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Potential use of thermosensitive genetic male sterility for hybrid development in safflower

Vrijendra Singh, S. R. Deshmukh, M. B. Deshpande and N. Nimbkar
Nimbkar Agricultural Research Institute (NARI), Lonand Road,Phaltan 415523, Maharashtra, India, nariphaltan@gmail.com

Abstract
During the course of development of cytoplasmic male sterility (CMS) in safflower, in winter 2003-04 the evaluation of some CMS-based sib-mated crosses and their corresponding parents showed complete male sterility. To examine the causes of male sterility in them, leftover seeds of all the entries were sown during summer 2004. Evaluation of all the crosses and their parents for sterility/fertility under summer conditions revealed 100% restoration of fertility. Evaluation of the seed obtained from these genotypes, in subsequent winter and summer seasons showed complete male sterility in winter and restoration of fertility in summer as before. Thus the male sterility in these genotypes can be said to be thermosensitive in nature. The average minimum and maximum day temperatures during reproductive phase of safflower were recorded to be < 13 and 32^{0}C respectively in winter and > 21 and 39^{0}C respectively in summer.

Thermosensitive male sterility was observed to be digenically recessive in nature and indicated the role of inhibitory gene action. Due to this these male steriles on crossing with different cultivated safflower genotypes gave fertile F_1 hybrids in all the seasons. Evaluation of the thermosensitive male sterility-based hybrids under rainfed conditions during winter 2007-08 recorded an increase of as much as 45% in seed and 55% in oil yield over the recently released CMS-based hybrid MRSA-521. This shows the potential for commercial scale exploitation of hybrid vigour in safflower. Varied climates in which safflower is grown in different parts of India would enable the production of hybrid as well as parental line seed in the same season.

Key words: Thermosensitive genetic male sterility - CMS - hybrid - inhibitory gene action

Introduction

India, since 1997 has released four genetic male sterility (GMS)-based hybrids comprising of three spiny and one non-spiny hybrid for commercial cultivation. Recently in the year 2006, a cytoplasmic-genetic male sterility (CMS)-based hybrid MRSA-521, bred by a private seed company “Mahyco”, has been released for commercial production. The hybrids based on both GMS and CMS systems, in general give an average increase of 15-20% in seed and oil yield over the varieties. The commercial success of GMS-based hybrids has been limited due to inherent problems associated with commercial scale hybrid seed production in them. The commercial success of CMS-based hybrid MRSA-521 is yet to be observed.

Since the GMS system had inherent problems in commercial scale seed production, we like others resorted to the development of CMS system which is considered to be suitable for hybrid seed production in a spiny crop like safflower. During the course of CMS development we came across a sib-mated CMS cross exhibiting thermosensitive nature of male sterility. The thermosensitive genetic male sterile (TGMS) in safflower exhibited complete male sterility during winter (average minimum and maximum day temperature < 13 and 32^{0}C) and restoration of fertility under summer conditions (average minimum and maximum day temperature > 21 and 39^{0}C). Since safflower is grown in India mainly in winter, the identification of thermosensitive male steriles expressing male sterility in winter will be of great use for producing commercial scale hybrid seed at the lowest possible cost. The seed of TGMS lines can be produced either in the winter season itself at the locations where temperatures are observed to be high or in the summer season. This is the first report of thermosensitive male sterility in safflower. However, it has already been identified in several cereal crops including rice (Si and Deng 1986, Wang et al. 1995), maize (He et al. 1995), wheat (Xing et al. 2003) and pearl millet (Shinde and Mehetre

2003, Kaushal et al. 2004). The inheritance and potential of thermosensitive genetic male sterility for hybrid development in safflower is discussed in the present paper.

Material and Methods

During the course of development of CMS system in safflower at NARI, in winter 2003-04, some of the sib-mated CMS crosses as well as their pollinator parents showed 100% male sterility. The leftover seeds of the said entries when sown in summer 2004, surprisingly showed 100% fertility, thereby suggesting thermosensitive nature of male sterility in these genotypes. Further screening of the said genotypes in subsequent summer and winter seasons from 2005 to 2008 confirmed the male sterility to be of thermosensitive and stable nature.

To study the inheritance of thermosensitive genetic male sterility in safflower, uniform and stable TGMS lines were crossed with the self-pollinated genotypes NARI-10, D-149-37-2-4, NARI-36-1 and GMU-701 during winter 2005-06. The F_1 generation of each cross was raised in a 2-row plot of 5 m length in summer 2006 as well as in winter 2006-07. All the F_1 plants of each cross were fertile in both the seasons. Two plants of each cross grown in summer 2006 were bagged before flowering to get selfed seed. The selfed F_1 seed of each cross was used to raise the F_2 generation during winter 2006-07. Each F_2 was sown in 24-row plots of 5 m length and screened for presence of male sterile plants during flowering of the crop. The identification of sterile plants was carried out by inspecting the flowered capitulum of each F_2 plant for the absence of anthers or the presence of rudimentary anthers. The fertile plants on the other hand contained fully developed anthers full of pollen grains. Data of segregation of F_2 populations into sterile and fertile plants were subjected to χ^2 test. To assess the potential of TGMS lines for hybrid development in safflower they were crossed with different promising fertile genotypes to produce 45 TGMS-based hybrids during winter 2006-07. All the 45 TGMS-based hybrids along with the GMS and CMS-based standard checks were evaluated in a randomized block design with two replications under rainfed conditions during winter 2007-08.

Results and Discussion

The fertile F_1 plants in all the four crosses made with the TGMS lines suggest the recessive nature of male sterility in thermosensitive male sterility system in safflower. Similar observations have also been made in rice (Maruyama et al. 1990) and maize (Tang et al. 2006). The F_2 generation of all the four TGMS-based crosses segregated in the ratio of 13 fertile: 3 sterile plants indicating thereby the digenic control with inhibitory gene action for male sterility (Table 1). Digenic recessive thermosensitive male sterility has also been reported in maize (Fu et al. 2004) and rice (Reddy et al. 2000).

Table 1: Inheritance of thermosensitive genetic male sterility in F_1 and F_2 generations of different crosses in safflower

Sr. No.	Cross	Generation	Total no. of plants	Male sterility			
				No. of plants		Fit to 13:3 ratio	
				Fertile	Sterile	χ^2	P
1.	TGMS-1 X NARI-10	F_1	90	90	-	-	-
		F_2	365	290	75	0.774	0.5-2.0
2.	TGMS-2 X D-149-37-2-4	F_1	80	80	-	-	-
		F_2	526	434	92	0.547	0.5-0.2
3.	TGMS-3 X NARI-36-1	F_1	110	110	-	-	-
		F_2	685	540	145	2.628	0.2-0.1
4.	TGMS-1 X GMU-701	F_1	65	65	-	-	-
		F_2	367	295	72	0.182	0.7-0.5

The evaluation of 45 TGMS-based hybrids showed that 12 of them recorded significantly higher seed yield than the CMS hybrid check MRSA-521 (Table 2). Hybrid TGMS-H-42 recorded the maximum increase of 45% in seed and 55% in oil yield. It was followed by the hybrids TGMS-H-

39 (39%, 53%), TGMS-H-38 (38.5%, 51%) and TGMS-H-41 (31%, 41%). This clearly demonstrates the potential and suitability of TGMS lines for commercial scale exploitation of hybrid vigour in safflower.

The CMS system which is commonly known as three line system is the most widely used system for producing F_1 hybrids in sorghum, rice, maize, pearl millet, sunflower, rape and cotton. This system is labour-intensive, since CMS lines need specific maintainer and fertility restorer genotypes, thereby restricting the choice of parents for hybrid development. The thermosensitive male sterility on the other hand offers significant advantages not only because a single genotype is used as the male sterile as well as the maintainer, but there is an opportunity to use the entire compatible germplasm as fertility restorers for hybrid development. Another advantage is also that it lacks adverse effect of sterile cytoplasm.

Maintenance of male sterility to the extent of 100% is very important for successful utilization of TGMS system for hybrid development. Therefore it is important to know exactly the critical temperature causing male sterility and fertility in the TGMS line. Identification of proper locations for seed production of hybrid as well as of the TGMS line itself would play an important role in commercial success of this system. Therefore hybrid seed production needs to be done at a place where a sudden rise in temperature above normal does not cross the limit identified for restoration of fertility in the TGMS line. India being a country with varied climates an array of temperature regimes is available in a given season, thus offering conditions suitable for the exploitation of TGMS system for hybrid development in safflower.

Table 2: Yield performance of TGMS-based promising hybrids as compared to GMS and CMS-based hybrid checks

Hybrid	Seed yield (kg/ha)	% increase in seed yield over MRSA-521	Oil yield (kg/ha)	% increase in oil yield over MRSA-521
Rainfed				
TGMS-H-23	2047*	21.63	672	42.98
TGMS-H-24	1940*	15.27	693	47.45
TGMS-H-29	2001*	18.89	613	30.42
TGMS-H-35	2076*	23.35	578	22.98
TGMS-H-38	2331*	38.50	709	50.85
TGMS-H-39	2334*	38.68	720	53.19
TGMS-H-41	2207*	31.13	663	41.06
TGMS-H-42	2436*	44.74	729	55.11
TGMS-H-46	2174*	29.17	545	15.96
TGMS-H-54	1972*	17.17	574	22.13
TGMS-H-58	2002*	18.95	677	44.04
TGMS-H-60	2034*	20.86	549	16.81
NARI-H-15 (GMS hybrid check)	1451	-	434	-
NARI-NH-1 (GMS hybrid check)	1798	-	636	-
MRSA-521 (CMS hybrid check)	1683	-	470	-
CD at 0.05	256	-	-	-
C.V.%	16.65	-	-	-

* Significant at 5% level.

References

Fu, Z. Y., Zhao, G. Y., Tang, J. H., Hu, Y. M., He, Z. Y. and He, J. 2004. Comparison about traits related to fertility between two maize thermo-sensitive genic male sterile lines and primary mapping the genes for TGMS. Mol. Plant Breed. 2: 633-636.

He, Z. Y., Li, Y. B., and Tan, S. Y. 1995. The discovery and preliminary study of the thermo-sensitive genic male sterile maize. Crops (2): 1-2.

Kaushal, P., Roy, A. K., Zadoo, S. N. and Choubey, R. N. 2004. Cytogenetic analysis of thermosensitive genic male sterility (TGMS) recovered from a *Pennisetum glaucum* (L.) R.
Br. X *P*. v*iolaceum* (Lam.) L. rich cross. Cytologia. 69 (4): 409-418.
Maruyama, K., Araki, H. and Kato, H. 1990. Thermosensitive genic male sterility induced by irradiation. Proceedings of the Second International Rice Genetic Symposium. International Rice Research Institute, P.O. Box 933, Manila 1099, Philippines. May 14-18, 1990.
Reddy, O. U. K., Siddiq, E. A., Sarma, N. P., Ali, J., Hussain, A. J., Nimmakayala, P.,
Ramasamy, P., Pammi, S. and Reddy, A. S. 2000. Genetic analysis of temperature-sensitive male sterility in rice. Theor. Appl. Genet. 100 (5): 794-801.
Shinde, G. C. and Mehetre, S. S. 2003. Cytological studies of temperature-sensitive genic male sterile lines of pearl millet [*Pennisetum glaucum* (L.) R. Br.]. Caryologia. 56 (4): 399-403.
Si, M. S. and Deng, J. Y. 1986. The discovery, determination and utilization of the Hubei photo-sensitive genic male-sterile rice (*Oryza sativa* subsp. japonica). Acta. Genet. Sin. 13 : 107-112.
Tang, J. H., Fu, Z. Y., Hu, Y. M., Li, J. S., Sun., L. L. and Ji, H. Q. 2006. Genetic analyses and mapping of a new thermo-sensitive genic male sterile gene in maize. Theor. Appl. Genet. 113: 11-15.
Wang, B., Xu, W. W., Wang, J. Z., Wu, W., Zhang, H. G., Yang, Z. Y., Ray, J. D. and Nguyen,
H. T. 1995. Tagging and mapping the thermo-sensitive genic male-sterile gene in rice with molecular marker. Theor. Appl. Genet. 91: 1111-1114.
Xing, Q. H., Ru, Z. G., Zhou, C. J., Xue, X., Liang, C. Y., Yang, D. E., Jin, D. M. and Wang, B. 2003. Genetic analysis, molecular tagging and mapping of the thermo-sensitive genic male-sterile gene (wtms1) in wheat. Theor. Appl. Genet. 107: 1500-1504.