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Short Communication

Development of cytoplasmic-genic male sterility in safflower

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With 2 tables

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Abstract

An interspecific cross was made between *Carthamaus oxyacantha* and the cultivated species *C. tinctorius* to develop a cytoplasmic-genic male sterility (CMS) system in safflower. *C. oxyacantha* was the donor of sterile cytoplasm. The 3 : 1 segregation pattern observed in BC_1F_2 suggested single gene control with dominance of male-fertility over male-sterility. The information obtained from crossing male sterile × male fertile plants in BC_1F_3 and BC_1F_4 generations showed statistically significant single gene (1 : 1) segregation for male sterility vs. male fertility. The results demonstrated that *C. tinctorius* possesses a nuclear fertility restorer gene and that a single dominant allele restored fertility (Rf) in progeny carrying CMS cytoplasm of *C. oxyacantha*. Male sterility occurred with the homozygous recessive condition (rfrf) in a sterile *C. oxyacantha* cytoplasm background and not in the normal cytoplasm of *C. tinctorius*. The genetic background of different restorer lines of *C. tinctorius* having normal cytoplasm did not effect fertility restoration. The absence of male sterile plants in *C. tinctorius* populations ruled out the possibility of genetic male sterility. Normal meiosis in F_1 and BC_1F_2 ruled out a cytogenetic basis for the occurrence of male sterility.

Key words: *Carthamus tinctorius—Carthamus oxyacantha*—fertility restoration—safflower-cytoplasmic-genic male sterility

Safflower (*Carthamus tinctorius* L.) is predominantly a self-pollinated crop; cross pollination to the extent of 5–40% has been reported (Claassen 1950). The development of genic male sterile (GMS) lines made safflower hybrids feasible (Anjani 1997a,b). Thus, the first GMS-based safflower hybrid DSH 129 was released (Anjani 1998) for commercial cultivation in India. However, hybrid seed production of DSH 129 at a commercial level is constrained because of incomplete or delayed rogueing of fertile plants from the female parent population. This necessitated the development of cytoplasmic-genic male sterile lines that would overcome these constraints and facilitate hybrid seed production.

Availability of CMS lines in safflower was reported first by Hill (1989). However, no published information is available about the source of the cytoplasmic male sterility in this CMS system, other than that the original system, developed by Hill was 'derived from some wild sources of germplasm and had several undesirable traits'. He reported that the time from 1983–1997 was spent getting rid of the load of 'other' genes that came along with the maintainer genes (Hill 1997). As the CMS system developed by Hill is not available for use in hybrid development programmes, another CMS system had to be developed in safflower. This paper reports the development of a cytoplasmic-genic male sterility system in safflower and preliminary information on the mode of inheritance.

The wild species *C. oxyacantha* from Punjab, India. (Anjani et al. 1999) and three true-breeding lines belonging to the cultivated species *C. tinctorius* namely, 'A1' (variety), 96-520, PI 537701 were used in the crossing programme. The initial crosses *C. oxyacantha* × 'A1' and 'A1' × *C. oxyacantha* were made by manual emasculation and pollination. The plants of *C. oxyacantha* and 'A1' used in the initial crossing programme were selfed to use their progeny in a backcross programme. Out of 72 F_1 plants of *C. oxyacantha* × 'A1' and 85 of 'A1' × *C. oxyacantha*, a few randomly-selected F_1 plants were selfed and harvested separately to grow the F_2s. These F_1 plants were backcrossed to a single self-progeny of the initial parents from both species to produce BC_1F_1s. Selected BC_1F_1 progeny of (*C. oxyacantha* × 'A1') × *C. oxyacantha*, (*C. oxyacantha* × 'A1') × 'A1', ('A1' × *C. oxyacantha*) × *C. oxyacantha* and ('A1' × *C. oxyacantha*) × 'A1' were selfed to produce BC_1F_2 generations.

After observing the occurrence of male sterile plants in the BC_1F_2 generation of (*C. oxyacantha* × 'A1') × *C. oxyacantha*, it was advanced further to produce BC_1F_3 and BC_1F_4 populations by sib crossing male-sterile and male-fertile progeny as well as by self pollinating the male-fertile individuals. The sterile plants were also pollinated by pollen collected from individual plants of self-progeny of both parents, bulk pollen collected from the 'A1' population and pollen from selected individual plants of 96-520 and PI 537701. The plants of 96-520 and PI 537701 used in crosses were also selfed. All generations were grown under mosquito nets to prevent cross pollination through honeybees. The selfed and backcrossed capitula were further covered with butter paper bags. Male sterility was assessed in main, primary, secondary and higher order capitula by visual observation of anther size and pollen production, and by squashing anthers prior to anthesis in aceto-carmine to observe absence or presence of pollen grains under a microscope. To study meiosis, floral buds were fixed in Piennar's fluid. PMC smears were stained with aceto-carmine. BC_1F_2s of other backcrosses were not advanced further as no sterile plants were observed in them.

The F_1, F_2 and BC_1F_1 plants of the crosses *C. oxyacantha* × 'A1' and 'A1' × *C. oxyacantha* were male fertile. The F_1 plants of both crosses were intermediate with respect to morphology. Eleven male-sterile and 60 male-fertile plants were observed in BC_1F_2 of (*C. oxyacantha* × 'A1') × *C. oxyacantha*. Phenotypically, eight male sterile plants had 'A1' traits while one resembled *C. oxyacantha* and two showed intermediate traits. Among fertile plants, 'A1' traits were predominant in 22 plants and *C. oxyacantha* traits were

predominant in 38 plants. The absence of sterile plants in F_2 of *C. oxyacantha* × 'A1' could be ascribed to either the very small population of F_2 families (10–16 plants) or the randomly-selected F_1 plants selfed to produce F_2 not possessing required non-restorer allele rf. Non-segregation for fertility in BC_1F_1 and BC_1F_2 generations of (*C. oxyacantha* × 'A1') × 'A1' could be because of the absence of the non-restorer allele rf in 'A1' plant ('A1'-1F-P2) that was used in backcrossing and production of BC_1F_2 from BC_1F_1 plants that did not posses the rf allele. The absence of the rf allele in 'A1'-1F-P2 was proved and is discussed later in the results. All flowers in the male-sterile plants were sterile and were smaller than fertile flowers, with very small or rudimentary anthers. Sterile flowers were mostly devoid of pollen grains; sometimes 1–10 small unstained pollen grains were seen under a microscope in a very few florets. Conversely, abundant pollen was present in fertile plants; these were big and darkly stained. The 3 : 1 segregation pattern of male-fertile and male-sterile plants in BC_1F_2 suggests dominance of male fertility over male sterility. Statistically significant single gene (1 : 1) segregation for male sterility vs. fertility was observed in BC_1F_3 populations, that were derived by sib crossing the male-sterile and male-fertile BC_1F_2 sister plants possessing *C. oxyacantha* cytoplasm (Table 1). The same segregation pattern was observed in some of the BC_1F_4 populations derived by sib crossing male-sterile and male-fertile BC_1F_3 sister plants (Table 2). From the segregation pattern of these sib crosses, the fertile plants were assumed to be heterozygous, Rfrf. The heterozygous nature of the fertile plants Nos. 2, 3, 8, 11 and 22, that were used in the sib crosses to produce BC_1F_4 populations, could be confirmed by the 3 fertile : 1 sterile segregation pattern of their self-progeny (Table 2). Non-segregation of self-progeny of some of the BC_1F_2 fertile plants (Table 1), BC_1F_3 fertile plants and sib crosses (Table 2) could be ascribed to the homozygous (RfRf) nature of the fertile parents.

In the test-crosses, when the sterile (rfrf) plants with *C. oxyacantha* cytoplasm were crossed to two randomly-selected self-progeny ('A1'-1F-P1 and 'A1'-1F-P2) of the initial plant of 'A1', the progeny of the crosses with 'A1'-1F-P1 showed a 1 : 1 segregation for male-sterile and male-fertile, whereas the progeny of the test-crosses with 'A1'-1F-P2 were all pollen fertile and their self-progeny were also fertile. This clearly indicates that 'A1'-1F-P1 was heterozygous (Rfrf) and 'A1'-1F-P2 was homozygous (RfRf) for the male fertility restorer and also confirms the heterozygous nature of the initial 'A1' plant used in the primary cross. The data presented suggested that male sterile individuals are genetically rfrf, while male fertile plants are either RfRf or Rfrf. Further, the progeny of a cross between a sterile plant and the bulk pollen collected from 'A1' plants segregated into 2 sterile and 6 fertile individuals. This suggests that in the self population of 'A1', the restorer gene exists in the heterozygous (Rfrf) form; as a result there is the possibility of the existence of dominant and recessive homozygotes (RfRf and rfrf) in the 'A1' population. Similar results were obtained when the male-sterile plants were crossed to different lines of *C. tinctorius* namely, 96-520 and PI 537701. Two sterile and 8 fertile progeny were produced when a sterile plant was crossed to 96-520, and 5 sterile and 17 fertile progeny were produced when another sterile plant was crossed to PI 537701.

In order to test a possible genome-plasmon interaction causing male sterility in BC_1F_2 material of (*C. oxyacantha* × 'A1') × *C. oxyacantha*, the F_1 of the reciprocal cross ('A1' × *C. oxyacantha*) and its F_2 and backcross progeny were observed for male sterility. Absence of male sterility in them suggested the occurrence of genome-plasmon interaction in the present material. To further confirm that the male sterility occurring in the material was due to interaction between the cytoplasm of *C. oxyacantha* and a recessive nuclear gene from *C. tinctorius*, 1958 individual plants of 'A1' were observed visually for pollen production as well as under a microscope. Presence of abundant fertile pollen and well-developed anthers in these plants confirmed that the recessive male sterility nuclear gene (rfrf) present in *C. tinctorius* ('A1') could not cause sterility in a *tinctorius* cytoplasm background. The above studies confirmed that *C. tinctorius* is the donor of a recessive nuclear allele that interacted with the cytoplasm of *C. oxyacantha* to produce male-sterile plants in BC_1F_2 of (*C. oxyacantha* × 'A1') × *C. oxyacantha*.

In order to rule out the role of chromosomal and meiotic aberrations in the expression of male sterility, meiosis studies were undertaken in the F_1 of *C. oxyacantha* (2n =

Table 1: The observed male-fertile and male-sterile plants in the segregation of sib crosses between sterile and fertile BC_1F_2 plants and self- pollinated fertile plants

Sib crosses between sterile and fertile plants of BC_1F_2 of (*C. oxyacantha* × *C. tinctorius*) × *C. oxyacantha* and self-pollinated fertile plants of BC_1F_2	Segregation in BC_1F_3		χ^2	Probability (1 : 1)
	Male fertile	Male sterile		
Sib crosses				
Sterile plant-5 × Fertile plant-2	24	20	0.363	0.50–0.70
Sterile plant-6 × Fertile plant-3	48	42	0.400	0.50
Sterile plant-6 × Fertile plant-4	51	40	1.328	0.25
Sterile plant-6 × Fertile plant-6	72	65	0.356	0.50–0.60
Sterile plant-5 × Fertile plant-1	54	48	0.352	0.50–0.60
Sterile plant-5 × Fertile plant-4	57	55	0.035	0.80–0.90
Fertile plants				
Plant-35	10	0	–	–
Plant-36	42	0	–	–
Plant-15	43	0	–	–
Plant-25	41	0	–	–
Plant-33	40	0	–	–
Plant-43	65	0	–	–
Plant-38	72	0	–	–
Plant-48	79	0	–	–
Plant-52	82	0	–	–

Table 2: The observed male-fertile and male-sterile plants in the segregation of sib crosses between sterile and fertile plants of BC_1F_3 and self-pollinated fertile plants

Sib crosses between sterile and fertile plants of BC_1F_3 of (*C. oxyacantha* × *C. tinctorius*) × *C. oxyacantha* and self-pollinated fertile plants of BC_1F_3	Segregation in BC_1F_4		χ^2	Probability (P)
	Male fertile	Male sterile		
Sib crosses				**P (1 : 1)**
Sterile plant-15 × Fertile plant-3	52	48	0.160	0.65–0.70
Sterile plant-10 × Fertile plant-8	67	58	0.648	0.30–0.50
Sterile plant-8 × Fertile plant-11	42	40	0.048	0.80–0.90
Sterile plant-20 × Fertile plant-22	67	62	0.190	0.50–0.70
Sterile plant-7 × Fertile plant-2	38	33	0.352	0.50–0.60
Sterile plant-8 × Fertile plant-1F′	79	0		
Sterile plant-10 × Fertile plant-1F′	82	0		
Sterile plant-1 × Fertile plant-5	65	0		
Sterile plant-15 × Fertile plant-7	89	0		
Sterile plant-20 × Fertile plant-7	79	0		
Fertile plants				**P (3 : 1)**
Fertile plant-2	56	14	0.933	0.30–0.50
Fertile plant-8	62	22	0.062	0.80–0.85
Fertile plant-11	48	15	0.046	0.80–0.90
Fertile plant-22	58	16	0.449	0.50
Fertile plant-3	69	18	0.861	0.30–0.50
Fertile plant-1F′	43	0		–
Fertile plant-5	68	0		–
Fertile plant-7	72	0		–
Fertile plant-10	65	0		–

24) × *C. tinctorius* (2n = 24) and BC_1F_2 of (*C. oxyacantha* × *C. tinctorius*) × *C. oxyacantha*. Meiosis was normal with 12^{II} at diakinesis and metaphase-I and 12^{I}-12^{I} segregation at anaphase-I. Chromosomal abnormalities such as aneuploidy, laggards, etc., were not found. This clearly rules out a cytogenetic basis of male sterility in the material.

The rationale of the approach was to search for a CMS system, based on the interaction of the cytoplasm with a restorer allele Rf and a maintainer allele rf at a nuclear locus, which showed stable and homozygous expression of male sterility. The genetic background of different restorer lines belonging to *C. tinctorius*, having normal cytoplasm did not effect fertility restoration. The heterozygous fertility restorer in an *oxyacantha* cytoplasm background produced male-fertile and male-sterile progeny in a 3 : 1 ratio upon sefing, and in a 1 : 1 ratio upon sib crossing to sterile progeny. The restorer gene in the recessive homozygous state (rfrf) had no effect in normal cytoplasm of *C. tinctorius*. Jones (1946) in onion and Peterson (1958) in *Capsicum* observed similar results. They demonstrated that a recessive gene (rf) when in a homozygous state in a certain type of cytoplasm (S) caused plants to be male sterile. The same gene in a second type of cytoplasm (N) had no effect. The presence of pollen fertility restorer genes in a cultivated species, which restored male fertility in *petiolaris* cytoplasm, is well known in sunflower (Vranceanu and Stoenescu 1971). The results showed that the restorer locus with alleles Rf and rf exists in dominant homozygous and heterozygous conditions in *C. tinctorius*, and suggests the homozygous recessive (rfrf) form of the locus would also exist in *C. tinctorius*, though at low frequency. Therefore, it is postulated that further extensive crossing of individual *C. tinctorius* ('A1') plants to male sterile plants and their progeny-row observation for complete male sterility should identify *C. tinctorius* ('A1') plant with rfrf in normal cytoplasm, which would be the maintainer of the CMS system. The 'A1' plants having a heterozygous maintainer gene were identified and self-pollinated to isolate homozygous recessive genotypes of maintainers in the progeny generation.

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References

Anjani, K., 1997a: Feasibility of recessive genetic male sterile lines in safflower hybrid seed production. Proc. IV Int. Safflower Conference, Bari, Italy, 278—280.

Anjani, K., 1997b: Safflower hybrids developed using recessive genetic male sterility system. Proc. IV Int. Safflower Conference, Bari, Italy, 281—282.

Anjani, K., 1998: DSH 129: The first safflower hybrid released for commercial cultivation. Proc. Nat. Seminar on Heterosis: Its Commercial Exploitation. Nagpur, India, 32.

Anjani, K., W. S. Yadav, and S. S. Duhoon, 1999: Safflower germplasm collecting in northwestern India. IPGRI Newsletter for Asia, The Pacific and Oceania **28,** 8—9.

Claassen, C. E., 1950: Natural and controlled crossing in safflower (*Carthamus tinctorius* L.). Agronomy J. **42,** 381—384.

Hill, A. B., 1989: Hybrid safflower breeding. Proc. II Int. Safflower Conference, Hyderabad, India, 171.

Hill, A. B., 1997: A brief talk on safflower hybrids. Proc. IV Int. Safflower Conference, Bari, Italy, 261.

Jones, H. A., 1946: Cytology, genetics and breeding. Problems and progress in onion breeding. Herbertia **11,** 275—294.

Peterson, P. A., 1958: Cytoplasmically inherited male sterility in *Capsicum*. The American Naturist **92,** 111—119.

Vranceanu, V. A., and F. M. Stoenescu, 1971: Pollen fertility restorer gene from cultivated sunflower (*Helianthus annuus* L.). Euphytica **20,** 536—541.

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