early stage (Siddique and Ali 1999). The pioneering discovery of photoperiod sensitive genetic male sterility by Shi (1981) and temperature sensitive genetic male sterility by Yang and Wang (1989) (together called environmental sensitive genetic male sterility) in rice led to two line method of hybrid breeding. Environmental sensitive genetic male sterility is considered a promising approach to combat limitations of male-sterility based three line method and has assumed field reality in rice in China (Yuan 1998). Such lines have also been reported to be available in cotton (Laxman 2008), as well as other crops such as maize (Tang *et al*. 2006), wheat (Xiaodong *et al*. 2010), barley (Gupta and Singh 2000), safflower (Singh *et al.* 2008), soybean (Ryan *et al*. 2010) etc. EGMS lines are sterile during certain weather condition in which it could be used for hybrid seed production and fertile during certain other weather regime, in which it can be multiplied by mere selfing. The other advantages include wider choice of parents since restoration of such sterility is not a problem and easy transfer of the trait to any new line without the negative effect of sterile cytoplasm (Virmani *et al.* 2003). However, there is need to determine sterile, fertile and the fertility/sterility transition phases of these lines in different ecological areas, so that the proper seasons and locations for multiplication and hybrid seed production can be recommended.

Environmental sensitive genetic male sterility sensitive

[1]Scientist (SS) (santhy100@gmail.com), [2]Principal Scientist; [5]Dean (PGS), UAS, Dharwad

predominantly to temperature and termed as TGMS line was identified in diploid cotton (*Gossypium arboreum* L. race *bengalense*). The TGMS line (CATS 18) obtained as a spontaneous mutant from an existing genetic male sterile line of *G. arboreum*, showed reversion to fertility at minimum temperature less than 18°C under field conditions in experimental fields of CICR farm. The line showed male sterility under high minimum temperature conditions when continued as rattoon crop. For successful utilization of this novel male sterile system in hybrid seed production, knowledge on critical fertility period (CFP) as well as critical sterility period (CSP) under field conditions is highly essential. This innovative method could be exploited successfully in *desi* intra-arboreum cotton hybrids, as only GMS system is utilized here and conventional system cannot work due to small and delicate flower structure. Thus the objective of present study was to characterize and study the inheritance of TGMS trait which would enable in breeding new TGMS lines with diverse genetic backgrounds.

MATERIALS AND METHODS

A group of similar plants observed under the TGMS line CATS 18 were further stabilized by repeated selfing, evaluation for pollen sterility/fertility on selfed progenies followed by selection of true to types. The individual plants were observed daily for pollen sterility/fertility when the flowering was initiated. During the first year, the plants which showed sterility-fertility reversion were tagged and others removed. The selected plants were selfed and seeds were collected boll wise from selected labeled plants. The reversion from fertility to sterility was confirmed by continuing the plants during summer as rattoons. Boll to row progeny was raised during successive two years which were thoroughly monitored for alterations till they were found stable with respect to the TGMS trait.

The flower behaviour during normal flowering season was observed continuously during September – November in the stabilized TGMS line, 1-1 for four consecutive years (2008, 2009, 2010 and 2011) and correlated with the prevailing weather conditions especially mean minimum temperature and sunshine hours during the period. The sterility/fertility of flowers were confirmed by visual observation of flowers as well as by pollen staining studies under microscope. The flowers showed white anthers during sterility phase and yellow anthers during fertility phase.

The line was also grown under phytotron conditions for confirming the phenomenon. Varying minimum/night temperature, fixed maximum temperature and fixed period of light was provided in the phytotron to study pollen fertility/sterility alteration in *G. arboreum* TGMS line 1-1.

The stabilized line (TGMS 1-1) was crossed with *G. arboreum* released variety PA 255 during 2008-09 crop season. The F1 hybrid obtained was grown in the following summer as off-season crop to provide the sterility inducing condition and study the dominance/recessive effect of the trait. The F1 progeny was selfed during the season to obtain F2 seeds. A total of 158 plants were grown to ascertain the TGMS trait inheritance. The individual F2 plants were observed for their flower fertility/sterility morphologically as well as by acetocarmine staining of pollen grains followed by microscopic observation.

RESULTS AND DISCUSSION

The presence of EGMS phenomenon has been reported earlier in *G. hirsutum* cotton by Laxman *et al.* (2008). The existence and characterization of EGMS system in *desi* cotton (*G. arboreum)* has not been reported. The system would significantly benefit hybrid seed production in *desi* types where conventional method consisting emasculation is difficult to employ due to small flowers and non-availability of CGMS restorers. The *intra-arboreum desi* cotton hybrids are based on GMS system which carries with it the problem of maintenance of female parent. Success to some extent depends on linking the trait with an easily identifiable seedling marker for GMS. Kopulwar *et al.* (2007) has reported use of an RAPD marker OPL-19 to determine male sterility in cotton (*G. hirsutum*) which could be beneficial in GMS parental line maintenance. Till such markers are identified in *desi* cotton, observing flowers for fertility and rouging of such plants will have to be undertaken during GMS parent seed multiplication.

Stabilization of the system

In the present study, during the years, 2008 and 2009, flowers produced at the onset (i.e during first week of September) were sterile with very small white anthers. The mean minimum temperature during the period was 24°C with continuous good sunshine hours (Fig 1). In rice, the TGMS line T 29 was male sterile when exposed to daily mean temperature of 24.1°C (Cuong *et al.* 2004). Mean temperature has been observed to be the primary factor influencing fertility alteration in TGMS lines of rice (Latha and Thiyagarajan 2010). The percentage boll set after selfing was zero in the TGMS line during initial flowering stage, i.e. September. As the temperature reduced towards the end of September and beginning of October, the flowers started turning fertile with yellow anthers (Fig 1). The flowers turned completely fertile with almost full yellow anthers when the mean minimum temperature reduced to 18°C during the following months. These plants when continued beyond February started turning sterile and became completely sterile by the end of April under high temperature and continuous good sunshine hours. TGMS lines causing male sterility at high environmental temperature and fertility under low temperature have been reported in rice (Virmani 1992, Ramakrishna *et al.* 2006), and barley (Gupta and Singh 2000) The pollen sterility was confirmed by visual observation of flowers as well as by pollen staining studies under

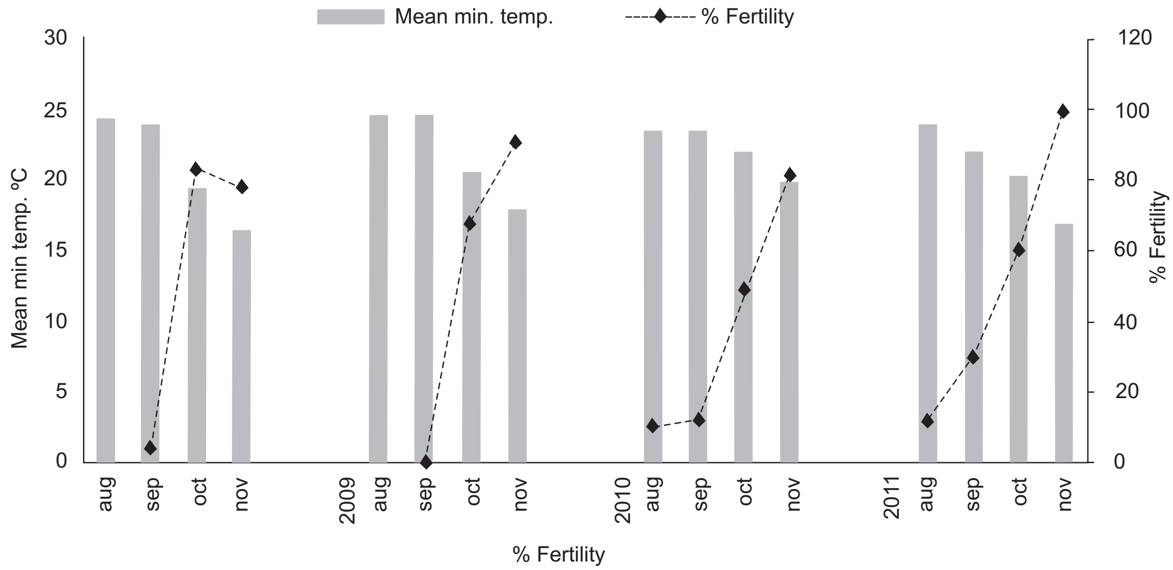


Fig 1 Relationship between mean minimum temperature and percentage fertility during normal flowering period in TGMS 1-1

microscope (Fig 2). The period in between the completely fertile and completely sterile phase which produced partially fertile/sterile flowers were observed to be the sensitive stage. Thus three stages critical fertility phase, critical sterility phase and sensitive phases were identified in the TGMS line 1-1. The existence of different phases has similarly been observed in wheat where, TGMS wheat line, C 412 S exhibited complete sterility, half sterility to normal fertility and complete fertility with changes of temperature (Zhang *et al.* 2009).

Phytotron study for confirmation

Since many factors associated with temperature directly or indirectly influence the fertility/sterility of TGMS lines, influence of any single factor should be determined by evaluating the line in phytotron keeping other factors constant (Latha *et al.* 2003). The *G. arboreum* TGMS line 1-1 was hence grown under phytotron conditions under varying minimum/night temperature, fixed maximum temperature and fixed hours of light. The increase in fertility was observed when minimum/night temperature was reduced from 22°C to 20°C and flowers showed 20-50% fertility (Table 1). As the minimum temperature was reduced further to 16°C, fertility increased further and reached 100% under artificial controlled conditions. The transition temperature from sterility to fertility was observed to be 22°C in the TGMS line under phytotron condition. Similar study was done in rice TGMS lines in a growth chamber providing varying night temperature to confirm the sterility/fertility alteration behavior (Ramakrishna *et al.* 2006)

Table 1 Effect of night temperature on pollen viability in TGMS line of *G. arboreum*

Temperature regime	% male fertile flowers	Pollen viability (%)	No. days tested
30°C day/ 26°C night	0	0	10
30°C day/ 22°C night	0	0	7
30°C day/ 20°C night	9	20-50	7
30°C day/ 18°C night	37	20-50	7
30°C day/ 16°C night	41	20-100	7

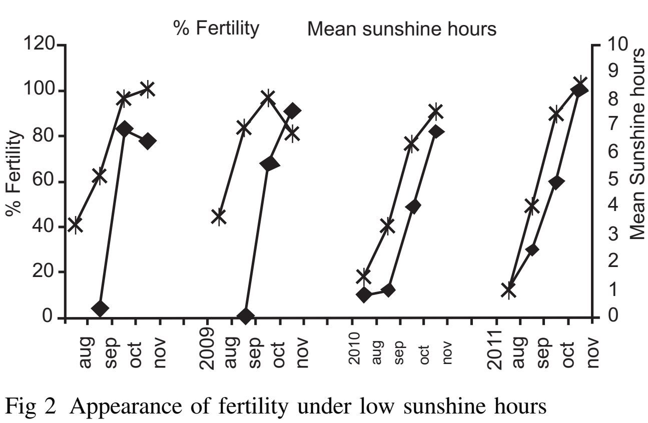


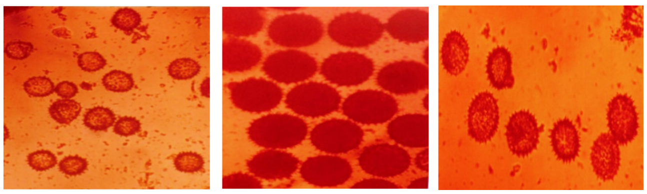
Fig 2 Appearance of fertility under low sunshine hours

Inheritance study of the trait

The F1 hybrid obtained between the cross of TGMS line 1-1 and PA 255 (direct as well as reciprocal) when grown as offseason during summer month showed all plants to be completely fertile with yellow and filled anthers indicating the complete dominance of fertility over sterility (Fig 3). Environment – influenced male-sterility systems are known to be governed by specific temperature–or daylength-sensitive nuclear genes in the recessive state (Yuan 1992, Zhang *et al.* 1992). Studies performed in various crops including cotton indicated the recessiveness of trait (Laxman *et al.* 2009, Li *et al.* 2005, Singh *et al.* 2008). The selfed progenies (F2 plants)

Table 2 Inheritance pattern in F2 obtained from TGMS 1-1 × PA 255

	No. of sterile plants altered to fertility	No. of fertile plants	Total no. of plants
Observed	42	116	158
Expected	40	118	158
Chi-square value	0.1	0.033	0.133



Sterile Fertile Partial fertile

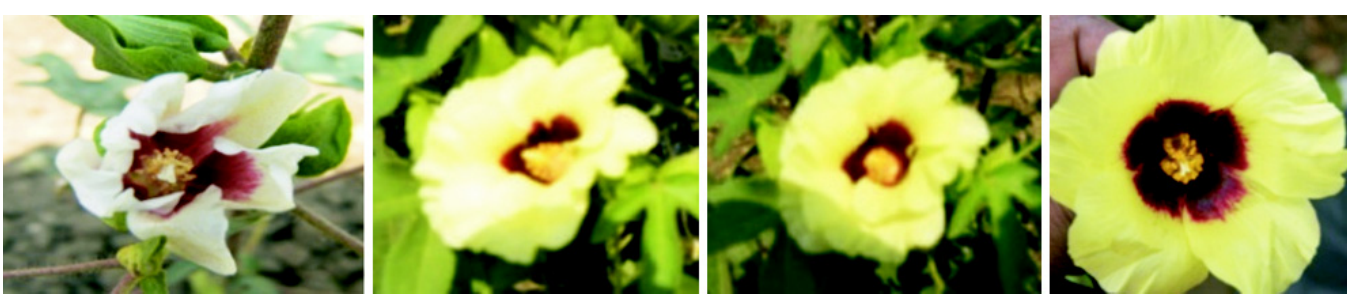
Fig 3 Appearance of pollen under microscope after acetocarmine staining

raised in the next season evaluated for pollen fertility/sterility behavior revealed two groups, one always showing partially fertility-complete fertility and other showing complete sterility. Similarly, inheritance study of TGMS in rice revealed three groups and, partially fertile plants were included in the fully fertile group during the study (Li *et al.* 2005). The ratio of fertile to sterile plants in the F2 fitted well with 3:1 monogenic recessive inheritance after chi-square test (Table 2) and was in agreement with the earlier report on TGMS inheritance in *G. hirsutum* cotton by Laxman *et al.* (2010). Various other studies on genetic inheritance in TGMS lines of rice revealed the trait to be governed by single recessive gene (Borkakati and Virmani 1996, Shankar *et al.* 2007). In wheat, Tang *et al.* (2006) through classical genetics and QTL mapping in F2 derived from a single cross indicated that the TGMS trait is governed by a single recessive gene. Digenic recessive inheritance for TGMS trait was reported in safflower (Singh *et al.* 2008). Digenic and complicated type of inheritance has also been reported in rice (Li and Pandey 1998, Li *et al.* 2005).

Variation in sowing date to determine stable sterility period

With a view to increase the period of sterile flower production during *kharif* season (as the sterility was observed only during flower initiation in September during 2008 and 2009) the TGMS line was sown thrice, first in the month of May, second in the month of June, i.e. normal season during the year 2010 and third in the month of February during the year, 2011. Staggered sowing method was adapted in rice to characterize the TGMS line and identify fertile phase, sterile phase and fertile/sterile transition phase (Latha *et al.* 2003). The flowers were observed for their behaviour at the onset of flowering during the beginning of August in the May sown crop. The flowers were partially fertile with 10% to 20% fertility even at mean minimum temperature of 24°C ascertained by pollen staining using acetocarmine and observation under microscope (Fig 2). The weather data revealed significant reduction in sunshine hours during the period compared to other years . Influence of sunshine hours and relative humidity on sterility/fertility alteration has been observed by Liu *et al.* (1997) in TGMS lines of rice. Occassional appearance of fertility at temperatures above the limit of critical sterility could be due to influence of other factors (Latha *et al.* 2010). Unstable sterility, easy fertility reversibility and influence of sunshine hours on sterility behavior in the present study material indicate to some extent the role of photoperiod too in controlling the sterility expression as observed by Haohua *et al.* (2005) in rice. The existence of P(T)GMS lines influenced by both temperature and photoperiod in different degrees (i.e. influenced more by temperature and less by photoperiod or vice-versa) was revealed earlier in rice (Wan and Deng 1990, He *et al.* 2007, Zhang *et al.* 1993, 1994) and wheat (Rui-Xing *et al.* 2006).

The third sowing date which was in the end of January 2011 started flowering by the end of April and was at peak flowering by the month of May. The flowers were completely male sterile with unstained and deformed pollen observed under microscope after acetocarmine staining. Simultaneously selfing was performed in the TGMS line as well as normal fertile line. The selfed boll set was zero in the TGMS line till the month of June whereas it was 60-70% in the normal fertile line. A similar TGMS line exhibiting full sterility during summer was discovered in maize (Tang *et al.* 2006). However, during the months of July–August, similar to the previous year mean sunshine hours reduced significantly (less than 2.0 with no sunshine for 4-5 days continuously)



TGMS parent TGMS 1-1 × PA 255 PA 255 × TGMS 1-1

Fig. 4 Dominance of fertility over sterility in F1 crossess (direct and reciprocal) resulting in 10-20% fertility (Table 3). The environment sensitive genetic male sterility such as P (T) GMS system that affect the critical sterility temperature has been well studied in rice (Chen *et al.* 2010). They suggested that there is interrelationship between temperature and day length on P(T)GMS and that the critical temperature of sterility (temperature at which complete sterility observed) becomes lower under long day length whereas higher under short day length conditions. For pure TGMS, the critical temperature of sterility remains relatively stable. This could explain for obtaining a low CSP (22°C) under phytotron condition where constant 12 hours day length was provided on all days and higher during August of 2010 and 2011 when sunshine hours were almost nil for consecutively 4-5 days.

Table 3 Mean maximum, minimum temperatures and sunshine hours during February–September 2011

Month	Weeks	Mean maximum tempe-rature	Mean minimum tempe-rature	Mean sun-shine hours	Floral expression
February	1st week				Crop sown
	2nd week				
	3rd week				
	4th Week				
March	1st week	34.0	19.3	9.0	
	2nd week	35.6	18.2	9.2	
	3rd week	37.0	16.8	9.0	
	4th Week	38.2	19.0	9.0	
April	1st week	38.0	21.1	9.0	
	2nd week	37.5	21.5	8.0	
	3rd week	39.8	22.0	9.0	
	4th Week	40.6	24.3	10.0	Flowering initiated and all sterile
May	1st week	41.0	25.0	8.0	100% Sterile
	2nd week	43.5	27.5	9.2	100% Sterile
	3rd week	43.0	26.0	10.0	100% Sterile
	4th Week	43.5	27.5	10.8	100% Sterile
					100% Sterile
June	1st week	40.1	28.0	8.8	100% Sterile
	2nd week	38.5	28.2	8.0	100% Sterile
	3rd week	35.4	26.0	3.2 (3 consecutive days no sunshine)	10% fertility
	4th Week	35.4	24.0	7.0	
July	1st week	35.2	24.8	2.80 (4 days no sunshine)	15% fertility
	2nd week	33.1	24.7	3.8	5–10% fertility
	3rd week	30.5	24.3	2.3 (4 days no sunshine)	15% fertility
	4th Week	32.0	25.2	0	5–10% fertility
August	1st week		24.8	0	18%
	2nd week		24.4	0.5	10%
	3rd week		23.6	2.2	20%
	4th Week		24.4	2.7	18%
Septem-ber	1st week		24.1	0	28%
	2nd week		20.6	3.9	Appeared fertile, percentage not recorded
	3rd week		24.2	7.1	Appeared fertile, percentage not recorded
	4th Week		23.5	8.0	25%

In the present study a period of 35-40 days with complete male sterility could be obtained in the third date of sowing unlike other two dates of sowing. The EGMS line developed and characterized was thus found to be beneficial for hybrid seed production only in May (100% male sterile) and for maintenance by selfing during the normal *kharif*. A higher critical sterility temperature is suggested to be beneficial as chances of sudden drop in temperature low enough for fertility appearance is very minimal (Ramakrishna *et al.* 2006).

The suitability of line as such for commercial exploitation and hybrid seed production seems limited. However, it is possible to utilize the present material as donor in developing new TGMS lines with different genetic backgrounds which could give stable sterility under normal cotton growing season. This could be possible since it is well indicated that the expression of sterile gene is affected by genetic background. The requirement of CST/CFT was found to vary for different lines developed from single TGMS source in rice (Zhang and Zhu 1991, Ali *et al.* 1995, Reddy 1997, Wu 1997).

It is concluded that the TGMS trait under present study showed unstable sterility during *kharif* season indicating role of sunshine hours or daylength in expression of sterility which needs to be ascertained further. The Critical Sterility Point under field conditions was determined as 24°C minimum temperature with continuous good sunshine hours and Critical Fertility Point was determined as 18°C minimum temperature. Exploitation of the phenomenon for hybrid seed production in *desi* cotton could be possible only during the month of May when all flowers show complete male sterility and maintenance by selfing during the normal cropping season especially when peak flowering reaches the month of October-November.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Director, CICR, Nagpur who is also Member Secretary, Technology Mission on Cotton, Mini Mission I for providing necessary funds and other facilities to carry out the research. Heartfelt thanks is also extended to Dr S R Bhat, Principal Scientist, NRCPB, New Delhi and his team for conducting the phytotron study of TGMS material.

REFERENCES

Ali J, Siddiq E A, Zaman F U, Abraham M J and Ahmed I M. 1995. Identification and characterization of temperature sensitive genic male sterile source in rice. *Indian Journal of Genetics* **55:** 243–59, 123–8.

Chen li-Yun, Xiao Ying-hui, Lei Dong-Yang. 2010. Mechanism of sterility and breeding strategies for photoperiod/thermosensitive genic male sterile rice. *Rice Science* 17 (3).

Coung Van Pham, Seitchi Murayama, Yuhio Ishimine Yoshinobu Kawamitsu, Keiji Motomura and Eiji Tsuzuki. 2004. Sterility of thermosensitive genic male sterile line, heterosis for grain yield and related character in F1 hybrid rice. *Plant Protection Science* **7**: 22–9.

Gupta S K and Singh Dalvir. 2000. Thermosensitivity in genic male sterile lines of barley (*Hordeum vulgare* L.). *Indian Journal of Genetics and Plant Breeding* **60**(3): 297–300.

Haohua He, Xiaoshong Peng, Huiming Gong, Changlan Zhu, Guoyou Ye. 2005. Fertility behavior of rice lines with dominant male sterile gene and inheritance of sterility and fertility restoration. *Field Crops Research* **98**: 30–8.

He Q, Chen l Y, Deng H F, Tang W B, Xiao Y H and Yuang L P. 2007. Fertility photo-thermo characteristics in PTGMS rice C815 S and its homologous plant lines. *Sci. Agric. Sin.* **33**(2): 262–8.

Kopulwar, Aeshwarya, Borase Tushar, Phalak Manoj and Dagaonkar Vipin. 2007. Identification and use of molecular markers for genetic male sterility in cotton (*G. hirsutum* L.). (In) *Book of Abstracts.* World Cotton Research Conference – 4 , 10-14 Sep.

Latha R and Thiyagarajan K. 2010. Fertility alteration behavior of thermosensitive Genetic Male Sterile Lines in rice. *Electronic Journal of Plant Breeding* **1**(4): 1 118–25.

Latha R, Sentrilvel S and Thiyagarajan K. 2004. Critical temperature and stages of fertility alteration in thermo-sensitive genetic male sterile lines in rice. *Procedings of International Crop Science Conference.*

Laxman S. 2008. Identification of the environmental - sensitive, genic male-sterile line in cotton (*Gossypium hirsutum* L.) - A preliminary study. *Tropical Agricuture* **82**.

Laxman S. 2009. Inheritance study of Environmental Sensitive Genic Male Sterile gene in cotton (*Gossypium hirsutum* L.). *Journal of Cotton Research & Development* **23**: 149–50.

Li R h and Pandey M P. 1998. Genetics of the thermo sensitive genic male sterility trait in rice. *International Rice Research Notes* **23**: 9–10.

Li Rongbai, Pandey M P and Sharma Pankaj. 2005. Inheritance of thermosensitive genic male sterility in rice (*Oryza sativa*). *Current Science* **88**(11): 1809–15.

Liu Y, He H, Shun Y, Rao Z, Pan X, Huan Y, Geo J and He X. 1997. Light and temperature ecology of photo-thermo sensitive genic male sterile rice and its application in plant breeding. (*In*) *Proceedings International Breeding in Crops*, 6-8 September, China Center, Changsha, China, pp 49–58.

Ramakrishna S, Mallikarjuna Swamy B P, Mishra B, Virakthamath V C and Ilyas Ahmed M. 2006. Characterization of thermosensitive genetic male sterile line for temperature sensitivity, morphology and floral biology in rice. *Asian Journal of Plant Science* **5**(3): 421–8.

Reddy O U K. 1997. Physiological and molecular characaterization and genetics of temperature sensitive genic male sterile source for heterosis breeding in rice. Ph D thesis, Osmania University, Hyderabad.

Rui-Xing Guo, Dong-Fa Sun, Xun-Dong Cheng, De-Fu Rong and Chengdao li 2006. Inheritance of thermo-photoperiod sensitive male sterility in wheat. *Australian Journal of Agricultural Research* **57**: 187–92.

Frasch Ryan M, Weigand Courtney, Perez Paola T, Palmer Reid G and Sandhu D 2010. Molecular mapping of 2 environmentally sensititive male sterile mutants in soybean. *Journal of Heredity* **102**(1): 11-6.

Shankar V G, Ansari N, Ahmed M I. 2007. Inheritance of thermo-sensitive genic male sterility (TGMS) in rice (*Oryza sativa* L.). *SABRAO of Breeding & Genetics* **39**(2):143–6.

Shi M S. 1981. Preliminary reports of breeding and utilization of late japonica natural double purpose line. *Journal of Hubei Agricultural Science* **7:** 1-3.

Siddiq E A and Ali Jauhar. 1999. Innovative male sterility systems for explitation of hybrid vigor in crop plants. *PINSA* **6:** 331–50.

Singh, Vrijendra, Deshmukh S R, Deshpande M B and Nimbalkar N. 2008. Potential use of thermosensitive genetic male sterility for hybrid development in safflower. 7th International Safflower Conference.

Tang J H, Fu Z Y, Hu Y M and Li J S. 2006. Genetic analysis and mapping of new thermosensitive genetic mala sterility gene in maize. *Theoretical and Applied Genetics* **113:** 11–5.

Virmani S S. 1992. Transfer and induction of thermosensitive genic male sterile mutant in indica rice. (*In*) *Proceeding II International Symposium on Hybrid Rice,* 21-25 April 1992, Manila, Phillipines, IRRI.

Virmani S S, Sun Z X, Mou T M, Jauhar Ali A, Mao C X. 2003. Two-line hybrid rice breeding manual. International Rice Research Institute, Los Baños (Philippines). p. 88.

Wan B H and Deng Y B. 1990. Observation on fertility changes in Hubei photoperiod-sensitive genic male sterile rice Nongken 58S. *Journal of South China Agricultural University* **11**: 1-6.

Wu X J. 1997. Genetic strategies to minimize the risk in exploiting heterosis in rice by means of thermosensitive genic male sterility system. (*In*) *Proceedings of International Symposium on Two Line System of Heterosis Breeding in Crops*, 6–8 September.

Xiao-dong Chen, Dong-fa-sun, De-fu Rong, Genlou, Sun and Jun-hua Peng. 2010. Relationship of genetic distance in hybrid performance in hybrids derived from a new photoperiod – thermosensitive male sterile wheat line 337S. *Euphytica* **175**: 365–71.

Yang R C and Wang N Y. 1989. 5460S an indica photosensitive genic male sterile rice. *International Rice Res. Newsletter* **13**: 6–7.

Yuan. 1998. Hybrid rice breeding in China, (*In*) *Advance in Hybrid Rice Technology*. Virmani S S, Siddique E A and Muralidharan K (Eds.). IRRI, Manila, Philippines.

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

Zhang Jian Kui, Dong Jing, Zong Xue-Feng, Yu Guo-Dong, DAI Xiu-Mei and Ruan Ren-Wu. 2009. Fertility alternation of thermo-photo sensitive genic male sterile (TGMS) wheat line C412S and its association with adenine phosphoribosyltransferase gene expression. *Acta Agronomica Sinica* **35**(4): 562–671.

Zhang Z, Zheng H, Yang J and Yuan S C. 1993. Fertility altering conditions and ecological adaptability of photosensitive genic male sterile rice. *Chinese Journal of Rice Science* **7**.