Identification of cytoplasm-induced male sterility in sesame through wide hybridization

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Wide hybridization was carried out in direct and reciprocal directions to identify cytoplasm-based male sterile sources in sesame. Among the seven different interspecific hybrids produced and analysed, reciprocal difference for male sterility was observed in a cross involving *Sesamum indicum* and *S. malabaricum* alone. Cytological investigation revealed that the cause of sterility was cytoplasmic differences rather than chromosomal abnormalities. This is indicative of interaction of the cytoplasm of *S. malabaricum* with the nuclear genome of *S. indicum* leading to male sterility. The evidence for cytoplasmic male sterility in sesame is reported for the first time and its utility in hybrid seed development is discussed.

GLOBALLY, the seed yields of sesame are the lowest of all the major oilseed crops. But its importance is proportionately higher than its share of production because of the multiplicity of its uses compared to other oil-yielding crops. The nonavailability of high yielding cultivars, in addition to the lack of resistance to biotic and abiotic stresses, is the major cause of low productivity in sesame. The yield improvement achieved through conventional hybridization followed by selection has been only marginal. However, earlier studies clearly established that heterosis large enough for production and utilization of F_1 hybrids is available in sesame[1, 2]. But the commercial exploitation of this phenomenon is feasible only if the means of producing hybrid seeds economically could be made available.

Even though hand emasculation and pollination are simple in sesame, they are labour-intensive, resulting in increased cost of hybrid seeds. This prompted male sterility to become an effective tool for use in hybrid seed development, eliminating the tedium of hand emasculation and pollination. Although many workers have reported genic male sterility in sesame[3, 4], cytoplasmic male sterility has not yet been reported. Many of the present-day commercially utilized male sterile lines of different crops were isolated either in species crosses or induced through mutagenesis[5]. Hence, an attempt was made to identify male sterile sources through wide hybridization in sesame.

Six wild species of *Sesamum* falling in three chromosomal groups, viz. $2n = 26$ (*S. alatum* and *S. malabaricum*), $2n = 32$ (*S. laciniatum* and *S. prostratum*) and $2n = 64$ (*S. radiatum* and *S. occidentale*), collected from different parts of the country, and four cultivars of *S. indicum* ($2n = 26$) viz. TMV 3, TMV 4, TMV 6 and Co 1, were utilized. Both direct and reciprocal crosses were effected between the cultivated and different wild species.

Seeds extracted from crossed capsules were sown along with selfed seeds of the parents for evaluation. At the time of flowering, all the hybrid plants were screened for pollen stainability using 1:1 glycerol–acetocarmine mixture and a total of 15 plants per combination were labelled for cytological studies. PMC smears were prepared with 1% acetocarmine and temporary slides were observed for different stages of meiosis.

Attempts to cross either of the two 64 chromosome species with *S. indicum* ($2n = 26$) were not successful, whereas the success of crossing *S. alatum* ($2n = 26$) and *S. indicum* ($2n = 26$) was very much limited. The failures were attributed to very early collapse of the hybrid endosperm, with subsequent starvation of the proembryo and early abortion of the young embryo[6].

Both direct and reciprocal crosses between the cultivated sesame and three wild species, viz. *S. malabaricum* ($2n = 26$), *S. laciniatum* ($2n = 32$) and *S. prostratum* ($2n = 32$), however, showed good seed set and viable hybrids were obtained. It is significant that while the species with the same chromosome number as that of *S. indicum*, like *S. alatum*, failed to cross, the species of different chromosomal group, viz. *S. laciniatum* and *S. prostratum*, exhibited a high degree of cross-compatibility. Conspicuously, it is indicative of different degrees of isolation mechanisms operating in different species of *Sesamum*. All the successful interspecific hybrids thus produced showed dominance of morphological features of wild species over the cultivated parent.

When the fertility status of the hybrids was examined, hybrids of the combination *S. malabaricum* × *S indicum* and both direct and reciprocal hybrids of *S. indicum* × *S. prostratum* and *S. indicum* × *S. laciniatum*, exhibited very high percentage of pollen sterility (Table 1). Hence, to study the cause of sterility cytological studies were carried out in the hybrids. Among the different hybrid combinations studied, three hybrids involving $2n = 26$ species showed a normal course of meiosis. The hybrid between *S. alatum* and *S. indicum* showed regular formation of 13 II at metaphase I and 13/13 anaphase I distribution, leading to high pollen fertility (Table 1). Similarly, both direct and reciprocal hybrids involving *S. indicum* and *S. malabaricum* exhibited complete genome homology and a regular course of meiosis till tetrad formation (Figure 1). Despite the normal meiosis in both direct and reciprocal crosses, pollen stainability revealed fertility differences between the two. The hybrids with *S. malabaricum* as ovule parent showed a

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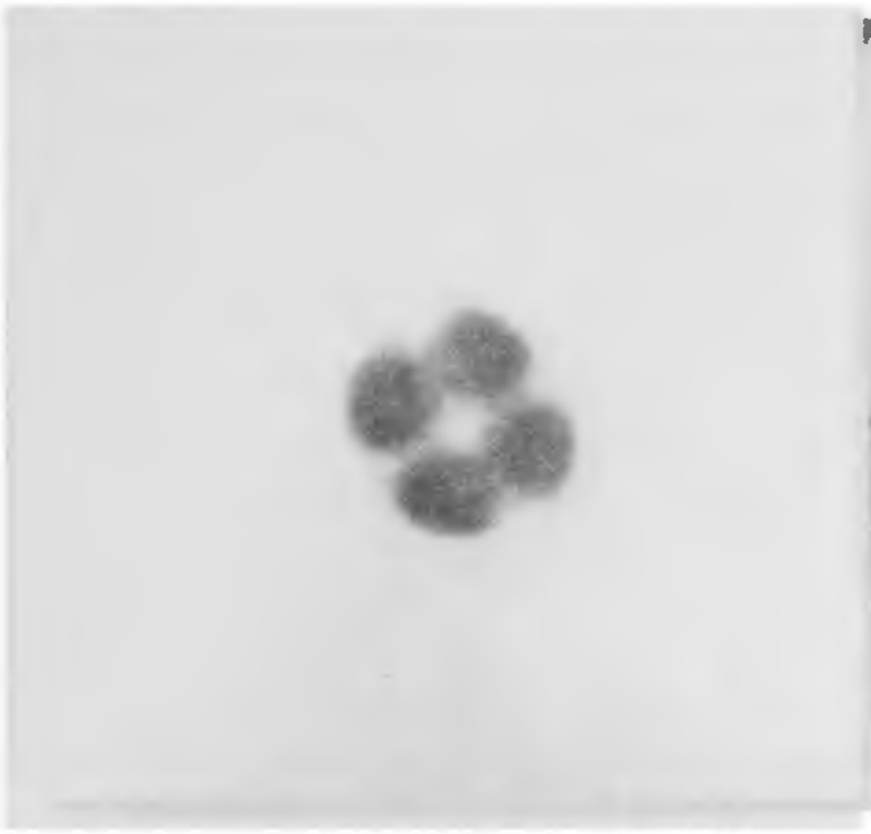

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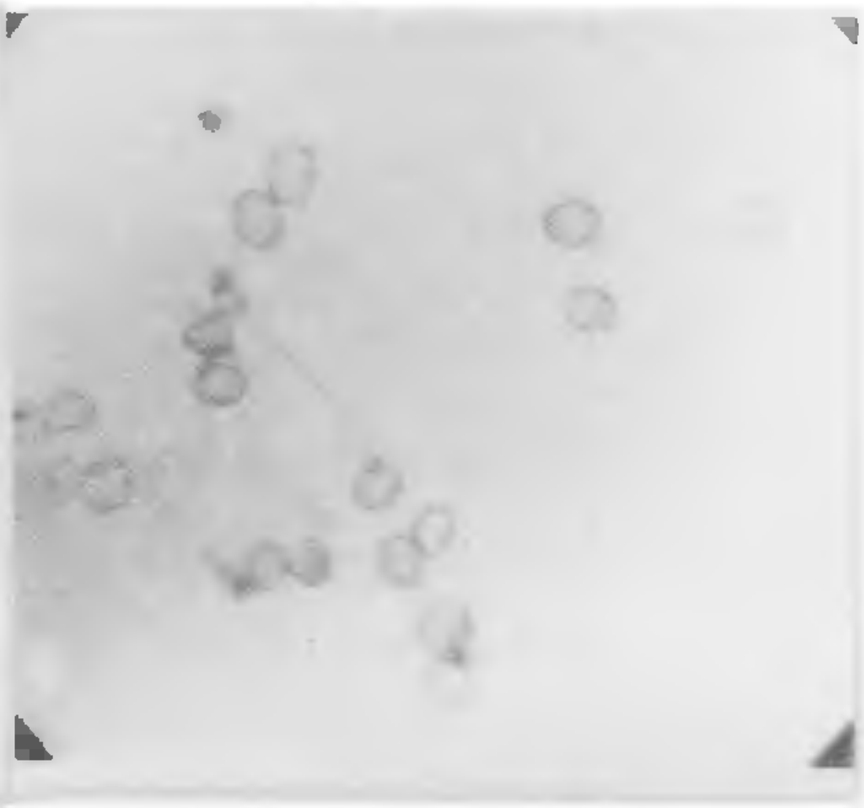

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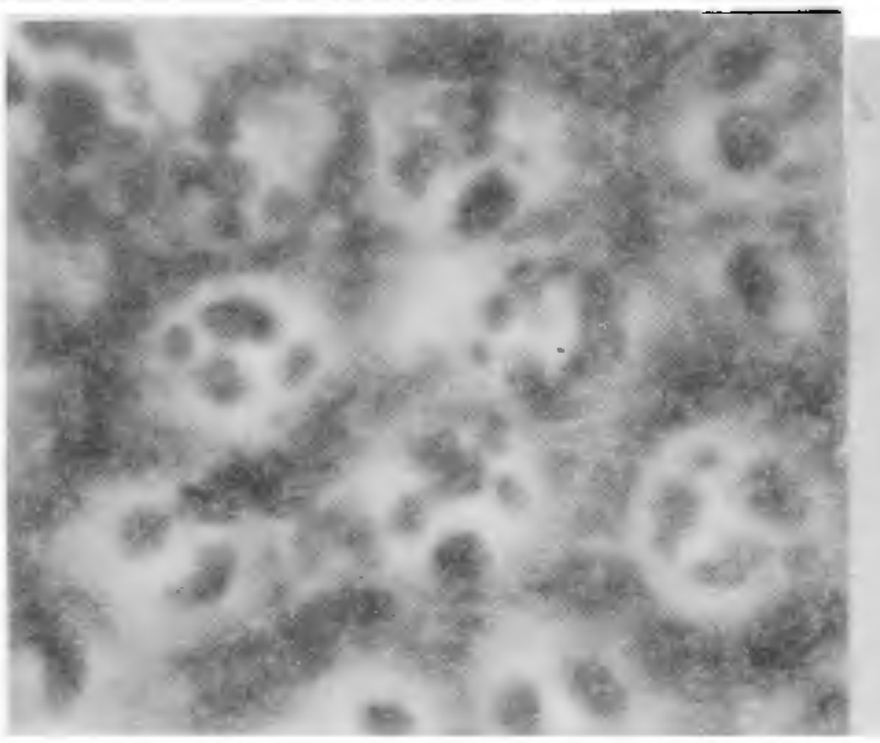

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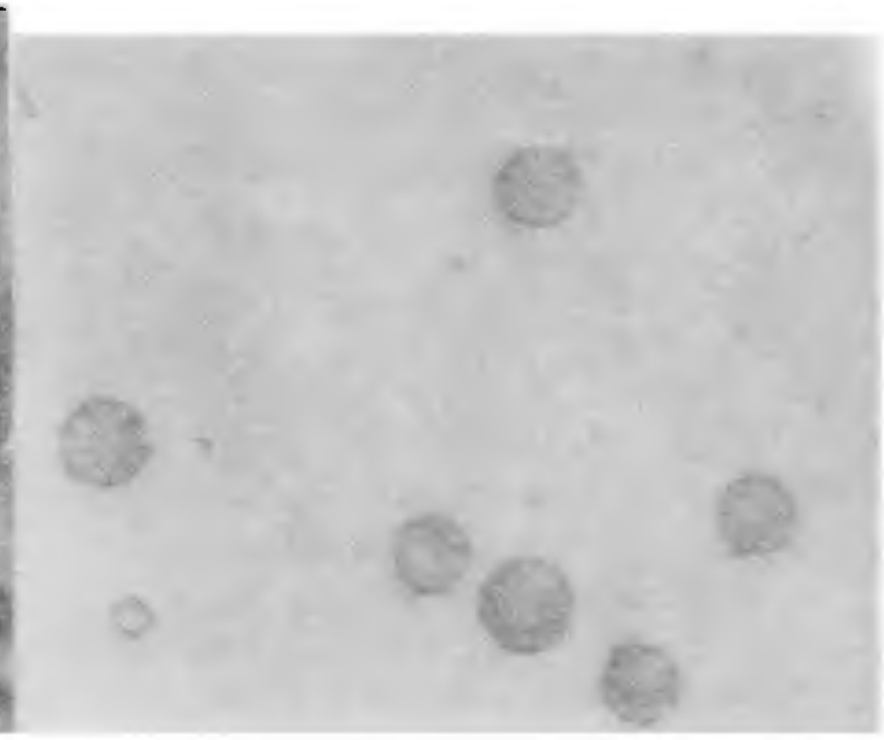

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Figure 1. Normal tetrad formed in *S. indicum* × *S. malabaricum* hybrids (× 450) **Figure 2.** Sterile pollen grains of *S. Malabaricum* × *S. indicum* hybrids (×120). **Figure 3.** Abnormal sporads formed in *S. indicum* × *S. prostratum* hybrids (×450) **Figure 4.** Sterile pollen grains of *S. indicum* × *S. laciniatum* hybrids exhibiting heteromorphism (×450) **Figure 5.** The male sterile plant

Table 1. Chromosome association and meiotic abnormalities in interspecific hybrids

Hybrid	F_1 2*n* no	Chromosome association* at metaphase I				Number of PMCs observed	Anaphase I separation	Sporad formation	Pollen* fertility percentage
		IV	III	II	I				
S alatum × *S indicum* (2*n* = 26) (2*n* = 26)	26	–	–	13	–	118	Normal	Tetrad normal	94 05
S. indicum × *S malabaricum* (2*n* = 26) (2*n* = 26)	26	–	–	13	–	143	Normal	Tetrad normal	73.06–79 85 (75 21)
S malabaricum × *S indicum* (2*n* = 26) (2*n* = 26)	26	–	–	13	–	109	Normal	Tetrad normal	6 15–23 25 (15 05)
S indicum × *S laciniatum* (2*n* = 26) (2*n* = 32)	29	–	0–1 (0 05)	2–9 (6 34)	11–25 (16 16)	97	Unequal separation like 17/12, 16/13, occasionally few laggards (1–3)	2–10 sporads	2 81–3 58 (3 15)
S laciniatum × *S. indicum* (2*n* = 32) (2*n* = 26)	29	–	0–1 (0.07)	2–9 (6 48)	11–25 (15 84)	121	Unequal separation like 17/12, 16/13, occasionally few laggards (1–3)	2–10 sporads	2 16–3 81 (3 22)
S. indicum × *S prostratum* (2*n* = 26) (2*n* = 32)	29	–	0–1 (0 08)	2–8 (6 06)	13–25 (16 66)	106	Unequal separation like 17/12, 16/13, occasionally few laggards (1–8)	2–10 sporads	2 42–3 09 (3 06)
S prostratum × *S indicum* (2*n* = 32) (2*n* = 26)	29	–	0–1 (0 01)	2–8 (5 98)	13–25 (16 80)	102	Unequal separation like 17/12, 16/13, occasionally few laggards (1–8)	2–10 sporads	2 20–3 15 (2 93)

*Values in the parentheses indicate mean values

high amount of pollen sterility (76.75–93.85%), whereas the reciprocals showed fairly good pollen fertility (73.06–79.85%). However, the pollen grains were identical in shape (Figure 2). Hence, the sterility observed in the hybrids with *S. malabaricum* as ovule parent was presumed to be due to cytoplasmic differences as there were no chromosome irregularities. This is indicative of interaction of the cytoplasm of *S. malabaricum* with the genome of *S. indicum* resulting in male sterility. Similar type of sterility observed in the species crosses of different crop species was successfully used in the development of alloplasmic lines and is being utilized in commercial hybrid seed production of sunflower, maize, *Sorghum*, *Brassica*, etc.[7–10].

The remaining four combinations involving *S. indicum* and the two 32-chromosome species showed irregular chromosome association at metaphase I, such as the formation of trivalents and univalents in addition to normal bivalents. In respect of anaphase I separation, abnormalities such as unequal separation and laggards were observed (Table 1). As a result, sporads of two to ten cells were formed, leading to high percentage of pollen sterility, and the pollen grains exhibited heteromorphic nature (Figures 3 and 4). Hence, the pollen sterility was concluded to be due to chromosomal abnormalities in the hybrids since the two species crossed possessed different chromosome numbers and the chromosomes were only partially homologous[6, 11].

It would be important to mention that the present investigation has thrown much light on the cytoplasmic genic male sterility in sesame for the first time. The sterile hybrids of *S. malabaricum* × *S. indicum* (Figure 5) were backcrossed to the cultivated parent and are being evaluated in backcross generations. The results obtained so far have indicated that the degree of male sterility increased by the backcross (unpublished result). After five to six substitution backcrosses, male sterility will be almost complete and stable. Intensified efforts are being made to isolate highly pollen-sterile but female-fertile lines by enhancing the expression of pollen sterility in each backcross generation by appropriate selection of sterile plants and backcrossing to *S. indicum* as pair crosses. Thus, the present study was successful in identifying for the first time an appropriate gene–plasmon combination in *S. malabaricum–indicum* hybrid that would result in alloplasmic sterile line in sesame lending for commercial exploitation.

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Is there dimorphism for style lengths in monoecious figs?

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Contrary to the expectation, style lengths of flowers of seven tropical monoecious fig species exhibited normal distribution with a single mode; none of the species showed the expected bimodal distribution. In four of the species studied, nearly 100% of the flowers in a syconium had styles shorter than the mean length of the ovipositor of their pollinator wasp, indicating that the wasps can potentially usurp a greater proportion of the flowers than is generally thought. Thus, our results do not support the belief held for almost three decades that using style length as a strategy, figs can guard their flowers against complete depredation by wasps. The style lengths showed 3–4 times greater variation compared to the ovipositor length of their pollinator wasp. We suggest this to be a consequence of the evolutionary conflict between the fig and the pollinator over the allocation of flowers to wasp production and to seed production.

FOR almost three decades the interaction between figs (*Ficus* spp. Moraceae) and their species-specific pollinating wasps (Agaonidae, Hymenoptera) has been cited as one of the perfect examples of plant–pollinator mutualism[1–3]. The flowers of figs are enclosed in an urn-shaped inflorescence, the syconium. The pollen-laden female wasps enter the receptive syconium through a specialized opening, the oestiole. These wasps can reproduce only within the syconium by ovipositing in the ovaries, on which the wasp larvae feed. It was believed that monoecious figs bear two distinct kinds of female flowers, those with short styles into which wasps can lay eggs and those with long styles into which they cannot as their ovipositor does not reach the ovary; the latter,

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