J. Cotton Res. Dev. **34** (1) 46-49 (January, 2020) **ISSN No. 0972-8619**

GMS-CISG 20 - A new genetic male sterile line of diploid cotton (*Gossypium arboreum* L.) with marker trait

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Cotton is the leading commercial cash crop of the world. In India, cotton is grown in area of 12.3 million hectares with total production of 370 lakh bales (1 bale = 170 kg of lint) (ICAR-CICR Annual Report 2017-2018). All the four species, tetraploid and diploid viz., *Gossypium hirsutum*, *G. barbadense*, *G. arboreum* and *G. herbaceum* are being cultivated in India. The majority of cotton area (more than 90%) is under *G. hirsutum* while <10 per cent constitute the other three species (Mehetre, 2015). The *G. arboreum* popularly known as Asiatic cotton were occupying about 97 per cent area in 1947 and the American cotton (*G. hirsutum*) about 3 per cent. At present, the scenario is just opposite (Mehetre, 2015). The diploid cotton, both varieties and hybrids, are relatively tolerant to insect-pest and diseases and they are well adapted under low input conditions. Conventional intra *arboreum* hybrids were developed and released viz., G. Cot. DH-7 in 1984 and G. Cot. DH-9 in 1989 in Gujarat, DDH-2 in 1992 from Karnataka, MDCH-201 by Mahyco, Maharashtra and LDH-11 from Punjab (Patel *et al.*, 2008) but could not sustain due to high cost of hybrid seed produced through conventional emasculation and crossing method (Srinivasan and Guirurajan, 1974; Kajidoni and Patil, 2003). Later, the identification and registration of first genetic male sterile line DS 5 in *G. arboreum* by Singh *et al.* (1993) facilitated male sterility based seed production of intra-*arboreum* hybrids and their release. Thereafter, number of genetic male sterility based hybrids such as AAH 1, CICR 2, Raj DH 9, Moti, KR 64 were released using DS 5 male sterile line. Subsequently Mehetre and Patil (2001, 2004), Tuteja *et al.*, (2005) and Gedam *et al.*, (2012) identified number of new genetic male sterile lines such as GAKA 423, GAKA 423A, Million GMS, NCAGA 4, NCAGA 26, CISA-2 (GMS), MSD 10 and MSD 11. Khadi *et al.*, 2003 reported Temperature Sensitive Genetic Male Sterile (TGMS) lines in diploid cotton. Several reports on male sterility in American cotton are also available (Justus and Leinwaber, 1960; Richmond and Kohel, 1961; Weaver, 1968; Hutchinson and Gadkari, 1935).

The new genetic male sterile line has been identified in the seed multiplication plot of CISG 20 at ICAR-Central Institute for Cotton Research, Regional Station, Sirsa. The GMS line CISG 20 is a spontaneous mutant identified from agronomically adapted line CISG 20 during 2008-2009 crop season. It was maintained by sib mating. The line CISG 20 (GMS) has complete pigmentation i.e. red flower with red petal spot and red plant body which can be used as a marker trait for male sterility genotype identification and grow out test. The genetic male sterile line CISG 20 is pollen sterile throughout its

reproduction stage, no pollen shedders are reported which rules out the selfed seed during hybrid seed production programme thus enhances the boll setting percentage (Patil *et al.*, 2018)

Inheritance of male sterility: The original male sterile plant was identified in the seed multiplication plot of CISG-20 *(Gossypium arboreum L.)* at ICAR-CICR, Regional Station, Sirsa. The male sterile plants were maintained through sibmating with male fertile plants and ratio of 3:1 was observed. For genetic study, the male sterile plants and male fertile (homozygous) plants of CISG 20 were crossed during 2015 and 2016 to obtain F1 (fertile). During 2016 and 2017 BC1 (male sterile x F1) were attempted and F1 (fertile) selfed to obtain F2 generation. During 2017 and 2018, F2 and BC1 were raised to study the segregation pattern. The segregating plants for male sterility were grouped in male sterile and fertile in BC1 and F2. The test of goodness of fit to an expected segregation ration was performed by calculating the probability in Chi square test (Table 1). In F2 and BC1, the fertile-sterile plants ratio of 3:1 and 1:1 showed goodness of fit and non-significant confirming that a single-recessive gene is responsible for male sterility in line CISG 20.

Table 1. Segregation pattern for male and fertility and sterility in F1, F_2 and BC1 generation of GMS - CISG 20

Year	Generation	Ratio	Segregation observed		S«2	P=0.05
			Fertile	Sterile		
2016-2017	F1	-	38	-	-	-
2017-2018	F1	-	43	-	-	-
2017-2018	F_2	3:1	1128	398	1.011	0.31468
2018-2019	F_2	3:1	1236	442	1.683	0.19457
2017-2018	BC_1	1:1	211	183	1.990	0.15836
2018-2019	BC_1	1:1	215	191	1.419	0.23361

Gene controlling male sterility: The male sterile plants of CISG 20 were crossed with heterozygous male fertile plants of DS 5 (GMS) line, an existing source of GMS in *G. arboreum,* having genetic constitution of aMs1 ams1 to ascertain whether the gene responsible for male sterility is the same or different as that of DS (GMS) which has ams1 ams1(Singh and Kumar, 1993). If the gene governing the male sterility in new source is same, then the test cross ratio among the male fertile and male sterile plants would have been 1:1 but the segregation was 3:1 ratio (Table 2). Therefore, the genetic male sterility observed in the present study is

Table 2. Studies on identification of gene controlling genetic male sterility through test cross analysis

Test Cross	Segregation observed		S«2	p=0.05
	Fertile	Sterile		
CISG 20 x DS 5 (Fertile)	197	59	0.521	0.47049
Ratio	3:1			

governed by a single pair of alleles, which are different from the gene ams1 ams1 governing male sterility in DS5 (GMS) line. The gene for male sterility is designated as ams^2 ams^2 expressing completely male sterility condition and the heterozygous F_1 (male fertile) is designated as aMs^2 ams^2.

Although a number of male sterile lines have been reported, most of them cannot be used in hybrid seed production due to incomplete male sterility, poor agronomic traits, or poor general combining ability (GCA) and specific combining ability (SCA) of the genotype. The line CISG 20 (GMS) has open red flower with red petal spot using prominent marker trait and is easily maintained through sibmating (1:1 sterility and fertility ratio). Due to its red plant body the male sterility genotype can be easily identified. The genetic male sterile line CISG 20 is pollen sterile throughout its reproduction stage, which rules out the selfed seed during hybrid seed production programme with an added advantage of higher boll setting.

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Received for publication : June 26, 2019
Accepted for publication : November 16, 2019