



Identification and characterization of new fertility restorers in cytoplasmic genetic male sterility (CGMS) of cotton [*Gossypium hirsutum* (L.)] derived from *Gossypium harknessii*

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Cytoplasmic-nuclear male sterility (CGMS) is a maternally inherited trait, in conjunction with nuclear genome, suppresses the production of viable pollen grains, while not affecting the female fertility. Male sterile plants are able to set seeds as long as viable pollens are provided. The presence of certain nuclear genes, Rf (restoring fertility), can effectively suppress the male-sterile cytoplasm and restore pollen fertility. The application of CMS/Rf system has proved to be an effective means to produce commercial F₁ hybrid seed in a wide range of more than 150 plant species. Most CMS types have occurred naturally or in interspecific crosses [1]. The success in development of cotton hybrid largely depends on availability of the effective restorer and precise basic knowledge on the genetics of fertility restoration of such lines. The first F₁ line of commercial cotton was introduced by crossing an upland cotton (*G. hirsutum*) as a male parent to a wild species *G. harknessii* [2]. Therefore cytoplasmic male sterility (A), maintainer (B) and restorer (R) line, system can be used to develop hybrid cotton varieties. Reduction in cost of hybrid seed production is possible by using male sterility. The best-known sterile cytoplasmic source available for heterosis breeding in cotton is from *G. harknessii* developed by Meyer [3]. *Gossypium harknessii* Brandagee (D2-2) which is a diploid (2n = 26) was used as female by Meyer [4] to transfer *G. hirsutum* genome in the cytoplasm of *G. harknessii*. The resultant triploid was made hexaploid (2n = 78) using colchicine. Male sterile tetraploid plants were recovered from cross between hexaploid and tetraploids. The use of this CGMS source is limited for want of potential restorers. In case of cytoplasmic male sterility system the abnormality of disintegration before pollen mother cell undergo meiosis has been reported by Khadi *et al.* [5]. Whereas in case of genetic male sterility system the abnormality is post meiotic. Therefore, the scope of CGMS system will be greater if more number of divergent restorer lines are identified. It was

thought worthwhile to evaluate the CGMS lines for their potentiality as parents for hybrids and also to identify suitable restorers from the *G. hirsutum* accessions available in the gene bank.

Nine cytoplasmic genetic male sterile (CGMS) lines, LRA 5166 CMS, CSH 19CMS, RB 281CMS, Pusa 31 CMS, LH 1134CMS, F 505 CMS, K 34007CMS, F 1183CMS, and SH 2379CMS were developed at Central Institute for Cotton Research, Regional Station, Sirsa using IH 76 carrying *G. harknessii* cytoplasm as donor by back cross breeding. To identify the restorer lines, the CMS lines were crossed to nine *G. hirsutum* male parents *viz.* CIR 8, CIR 10, CIR 12, CIR 23, CIR 26, CIR 32, CIR 38, CIR 47 and CIR 70 selected from the germplasm collections during 2001-02. Eighty one crosses were grown in replicated trial of two row plot of 5.4 m length having 100 × 45 cm spacing between row to row and plant to plant during the *kharif* 2002-03. On the basis of pollen dehiscence plants were classified as male fertile or male sterile. It was also confirmed in the laboratory with 1% acetocarmine test. After identification of germplasm line as restorer, the fertility restorers were tested in three rows of 6 m row length for two years. The restorers, which were found stable for fertility restoration, were characterized for agro-morphological characters. The observation recorded on five randomly selected plants in each restorer for two years (2003-2004) is presented.

Out of 81 CMS hybrids, 72 (88.89 %) were fertile as CIR 8, CIR 10, CIR 23, CIR 26, CIR 32 and CIR 70 could restorer fertility in the 54 F₁s and CIR 12 could restore the fertility for 6, CIR 38 for 8 and CIR 47 for 4 CMS lines. The results revealed that 6 pure lines CIR 8, CIR 10, CIR 23, CIR 26, CIR 32 and CIR 70 acted as fertility restorer for all the 9 CMS lines. The *G. harknessii* cytoplasm of CMS lines LRA 5166 CMS, CSH 19CMS, RB 281CMS, Pusa 31CMS,

LH 1134CMS, F 505CMS, K 34007CMS, F 1183CMS, and SH 2379CMS may be same but there were differences in fertility restoration. Weaver and Weaver [6] also observed that a single gene, probably expressing partial dominance, controls fertility restoration in cytoplasmic male sterile cotton. F₁ hybrid population showed a wide range of male fertility expression. Pollen production in the F₁ (heterozygous for the restorer gene) was found to be much more variable and influenced by environmental conditions than in the homozygous parent. Tuteja et al. [7] also reported that a maintainer of one CMS sometimes could not maintain the sterility of other CMS line, having the same cytoplasmic background. The identified restorers were maintained through selfing.

distorted terminal leaves. Therefore, this gives the clue to intermate the different newly identified restorer lines, so that level of fertility restoration can be enhanced due to recombination of different genes [7]. These restorer lines, which have shown stable restorability and are agronomically desirable types, can be utilized for development of cotton hybrids based on cytoplasmic genetic male sterility system. However, no such deleterious effects were observed in the present study. The restorer lines CIR 8, CIR 10, CIR 23, CIR 26, CIR 32 and CIR 70 identified from the present study should permit the commercial production of completely fertile F₁ cotton hybrids. Moreover, these lines could also be used for intercrossing to develop base population for breeding better restorer lines.

Table 1. Characteristics of identified fertility restorer lines of cotton

S. No.	Restorer	Characters												
		Days to 50% flowering	Leaf shape	Petal colour	Anther colour	Plant type	Reaction to CiCuV	Plant height (cm)	Number of monopods	Number of sympods	Bolls/plant	Boll weight (g)	Seed fuzz colour	Seed index (%)
1	CIR 8	69	N	Bicolour	R	R	R	145	11.0	7.7	74.7	2.2	Grey	9.2
2	CIR 10	82	N	Bicolour	R	R	R	133	4.3	15.0	55.3	2.7	White	9.5
3	CIR 12	72	N	Y	Y	G	R	145	4.3	17.3	72.0	3.3	Grey	10.5
4	CIR 23	75	N	Y	C	G	R	131	5.0	12.7	60.3	2.5	White	9.5
5	CIR 26	70	O	C	C	G	R	128	4.0	13.3	53.7	2.6	Grey	9.3
6	CIR 32	65	N	Y	C	G	R	108	1.0	11.3	34.7	3.4	Grey	10.4
7	CIR 38	83	O	Y	Y	G	R	137	7.3	6.3	54.7	3.0	Grey	10.6
8	CIR 47	73	N	Y	Y	G	R	134	3.3	13.0	49.0	3.5	Grey	10.0
9	CIR 70	85	O	Bicolour	R	R	R	143	6.0	13.7	53.3	2.5	White	9.0

N = Normal; O = Okra leaf; C = Cream; Y = Yellow; R = Red; G = Green; R = Resistant

Nine phenotypically diverse restorers were characterized for agro morphological characters (Table 1) and designated as Cotton Institute Restorers (CIR 8, CIR 10, CIR 12, CIR 23, CIR 26, CIR 32, CIR 38 and CIR 70). The range in flowering time among restorers was 20 days, from 65 days (CIR 32) to 85 days (CIR 70). Among restorers, 2 were early flowering (65-69 days), 3 were medium flowering (70-73 days) and 4 were late flowering (75-85 days). Boll weight range was 2.2 g (CIR 8) to 3.5 g (CIR 47). The range in boll number per plant and seed index was also from 34.7 (CIR 32) to 74.7 (CIR 8) and 9.0 g (CIR 70) to 10.6 g (CIR 38) respectively (Table 1). Three restorers CIR 26, CIR 38 and CIR 70 have okra type leaf shape whereas remaining have medium leaf shape. Similarly the restorer lines CIR 8, CIR 10 and CIR 70 have red plant type body and bicolour flowers, which can serve as marker character for hybrid seed production.

Weaver and Weaver [6] observed two deleterious traits in population carrying gene for fertility restoration. One of these was cracked root, which causes the roots to be severely cracked and underdeveloped. Another deleterious factor was characterized by dwarfism and

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