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Male Sterility and Hybrid Development in Sorghum

Karthik R. and Gaganashree K. P.

[1]PhD scholar, Department of Genetics and Plant Breeding, University of Agricultural Sciences, Dharwad -580005.

*Corresponding Author: karthikrj1611@gmail.com

Sorghum, a member of the Poaceae family, possesses a chromosome number of 2n=20 and ranks as the fourth most significant cereal globally, following wheat, rice, and maize. Flourishing in tropical and subtropical regions, it is commonly referred to as Jowar in India. Sorghum cultivation spans three distinct seasons: Kharif, which aligns with the monsoon period from June-July to September; Winter, extending from September to February, encompassing approximately 35% of the national sorghum acreage; and summer sorghum, cultivated from February to June with the aid of irrigation. While *Rabi* sorghum grain holds high esteem as a staple food, *Kharif* sorghum primarily serves industrial purposes such as poultry and animal feed, as well as for alcohol distillation. Despite predominantly being a self-pollinating crop, with the development of new varieties being a natural means of crop enhancement, there exists a notable degree of outcrossing in sorghum, ranging from 5 to 15%, influenced by factors such as wind direction, genotype characteristics, and humidity. This outcrossing potential renders sorghum amenable to population enhancement and hybridization strategies, leveraging heterosis for improved agricultural outcomes.

Male sterility in sorghum

The initial documentation of male sterility in sorghum was provided by Stephens and Holland in 1954, wherein they identified the A1 CMS source in sorghum within the F2 population resulting from the cross between Double Dwarf Yellow Sooner Milo and Texas Blackhull Kafir. In this cross, twenty-five percent of the plants exhibited male sterility in the F2 generation when milo served as the female parent. The male-sterile segregants from this cross produced male-sterile hybrids upon crossing with the kafir parent and fully fertile hybrids when crossed with the milo parent. This discovery indicated that kafir could function as a maintainer source of CMS. The hypothesis arose that the milo parent possessed a male sterility-inducing cytoplasm alongside dominant nuclear genes for pollen fertility, while the kafir parent harboured a normal (fertile) cytoplasm coupled with recessive nuclear genes for male sterility. All progenies resulting from the milo × kafir cross carried milo (sterility-inducing) cytoplasm, yet those inheriting homozygous recessive genes from the kafir parent exhibited male sterility. Male-sterile plants from the milo × kafir cross were utilized as females in successive backcrossing with kafir as the male parent. Following seven backcrosses, the entire genome of kafir was transferred into the milo cytoplasm. This process yielded two morphologically similar versions of the combined kafir (CK 60) parent: a male-sterile combined kafir (CK 60A) and a male-fertile combined kafir (CK 60B). The male-sterile lines were designated as A lines, while their maintainer lines were labelled as B-lines.

Diversification of male sterility

Widespread adoption of a single male sterility cytoplasm source for hybrid production could lead to adverse consequences, such as increased susceptibility to pests and diseases, as seen in instances like CMS-T of maize becoming vulnerable to southern leaf blight and A_1 of pearl millet. Therefore, maintaining diverse sources of male sterile cytoplasm for hybrid development is crucial. Apart from the A1 source, numerous other cytoplasmic sources, including A2, A3, A4, Indian A4 (A4M, A4VZM, A4G), A5, A6, 9E, and KS cytoplasm, have been identified in sorghum (Reddy *et al*., 2010), each distinct from one another and from the A1 CMS system. The development of new male sterility cytoplasm sources can be achieved through techniques such as mutation breeding or hybridization programs aimed at transferring the genetic factors for sterile cytoplasm into a new genetic background.

Development and maintenance of A-, B- and R-lines

Improved breeding lines, released varieties, and landraces are viable options to serve as pollen parents or pollinators. Lines that yield fertile F1 offspring when crossed with A-lines are termed

restorer lines or R-lines. A variety of R-lines can be cultivated through R×R crosses, followed by the identification of the most suitable line within the segregating population, or alternatively, directly selected from the available germplasm resources for the crop. To utilize any line as a male parent (R-line), it is crucial to ascertain its restorability. This assessment can be conducted by enclosing the hybrid produced by specific R-lines (known as testcrosses) in bags i.e. Bagging test will be carried out, wherein 4-6 panicles are covered with paper bags before anthesis, and seed-set is observed after 2-3 weeks (similar to enclosing panicles in selfing bags) (Fig 1). Line with complete restorability can be used as pollen parent for hybrid seed production (Murty *et al*., 1994). Based on the Testcross results following inferences may be drawn (Reddy *et al*., 2008)

- Testcrosses showing no seed-set on all bagged panicles, indicating maintained male sterility in these hybrids. The corresponding pollinator is categorized as a maintainer, non-restorer, or B-line, potentially serving as a source for a new A-line.
- Testcrosses with complete seed-set on all bagged panicles, suggesting the pollen parents are potential restorers or R-lines, suitable for producing hybrids.
- Testcrosses displaying partial seed-set on all bagged panicles, leading to rejection of the corresponding male parents from the program, as they neither function as restorers nor maintainers.
- Testcrosses with full seed-set on some bagged panicles and no seed-set on others. The corresponding pollen parent of such hybrids is considered segregating for fertility restoration or sterility-maintainer genes. Typically, such parents are not further pursued in hybridization programs due to the additional work required to fix the genes for fertility restoration or sterility.

Genetic diversity, the individual performance of the lines, and general combining ability (GCA) serve as the primary criteria for selecting parents for A and R line development. In sorghum, shorter lines (typically 1.25-1.75 m) demonstrating high yield potential and sterility-maintenance capability are chosen for conversion into male-sterile lines. Conversely, taller lines (usually 1.75−2.50 m) exhibiting restorer reactions are selected as R-lines. The maintainers identified through the bagging test harbor recessive genes for fertility restoration but possess a normal cytoplasm. These selected B-lines can be crossed with any male-sterile line. The resulting F1 hybrids undergo repeated backcrossing with the respective maintainer lines for six or seven generations, using the corresponding maintainer lines as recurrent parents until male-sterile lines with an appearance identical to the recurrent B-line parent are obtained.

The A-lines thus obtained may be alternately sown with the respective B-lines, and the pollen (bulk) from the corresponding B-lines is collected in separate bags and applied over the male-sterile plants. Before pollination, these male-sterile panicles should be bagged as in selfing to prevent outcrossing with pollen from unwanted parents. Similarly, the B-lines should be self-pollinated. The seed bulked within the A-lines will form the A-line seed, while the seed bulked within the B-lines will form the B-line seed, thus maintaining the A- and B-lines (Reddy *et al*., 2008).

Fig 1: Bagging test for ascertaining restorability of pollen parent

Hybrid development

The process of sorghum hybrid production includes crossing between A (male sterile) line and R (restorer) line, further harvesting seeds from male

sterile line denoted as hybrid. For commercial hybrid seed production in sorghum usually two rows of R lines will be maintained for every four rows of male sterile parent to ensure the complete seed set. and maintenance of A line is achieved by crossing of male sterile plants to B (maintainer) line. (fig 2) Enhance in seed production of B line and R line is achieved by selfing and growing in isolation.

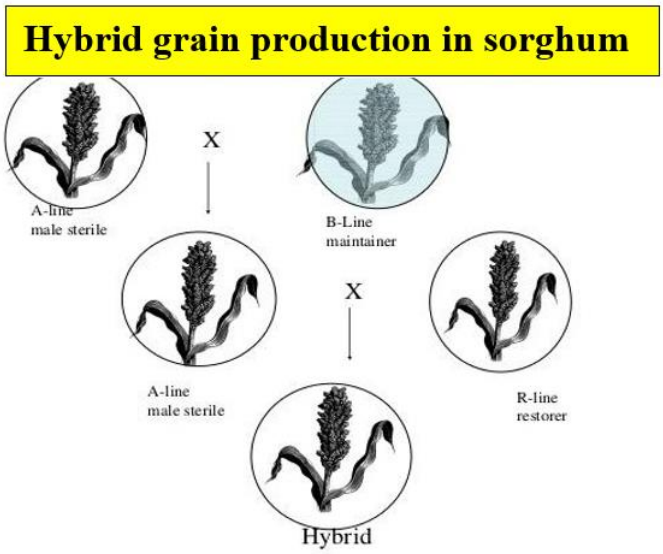

Fig 2: Hybrid seed production in sorghum using 3-line system (ABR)

Conclusion

Higher yields and improved crop resilience offered by hybrid varieties can translate into increased income for farmers. Many hybrid varieties are bred to be more resistant to pests and diseases. Male sterility Facilitates the production of large quantities of hybrid seeds. Allows for the efficient utilization of resources by eliminating the need for emasculation (removal of male reproductive organs) in the female parent. Enhances genetic purity and uniformity in hybrid seed production. Overall, male sterility is a crucial technique in hybrid seed production, enabling the development and dissemination of high-yielding and genetically superior crop varieties.

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