

Development of male-sterile lines in sorghum

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Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth important cereal crop in the world after wheat, rice, maize and barley. Of late, it has emerged as 'fuel' crop in addition to its food, feed and fodder utilities. Sorghum is predominantly a self-pollinated crop and development of new 'varieties' is a natural option for crop improvement. However, there is 5 to 15% outcrossing in sorghum depending upon the wind direction, nature of genotype, and humidity (House 1985), which makes it amenable for use in population improvement and hybrid development to exploit the heterosis. Discovery of genetic male sterility (GMS) and cytoplasmic-nuclear male sterility (CMS) facilitated the application of recurrent selection procedures and hybrid cultivar development methods, respectively, in sorghum improvement. In this article, male-sterile line development using CMS in sorghum is described.

Cytoplasmic-nuclear male sterility

CMS has been used extensively to exploit heterosis in hybrid development on a large scale for commercial cultivation since 1960s. In the pre-hybrid era of early 1960s, the average sorghum productivity was 0.49 t ha⁻¹ in India, 0.66 t ha⁻¹ in China, 0.76 t ha⁻¹ in sub-Saharan Africa, 1.48 t ha⁻¹ in Australia, and 2.8 t ha⁻¹ in the USA, respectively. In the USA, Northern and Central America, where commercial hybrids were exploited, there was a 40% increase in productivity from early 1960s to early 1990s. A similar trend was noticed globally. The productivity increases were 47% in China and by 50% in India from early 1960s to early 1990s. However, the productivity remained static in Africa from 1960s to early 1990s (FAO 1960–1996), which can be directly attributed to non-exploitation of heterosis through hybrid development in Africa.

Origin of CMS

CMS is a physiological abnormality, resulting from a disharmonious interaction between the cytoplasmic factors (now widely identified as mitochondrial genetic factors) and nuclear genetic factors leading to the production of degenerated or non-viable pollen grains or non-dehiscent anthers with or without functional

pollen grains. Understandably, this disharmonious interaction is likely to be more pronounced in populations incorporating divergent sources of cytoplasm and nuclear genes (Reddy et al. 2003). Sorghum is no exception to this. For example, the A₁ CMS source in sorghum was identified in the F₂ population of cross Double Dwarf Yellow sooner Milo × Texas Blackhull *kafir* by Stephens and Holland (1954), in which the milo inbred belongs to *durra* race and is from Sudan and the Ethiopian border (Duncan et al. 1991), and the *kafir* inbred from Eastern Africa (House 1985). Twenty-five percent of male-sterile plants were observed in the F₂ generation of this cross if milo was the female parent. The male-sterile segregants from this cross produced male-sterile hybrids if crossed with the *kafir* parent and fully fertile hybrids if crossed with the milo parent. Thus, it was recognized that *kafir* could be used as a maintainer source of CMS. Since the progeny received the cytoplasm from the female, it was hypothesized that the milo parent had a male sterility-inducing cytoplasm and dominant nuclear genes for pollen fertility, whereas the combine *kafir* parent contained a normal (fertile) cytoplasm but recessive nuclear genes for male sterility. All progenies of the milo × *kafir* cross contained milo (sterility-inducing) cytoplasm, but those that also inherited the homozygous recessive genes from the *kafir* parent were male sterile. The male-sterile plants in the milo × combine *kafir* cross were used as females in repeated backcrossing with *kafir* as the male parent. At the end of seven backcrosses, the entire genome of *kafir* was transferred into the milo cytoplasm. This resulted in two morphologically similar versions of the combine *kafir* (CK 60) parent: a male-sterile combine *kafir* (CK 60A) and a male-fertile combine *kafir* (CK 60B). The male-sterile lines are designated as A-lines and their maintainer lines as B-lines.

Development of new hybrid parents (A-, B- and R-lines)

The lines that produce fertile F₁s when crossed with A-lines are called restorer lines or R-lines. The development of hybrid parents involves two steps: (1) identification of potential B- and R-lines; and (2) development of A-lines and R-lines.

Identification of B- and R-lines: Improved breeding lines, named/released varieties and landraces from the pollinator collection are the sources that can be used as pollen parents or pollinators. The hybrids obtained by crossing these pollinators with a male-sterile line, the testcrosses, are evaluated for the sterility maintenance or fertility restoration in them (Murty et al. 1994). This evaluation is usually sown in small plots (one or two rows of 2 m length). Examination of anther morphology may be a basis for classifying the hybrids as male-sterile or male-fertile; but it is not a sure way. A more reliable method is the bagging test, ie, covering 4-6 panicles with a paper bag before anthesis, and observing the seed-set after 2-3 weeks. (Similar to enclosing the panicles in selfing bags). The testcrosses are of the following four types:

1. Testcrosses exhibiting absolutely no seed-set on all the bagged panicles, ie, male sterility was maintained in these hybrids. The corresponding pollinator is classified as a maintainer or non-restorer or B-line. This could serve as a source of a new A-line.
2. Testcrosses with complete seed-set on all bagged panicles. The corresponding pollen parents are classified as potential restorer or R-lines. They can serve as male parents to produce hybrids.
3. Testcrosses with a partial seed-set on all the bagged panicles. The corresponding male parents are rejected from the program as they serve neither as restorers nor as maintainers.
4. Testcrosses with a full seed-set on some bagged panicles and no seed-set in others. The corresponding pollen parent of such a hybrid is said to be segregating for fertility-restoration or sterility-maintainer genes. Usually, such parents are not pursued further in a hybridization program, as they involve additional work of fixing the genes for fertility restoration/sterility.

Development of new A- and R-lines: Three criteria are used in the selection of parents for this purpose: genetic diversity, the *per se* performance of the lines, and the average performance of a line in crosses with other lines [called general combining ability (GCA)]. Experience in sorghum has shown that parents of diverse origin produce highly heterotic hybrids. It has also been found that *per se* performance of parents is positively correlated with the performance of the hybrids (Murty et al. 1994). Further, the general combining ability is more important than specific combining ability (the deviation from performance predicted on the basis of general combining ability) in sorghum. Further, shorter (usually 1.25-1.75 m) and high-yielding lines with sterility-maintenance ability are chosen for conversion into male-sterile lines. Taller lines (usually 1.75–2.50 m) with restorer reaction are chosen as R-lines.

The maintainers identified through the bagging test possess recessive genes for fertility restoration/ sterility maintenance but have a normal cytoplasm. The selected B-lines can be crossed with any recognized male-sterile line. The resulting F_1 s and the corresponding maintainers are sown alternately in small plots, and the hybrids are backcrossed repeatedly with the respective maintainer lines for six or seven generations using the corresponding maintainer lines as recurrent parents until male-sterile lines with appearance identical to the recurrent B-line parent are obtained. It is important that plant-to-plant crossing should be attempted in the backcrossing phase. This involves crossing individual male-sterile plants with individual plants of the recurrent parent that are morphologically similar to each other. This plant-to-plant method is useful to select out the partial sterility maintainers from the program. Also, it enables faster realization of A-lines with morphological traits similar to the maintainer line.

The A-lines thus obtained may be sown alternately with the respective B-lines, and the pollen (bulk) from the respective B-lines collected in separate bags may be put over the male-sterile panicles with emerged stigmas. The bags should be shaken thoroughly. 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 selfed. The seed bulked within the A-lines will form the A-line seed. The B-line seed bulked within the line will form the B-line seed. Thus, A- and B-lines are maintained. It should be remembered that rouging should be carried out before selfing/pollination of A-/B-lines.

Once uniform A- and B- lines are produced, the stability of the male sterility in the A-lines may be evaluated by evaluating them in areas where the temperature at flowering reaches 42°C or more. Unstable A-lines become fertile at this temperature.

Seed production of A-, B- and R-lines

Small-scale production: R-line seed (identified through the bagging test of testcrosses) may be produced by sowing the seed in a plot of the desired size and selfing the plants after rouging out the off-types before and at flowering. Bulk harvesting of true-to-type panicles may be done. A plot of two rows of 4 m length, if maintained properly, may give about 2.0–2.5 kg seed.

Production of A- and B-lines involves several operations:

1. Sow A- and B-lines in the plot side by side. Usually, for every four rows of A-line, two rows of B-line are sown.
2. Carry out rouging regularly in the A-lines and B-lines before and during anthesis. Apart from off-types, pollen ‘shedders’ can be a problem in the A-lines [a pollen shedder is a fertile plant in the A-line that results from a breakdown of male sterility; in practice, however, B-line (fertile) plants which appear in the A-line plot due to mechanical mixing are also referred to as shedders]. These should be removed by inspecting the field everyday during anthesis.
3. Prune the florets of A-line with protruded anthers/stigmas at the tip of the panicles, and pull kraft paper bags over the panicles with the date of bagging recorded on them. Carry out a similar operation on the B-line.
4. After 4–6 days, collect pollen from the B-line panicles into the same bags used for selfing, and put these bags carefully over the respective A-line panicles, by slightly bending the A-line, and shake the panicles along with the pollen bags by holding the mouth of the bag tightly wrapped around the peduncle. Each pollen bag may be used to pollinate 2–3 panicles of the same A-line.

5. Cover the pollinated panicles with the same pollen bag or with a new one. The bag should carry information on the date of the first bagging and pollination, and an A×B mark indicating that it was pollinated by a B-line.
6. Pollination of A-lines with B-lines may be repeated again after the 6th or 7th day in order to pollinate all the florets in the entire panicle.
7. B-line panicles should be selfed by bagging after using their pollen to pollinate the A-line panicles.
8. Take out the bags 15–20 days after pollination/selfing, and staple them over the peduncle below the base of the panicles, as in selfing.
9. Rogue out plants at the time of harvest, and bulk harvest the panicles in A-lines and B-lines separately and label them clearly.

Precautions: Periodic replacement of damaged bags is essential.

Large-scale production: Large-scale production of A-, B- and R-lines is usually taken up in isolation plots (Chopra 1982).

1. **Production of R-line:** R-line is produced in an isolation field separated from other sorghum fields by at least 300 m. Periodic rouging of the off-types is essential. Bulk harvesting is done by taking true-to-type panicles.
2. **A- and B-lines:** Production of A- and B-lines is done by growing the A-line in four rows alternating with the corresponding B-line in two rows. Across all the rows in the entire field, it is recommended that a strip of 1 m length should be sown with the B-line. This is useful in providing pollen to the A-line panicles at the end of the rows. Rouging of the off-type plants and pollen shedders should be done during anthesis everyday. Open pollination by wind will ensure seed-set on the A-lines. Self-pollination takes place in the B-lines. Harvesting of A-line and B-line seed should be done separately. To avoid mechanical mixing, it is recommended that they should be harvested at different times, preferably one after the other.

Improving B-lines and A-lines

We have so far dealt with the procedure of developing A-lines from the B-lines identified from the pollinator collection through testcrossing. It is important to know the procedures involved in improving A- and B-lines in hybrid programs. It involves the following steps.

1. Identify the B-line(s) for improvement and the resistance source lines for stress factors or high yielding lines (depending on the objective) which may be fertility restorers/sterility maintainers.
2. Cross the B-line with the selected source line(s) and advance them to the F₂ generation.

3. Grow F_2 under the desired screening for resistance, and select for monogenic or oligogenic traits apart from resistance.
4. Grow selected F_3 progenies in head-to-rows under screening for stress factors of interest. Select plants with the desired combination of traits within the family selected for resistance and uniformity.
5. Testcross the selected segregants onto an A-line sown separately near the F_3 nursery under screening. Also self the selected segregants (pollinator) used in testcrossing.
6. Grow the testcross and the pollinator (F_4 s) in a block near the pollinator screening block. With experience, one can usually determine the male-sterile testcrosses by anther morphology at anthesis. Otherwise one should use the bagging test to identify male-sterile plants. Repeat step 4, ie, select families for resistance and select individual plants for crossing on the basis of agronomic desirability.
7. Backcross the male-sterile F_1 (A-line) panicles with pollen from the selected plants (as above) individual panicles as per the procedure outlines for pollination. The F_4 families selected for resistance should be used as pollinators. Harvest the backcrossed A-line panicles and selfed pollinators' panicles individually and pair them as per the pollination done.
8. Repeat steps 6 and 7 for six to seven generations. Care should be taken at every stage in the following areas: check male sterility on the basis of anther morphology and seed-set on a few panicles under bagging; also, always make plant-to-plant backcrossing, ie, the individual male-sterile panicles (2–3) selected for backcrossing should be similar in morphology to those individual plants of the pollinators selected for pollination.
9. At the stage when male-sterile lines resemble the respective maintainer lines and are uniform, they are called A- and B-lines. The B-lines may further be selected on the basis of their *per se* performance and resistance to the factors of interest.
10. Further selection of A-lines may depend on GCA tests for traits of interest. The selected A- and B-lines may thus be numbered with the year, followed by serial number and letters A or B to indicate male sterility or maintenance. For example, ICSA 95001 and ICSB 95001 indicate that these two represent one A and B pair, bred in the year 1995, and the line number is 1.
11. Maintenance of the selected A-lines is done as per the procedure outlined earlier.

The trait-based breeding approach followed at ICRISAT, Patancheru (1985–95) facilitated the use of lines of diverse origin and provided a range of male-sterile lines in varying genetic backgrounds. Resistance levels in each resistance group

vary from highly resistant to less susceptible. Grain yield level in these groups is compensated by resistance, and therefore they are on par with the best checks 296A/B, Tx 627 A/B or IC542/B 101 for grain yield and agronomic desirability. Efforts are underway for pyramiding resistance, assessing grain yield and grain characters of these lines to use them in hybrid development in a big way. Similarly, the ongoing programs (1999 onwards) on the race-specific and alternate (non-milo) CMS specific diversification of A-/B-lines is providing dividends in terms of increased diversity. By now 39 A-/B-lines with A₁ background belonging to different races and 46 A-/B-lines with A₂ background in different races have been developed by ICRISAT, Patancheru to thoroughly exploit the diversity for hybrid development. Efforts are underway at ICRISAT, Patancheru for utilization of A₃ and A₄ cytoplasm for further diversification of hybrid parents. Emphasis is also given on development of hybrid parents for post-rainy season adaptation.

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