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Development of an effective cytoplasmic genetic male sterility system through conventional breeding in safflower in India

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Abstract
The work on development of cytoplasmic genetic male sterility (CMS) system through conventional breeding was initiated in 1993 at the Directorate of Oilseeds Research, Hyderabad, India. The wild species *Carthamus oxyacantha* was identified as the source of male sterile cytoplasm. Using *oxy*-cytoplasm several spiny, non-spiny and sparsely spiny cytoplasmic male sterile lines (A-lines) were developed. Simultaneously, 77 restorer lines (R-lines), which could restore 100% male fertility on various CMS lines were developed. Through substitution backcrossing of putative CMS lines with cultivated safflower followed by sib crossing, near isogeneic maintainers (B-lines) that could maintain 88-100% male sterility in CMS lines were developed. The experimental CMS-based hybrids recorded 38 to 45% higher seed yield than the elite high yielding cultivars under dryland conditions in Vertisols. Testing of stability of maintainer lines in maintaining male sterility in diverse growing environments is currently under progress. With the availability of an effective CMS system, development of CMS-based public hybrids and their large scale commercial seed production are now feasible in India.

Key words: Cytoplasmic genetic male sterility

Introduction
Safflower is one of the major edible oilseed crops grown in winter season in India. The first safflower hybrid released in India for commercial cultivation in all safflower growing regions was based on genetic male sterility system (Anjani, 2000). To overcome the inherent problems associated with genetic male sterility system, the work on development of cytoplasmic genetic male sterility (CMS) system through conventional breeding was initiated in 1993 at the Directorate of Oilseeds Research, Hyderabad, India. Wide hybridization was attempted between the cultivated species (*Carthamus tinctorius* L.) and the two indigenously collected wild species namely, *C. oxyacantha* and *C. lanatus* (Anjani et al 1999) and four exotic species *viz., C. glaucus, C. lanatus, C. creticus* and *C. turkestanicus* in order to search for sources of male sterile cytoplasm among wild species. One of the indigenous collections of *C. oxyacantha* was identified as a source of male sterile cytoplasm (Anjani 2005). The genetic studies confirmed that the male sterility was due to interaction between cytoplasm of *C. oxyacantha* and a recessive nuclear allele from *C. tinctorius,* and the genotype of male sterile plants was *rfrf* with *oxy*-cytoplasm (Anjani 2005). Chromosomal imbalance or abnormalities were not observed in the interspecific F_1 between *C. oxyacantha* and the cultivated species as both the species were cross compatible and having same chromosome number (2*n*=24). Natural hybrids between these species could not be found due to great gap in their vegetative period and flowering time or phenology. With this background, this paper reports the success made in development of an effective CMS system in safflower through simple conventional breeding techniques.

Materials and Methods
The experiments were conducted at the research form of the Directorate of Oilseeds Research, Hyderabad, India from 1993 to 2007. Sowings were under taken during the 1^{st} and 2^{nd} week of October every year. The male sterile plants identified in BC_1F_2 of (*C. oxyacantha* x *C. tinctroius*) x *C. oxyacantha* were backcrossed repeatedly to the recurrent parent and the progeny generations were evaluated for male sterility percent. The backcross progeny were simultaneously advanced through sib crossing. In each backcross and sib cross generation, the

sterile sib and backcross progenies predominantly resembling cultivated species were selected. The recurrent parent plants involved in backcross were maintained through individual plant self-pollination. The fertile plants involved in sib crossing were maintained through self-pollination. All the experiments were conducted under white pollination-net to avoid pollen contamination by honeybee and other insects. The flower buds prior to pollination were covered with butter paper bags as an additional safeguard measure.

Male sterility was assessed by visual observation for pollen presence as well as under microscope. The high combining cross combinations (F_1 hybrids) were evaluated for seed yield and agronomic traits in a randomized block design with three replications along with the released hybrid DSH 129 and varieties 'A1', 'Manjira' and 'NARI-6' under rainfed conditions in Vertisols. In two years, 348 restorer lines were evaluated in an augmented block design along with three check varieties namely, 'A1', 'Manjira' and 'NARI-6'. The data were analyzed using INDOSTAT statistical software.

Results and Discussion

CMS-System: Pollen production was absent in cytoplasmic genetic male sterile (CMS) lines. There was no partial male sterility in CMS lines. All flower heads in a male sterile plant were completely male sterile and were not reverted to fertility. Flower morphology and seed production were not affected by male sterility. Honeybee made equal visits to sterile and fertile plants in the experimental plots. Seed set and production in male sterile plants were normal indicating female fertility. The *per se* yield performance of male sterile plants was two third of that of male fertile plants. The undesirable traits like late maturity, very small seed, black and brown colour seed, very small capitula and seed shattering that were introgressed from *C. oxyacantha* in the backcross and sib cross progenies were gradually eliminated through meticulous selection for male sterile progenies predominantly resembling the cultivated species while maintaining the desirable traits like basal branching, high number of branches and high number of capitula intogressed from *C. oxyacantha* in CMS lines. Thus spiny, sparsely spiny and non-spiny CMS with different flower colours were developed. Absence of male sterile plants in F_1 generations of crosses between CMS lines and genotypes of the cultivated species has further confirmed recessive nature of male sterility.

CMS lines were repeatedly backcrossed to selected recurrent parents as well sib crossed to fertile counter parts in backcross generation in order to isolate maintainer sources. Sharp increase in male sterility per cent was noticed in backcross and sib cross progeny generations from 62 to 100% in the last six years. The variability and increase in male sterility percent probably resulted from the disruption of cross-talk between genomic and cytoplasmic factors as a result of increased genomic proportion of *C. tinctorius* and proportionate decrease of *C. oxyacantha* nuclear genome after each backcross (Pallavi and Anjani 2006). It was evident that there was a progress towards homogeneity for male-sterility trait following each substitution backcrossing which caused 100% male sterility in progenies of crosses involving certain maintainer sources. These maintainer sources maintained 100% male-sterility in *oxy*-CMS lines. Sometimes reduction of male sterility from 100 to 88% was observed in some case of maintainers, this may be because of frequent change of mitochondrial genome in CMS lines that interact with the male sterility conferring nuclear allele from maintainer. Hence, testing of maintainer sources under diverse environments is currently being taken up.

The F_1s of a crosses between CMS lines and diverse genotypes belonging to *C. tinctorius* were examined for male sterility segregation per cent. The genotypes giving 100% fertile progeny in F_1 were selected as restorers. So far 77 high combining and high yielding restorer lines were identified.

Experimental hybrids: The heterosis for seed yield over the better parent has ranged from 35 to 120% in F_1 combinations. The experimental CMS-based hybrids recorded 38 to 45% higher seed yield than the elite high yielding cultivars under dryland conditions in Vertisols. The exotic cytoplasm in CMS parent has not caused floral abnormalities and male sterility in hybrid population. It has not exerted any detrimental effect on yield and other quantitative and qualitative traits of hybrid. Seed set was normal and seed shattering was absent in hybrids. The hybrids matured in 120 to 130 days and possessed 2 to 3 % higher oil content than the varieties.

Conclusion

The CMS-lines continued to be male sterile, maintainer sources could maintain high male sterility in CMS lines, fertility restoration in CMS lines was 100% by restorer sources, exotic cytoplasm in CMS parents did not exert detrimental effects, heterosis manifestation was high and seed set was normal in hybrids. These positive features ascribed the CMS system developed at the Directorate an effective and feasible system to produce CMS-based hybrids and their commercial seed production in large scale. This is the first CMS system developed in safflower in India.

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