

## Hybrid Sorghum Development Mechanisms to Enhance Production and Productivity

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**Abstract:** Estimating genetic gains in sorghum is necessary to assess whether the current rates of improvement will meet future production demands. In crop plants, the term “F1 hybrid” is usually reserved for agricultural cultivars derived from two different parent cultivars, each of which are inbred for a number of generations to the extent that they are almost homozygous. Crossing two genetically different plants produces a hybrid seed by means of controlled pollination. To produce consistent F1 hybrids, the original cross must be repeated for each season. The divergence between the parent lines promotes improved growth and yield characteristics, while the homozygosity of the parent lines ensures a phenotypically uniform F1 generation. Sorghum hybrid development involves development of parental lines based on a cytoplasmic male sterile system including the pollen parent (R-line) and seed parents (A- and B-lines). New parental lines are developed by recombining existing elite parental lines to create new breeding populations or by adding specific traits of interest to existing parental lines by crossing elite lines with donor parents. Male sterility has been generated in sorghum mostly through mechanical and genetic mechanisms, with chemical treatments used sparingly. Mechanical sterility production is limited to small-scale seed production, while genetic male sterility is restricted to certain germplasm. Large numbers of seed could be produced with a chemical hybridizing agent that was not limited by genotypes. To create new hybrid combinations, newly created parental lines are crossed with other elite parental lines or with each other. The evaluation of the parental lines used to create novel hybrids aids in determining the produce ability and prospective cost of goods, both of which have a direct impact on the commercial release of new hybrid items. The cytoplasmic male sterility system has been widely utilized to increase sorghum output by exploiting heterosis. To efficiently deploy male sterility inducing cytoplasm, restorers and lines those are suitable for conversion to male. The pollen parent (R-line) and seed parents (A- and B-lines) are developed as parental lines based on a cytoplasmic male sterile system in sorghum hybrid development. New parental lines are formed by combining existing elite parental lines to develop new breeding populations or by crossing elite lines with donor parents to add specific traits of interest to existing parental lines.

**Keywords:** Hybrids; Sorghum; Cytoplasmic male sterility; Genetic male sterility; R-line; A-line; B-line

### 1. INTRODUCTION

Sorghum [*Sorghum bicolor* (L) Moench,  $2n = 20$ ] is the fifth-highest produced cereal crop after maize, wheat, rice and barley in the world (FAO, 2021). It has recently become popular as a 'fuel' crop, in addition to its food, feed, and fodder uses. Sorghum is a cereal grain that is grown as a grain and forage crop in the subtropics and certain temperate regions. It is produced commercially as a hybrid crop in developed regions of the world, although it is still grown as an inbred variety in less developed places. Sorghum has a complete flower and is mostly self-pollinated, with natural out-crossing rates ranging from 0% to 26%, depending on the genotype and environmental conditions before to and during anthesis (Pedersen *et al.*, 1998), making it suitable for population improvement and hybrid generation to exploit heterosis. Sorghum is a C<sub>4</sub> cereal crop domesticated in Africa; it is adapted to water stress, low soil fertility and high temperature conditions. Sorghum is a staple crop for more than 500 million people in 30 sub-Saharan African and Asian countries (Ashok *et al.*, 2011), while it is primarily grown as feed grain in the developed world.

Sorghum is grown in harsh environments where other crops grow poorly, by farmers who are among the worlds poorest (Dalton and Zereyesus, 2013). Globally, when combined with other farming practices, the diffusion of well-adapted, improved seed has enhanced the productivity of major food

crops, including sorghum (Evenson and Gollin, 2003). However, sorghum productivity is constrained by different biotic and abiotic factors mainly drought and Striga (a parasitic weed) in the lowland and biotic stress in the highland and intermediate environments. The demand for improved varieties with both higher grain yield and farmer's preferred traits, primarily grain size and plant height is increasing due to the rapidly growing human population and changing standard of living. World population growth and development projected by 2050 will significantly increase the demand for food, feed, energy, chemicals, and bio-based products (Bruinsma J, 2009).

Hybrid technology could have the potential to increase productivity while retaining high biomass and large grain size. Sorghum hybrids have been grown by farmers in developed countries since the late 1950s after the discovery of a viable cytoplasmic male sterility system, allowing cost-effective hybrid production, and are increasingly being adopted in the developing world (Ashok *et al.*, 2011). In sorghum superiority of the  $F_1$ , or hybrid vigor, can result in a 30- 40% increase in grain yield, depending on the environment and the genotypes used (Reddy *et al.*, 2006). In addition to increasing yield, sorghum hybrid vigor has also been demonstrated to have increased yield stability over inbred lines, particularly in stressed environments (Reddy *et al.*, 2006). In sorghum improvement, the discovery of genetic male sterility (GMS) and cytoplasmic-nuclear male sterility (CMS) allowed the use of recurrent selection procedures and hybrid cultivar development methods, respectively.

Plant breeders must use controlled hybridizations to generate genetically segregating populations from which they can choose desirable genotypes in any crop improvement program. The methods used to create these hybrids are determined by each crop species' growth patterns and pollination preferences. Because most self-pollinated crops contain entire flowers, the breeder must first sterile the flower male before introducing pollen from another plant. Hand-emasculation, genetic male sterility, environmental conditioning, or chemical hybridizing agents are all used to create male sterility in these species (Poehlman and Sleper, 1995). Sorghum breeders have identified and developed a number of ways for cross pollination in order to facilitate breeding crosses and commercial seed production. The number of crosses to be made and the amount of hybrid varieties desired determine the approach a sorghum breeder uses. Hand- or plastic-bag emasculations (which limit anther dehiscence by physical removal or wetness, respectively) are useful if small quantities of hybrid seed are acceptable (Schertz and Clark, 1967).

Although genetic male sterility systems allow for large-scale seed generation and are commonly utilized in long-term recurrent selection programs, the number of germplasm segregating for genetic male sterility is restricted. Finally, a hot water treatment of the panicle can cause temporal male sterility of the entire panicle (Stephens and Quinby, 1939), although this method is not repeatable and is vulnerable to genetic and environmental variance. Cytoplasmic male sterility systems are used in commercial hybrid seed production (Stephens and Holland, 1954). While all of these strategies make hybridization easier, none of them is a foolproof method for sterilizing sorghum lines for hybridization.

Genetic gain in self-pollinated crops, including sorghum, can be enhanced through population improvement and hybrid breeding. Heterosis breeding has the potential to improve sorghum productivity by 40%, which will reduce the yield gap between potential and actual yield (Atokple, 2003). However, genetic gain in sorghum has stagnated because of limited genetic variation due to the autogamous nature of the crop, and a lack of effective and reliably cheap systems to produce hybrid seed. Sorghum has perfect flowers that are highly autogamous, with a low level of out crossing (20%) (Reddy *et al.*, 2008). The flowers are small-sized and numerous, necessitating the use of new technologies for selective sterilization of the pollen grain to facilitate artificial cross-pollination for hybridization.

There are several methods, broadly categorized as physical, genetic and chemical, used to circumvent autogamy to allow cross-fertilization in sorghum. Physical or mechanical emasculation is the most widely used method, but it is more applicable where a limited amount of hybrid seed is required for breeding or genetic analyses (Yahaya *et al.*, 2021). Thus, the physical method is not appropriate for producing large amounts of hybrid seed. The most common genetic approach to control autogamy in sorghum is the use of a cytoplasmic male sterility (CMS) technique. The CMS system has been used sparingly in Africa because it is difficult to develop, maintain and use the three lines required for hybrid seed production using the CMS technique (Reddy *et al.*, 2008). Also, there is a limit to

germplasm accessions that can be used for generating all possible heterotic cross combinations due to strict requirements for maintaining the three-line CMS system.

Chemical hybridization agents, also called gametocides, are synthetic chemicals that can induce temporary male sterility for developing breeding populations or producing large quantities of hybrid seed in sorghum (Sleper and Poehlman, 2006). Chemical hybridization agents (CHAs) can readily overcome the limitations posed by the physical and genetic techniques. The use of CHAs is time and labor effective and allows for the generation of more hybrid seed for developing test populations for genetic studies, combining ability analyses and backcross breeding programs. The CHAs induce physiological abnormalities in the male gamete that prevent healthy pollen development and shedding and, ultimately, reduce pollen viability (Sleper and Poehlman, 2006). Chemical hybridization agents prevent the flower from self-pollination, allowing for effective cross-pollination if the female stigma is receptive and compatible with foreign pollen. Various chemical hybridization agents have been used for hybrid seed production in wheat (Parodi and Gaju, 2009), sorghum (Boerman *et al.*, 2019), coriander (Kalidasu *et al.*, 2009), rice (Efisue *et al.*, 2010) and sunflower (Razaq *et al.*, 2016).

Commercial production of hybrid seed in sorghum became feasible economically after the discovery of a cytoplasmic-genetic male-sterility system (Stephens and Holland, 1954). The combination of cytoplasm from 'milo' sorghum and recessive nuclear factors for fertility restoration from 'kafir' types produced plants that were pollen sterile. Hybrid seed of sorghums has been produced for more than three decades by using seed parents with the milo cytoplasm (designated A<sub>1</sub>) and male parents that possessed dominant nuclear factors for pollen fertility restoration (R-lines) in that cytoplasm. Commercial hybrids worldwide are currently based on the A<sub>1</sub> CMS system. However, hybrids based on a single CMS system with narrow nuclear genetic diversity of both male-sterile (A-) lines and restorer (R-) lines often become vulnerable to insect pests and diseases as was evident from the outbreak of southern corn leaf blight on hybrids based on a Texas cytoplasm in 1970 (Tatum, 1971).

The demands for cereals, including grain sorghum is progressively increasing due to population growth, yet total production is not sufficient to cover the internal demands (Ali *et al.*, 2011). Furthermore, the demand for higher productivity over a unit area, increasing population growth and decrease in arable land are reasons for necessity for hybrid production. Due to these reasons, it will be important to increase the yield of sorghum. One of the best ways of increasing sorghum productivity is by the use of hybrids. Moreover, it is common knowledge that open pollinated varieties (OPVs) are generally lower yielding than hybrids. In sorghum, male sterility has been induced mainly using mechanical and genetic means with minimal use of chemical methods. Mechanical sterility induction is limited to producing small quantities of seed and genetic male sterility is limited to specific germplasm. Male sterility has played a major role in production of hybrid seeds in large scale to exploit the phenomenon of heterosis. The objective of the review was to understand the methods of sorghum hybrid development to improve the production and productivity per unit areas.

## 2. POLLINATION CONTROLLING SYSTEM AND ITS TYPES

Intensive hybrid breeding and seed production in many crop species were made possible by the identification and characterization of a stable and heritable cytoplasmic male sterility (CMS) mechanism. CMS is a maternally inherited defect where, as the result of specific nuclear and mitochondrial interactions, plants fail to produce functional pollen, or to ensure normal anther dehiscence, without affecting the female fertility (Duvick, 1959). It is attributed to abnormal transcripts usually coding for chimeric open reading frames (ORFs) (Hanson and Bentolila, 2004). A CMS system depends therefore on a set of male-sterility-causing cytoplasm's and dominant or recessive alleles in the nuclear genome, which either restore the fertility or maintain the sterility (Rooney *et al.*, 2000). Several male-sterility-inducing cytoplasm's were described in sorghum since the identification of a stable CMS system by Stephens and Holland (1954).

The A<sub>1</sub> (milo) cytoplasm was first documented (Conner A.B, 1927) and is most widely used in sorghum hybrid development worldwide, followed by the A<sub>2</sub> cytoplasm (Schertz and Ritchey, 1978). Other types of CMS, namely A<sub>3</sub> (Quinby, 1982), A<sub>4</sub> (Worstell *et al.*, 1984), A<sub>5</sub>, A<sub>6</sub>, 9E (Webster and Singh, 1964). However, their use in commercial hybrid breeding programs is limited by the negative effects on grain yield of A<sub>3</sub> cytoplasm (Moran and Rooney, 2003), the low environmental stability of the restoration and, consequently, the lack of elite male restorer lines. Taking into account the mechanism affecting pollen production, pollination control systems can be classified as follows.

## 2.1 Systems Determined By Genetic Control

### 2.1.1 Male Sterility

Male sterility has been defined as the failure of plants to produce functional anthers, pollen or male gametes (Kaul, 1988). Anther and pollen development can be considered as a pathway with several stages: anther cell specification comprising stamen primordial initiation and archaespore initiation, mature pollen formation in which pollen mother cell meiosis and microspore maturation take place and anther maturation and pollen release, when dehiscence occurs. Many of the key genes involved in anther and pollen formation have been identified (Wilson *et al.*, 2011). In this process, the tapetum plays a central role as supplier of nutrients, proteins, lipids and polysaccharides which are used in microspore release and pollen-wall formation (Parish and Li, 2010).

Male sterility can be conditioned by nuclear or cytoplasmic factors affecting any of the stages of microsporogenesis or gametogenesis, resulting in genetic male sterility (GMS) and cytoplasmic male sterility (CMS), respectively. Male sterility is generally characterized by the impairment of the male reproductive development as a result of underlying genetic causes and leads to the malformation of male gametes and/or pollen. Male sterility has tremendous scientific and economic importance in hybrid seed production. Identification and characterization of a stable male sterility gene will be highly beneficial for making hybrid seed production economically feasible.

#### 2.1.1.1 Genetic Male Sterility (GMS)

Genetic male sterility (GMS) has emerged as an ideal tool to accelerate hybrid breeding. Male sterility is generally characterized by the impairment of the male reproductive development as a result of underlying genetic causes and leads to the malformation of male gametes and/or pollen. Genetic male sterility (GMS) has emerged as an ideal tool to accelerate hybrid breeding. Male-sterility is caused by single recessive genes. Of these, *ms* are most widely used because its expression of male-sterility is good and it is stable over many environments. Genetic male-sterility is used primarily in composites to enhance the level of recombination.

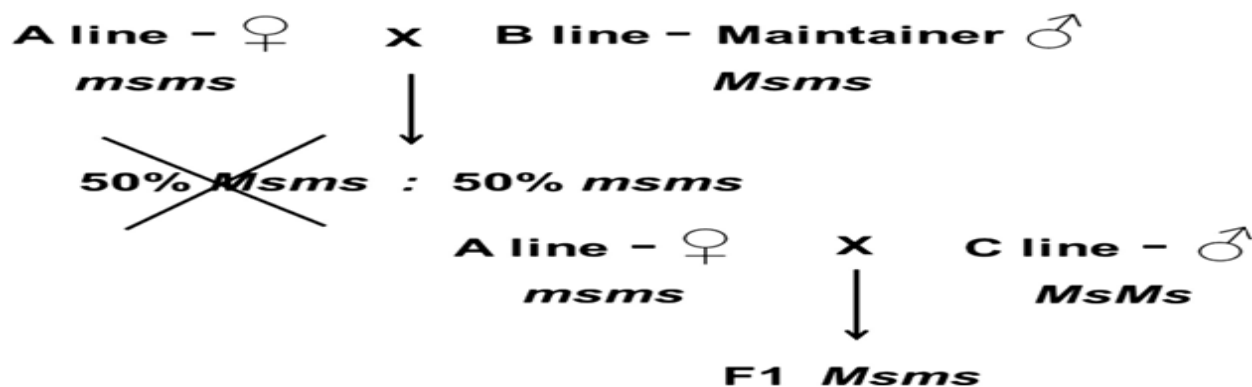


Figure 1: Hybrid seed production using recessive genetic male sterility

Some male sterility genes change their expression under different environmental conditions like temperature and photoperiod inducing thermo-sensitive genetic male sterility (TGMS) and photoperiod-sensitive genetic male sterility (PGMS), respectively. This means that *msms* plants will be male sterile at a particular temperature or photoperiod at a sensitive stage, whereas they will be male fertile at another condition (Virmani and Ilyas-Ahmed, 2001). When grown under restrictive environments, TGMS and PGMS lines serve as the male sterile female parent; the same lines grown in permissive conditions are fertile and allow the propagation of the sterile line. This reversible system eliminates the requirement of crossing to propagate the male sterile line and allows for the efficient development of the 'two-line' hybrids (Tang *et al.*, 2014).

#### 2.1.1.2 Spontaneous and Induced Mutants

The nuclear male sterility (NMS) trait, which is caused by mutations on the nuclear gene, is valuable for hybrid breeding and genetic studies. Male sterile mutants are key tools for hybrid breeding. Male sterility in flowering plants is often attributed to a failure in pollen production or shedding due to defective anther development or dehiscence, while the development of female floral organs is normal (Xin Z *et al.*, 2017). Genetic defect in the nuclear genome results in NMS, which is usually segregated as a recessive trait in the self-pollinated F<sub>2</sub> offspring. Most of the male sterile mutants have arisen spontaneously with a high frequency and some have been induced using physical or chemical

mutagens, singly or in combination. In all cases, the pattern of inheritance and expression of genetic male sterility is Mendelian (Kaul, 1988). The majority of the *ms* genes are recessive, in accordance with a loss-of-function mutation. However, some dominant genes have also been reported (Shu *et al.*, 2012).

### 2.1.1.3 Genetically Engineered Male Sterility

Genetic engineered male sterility has different applications, ranging from hybrid seed production to bioconfinement of transgenes in genetic modified crops. The impact of this technology is currently patent in a wide range of crops, including cereals, which has helped to deal with the challenges of global food security. Production of engineered male sterile plants by expression of a ribonuclease gene under the control of an anther- or pollen-specific promoter has proven to be an efficient way to generate pollen-free elite cultivars. Male sterility has been used by plant breeders to realize breakthroughs in the yield of different crops, through the development of hybrid cultivars. The impact of such technology is currently evident in some crops, including legumes (Saxena and Hingane, 2015), which has helped to deal with the challenges of global food security. Genes that are specifically expressed in the male reproductive organs could be used to obtain genetically engineered male sterile plants with potential applications in the production of hybrid seed, elimination of pollen allergens, or to avoid undesirable horizontal gene transfer in genetic modified crops.

Genetic cell ablation has been previously used to investigate male gametogenesis and as biotechnological tool to generate engineered male sterile plants using anther- or pollen-specific promoters fused to a cytotoxic gene (Yue *et al.*, 2017). Production of engineered male sterile plants by expression of the ribonuclease *barnase* gene (Hartley, 1988), under the control of anther- or pollen-specific gene promoters, has been proved to be a good approach to generate pollen-free elite cultivars without adversely affecting the respective phenotypes (Mishra and Kumari, 2018). Moreover, male fertility can be restored in plants showing barnase-induced sterility by crossing with a transgenic line harboring the *barstar* gene, which encodes a powerful inhibitor of barnase (Mariani *et al.*, 1992). Genetic engineering offers a precise way of manipulating plants to tailor specific traits. The large numbers of transgenics that are being developed bear a testimony to the potential of this new technology. Factors affecting the wide adoption of genetically engineered male sterility are as follows: availability of efficient gene constructs, possible dispersion of transgene to other related species, availability of efficient transformation techniques, very high initial investment and biosafety and regulatory matters (Ananthi *et al.*, 2013).

### 2.1.1.4 Cytoplasmic and cytoplasmic-genetic male sterility (CMS-CGMS)

Cytoplasmic male sterility (CMS) is determined by mitochondrial genes resulting from mitochondrial DNA rearrangements which disturb the normal development of pollen. This maternally inherited character has been described in more than 140 species of higher plants. Although the mechanism involved in CMS has not been elucidated, evidence supports roles for energy deficiency, programmed cell death (PCD) and reactive oxygen species (ROS) (Hu *et al.*, 2020). Cytoplasmic-genetic male sterility (CGMS) results from the interaction of mitochondrial genes causing male sterility and nuclear genes, which specifically restore male fertility *-Rf genes-*, thus representing an example of the crosstalk between mitochondrial and nuclear genomes.

All progenies of the milo × kafi r cross contained milo (sterility-inducing) cytoplasm, but those that also inherited the homozygous recessive genes from the kafi r parent were male sterile. The male-sterile plants in the milo × combine kafi r cross were used as females in repeated backcrossing with kafi r as the male parent. At the end of seven backcrosses, the entire genome of kafi r was transferred into the milo cytoplasm. This resulted in two morphologically similar versions of the combine kafi r (CK 60) parent: a male-sterile combine kafi r (CK 60A) and a male fertile combine kafi r (CK 60B). The male-sterile lines are designated as A-lines and their maintainer lines as B-lines.

Regardless of the type of sorghum, commercial seed production is based on hybrids produced using cytoplasmic male sterility (CMS) systems. A CMS system is essential because sorghum is a self-pollinated crop and hybrid seed production without a sterility system is not feasible. The first CMS was discovered, characterized and described by Stephens and Holland (1954). This system, designated as A1 CMS, is still used for hybrid sorghum seed production. The number of fertile (filled) and sterile (unfilled) grains per spike were counted after manual threshing and percent male sterility was computed using the formulae adapted from Chakraborty and Devakumar (2006).

Percent male sterility =  $\left(\frac{Sc-Sf}{Sc}\right) \times 100$ , Where,  $Sc$  = seeds per panicles in control plants  $Sf$ = seeds per panicles in bagged and treated plants Female fertility was recorded as a proportion of fertilized seeds in a plot relative to the total number of seeds in the control and treated plants. The number of seeds per panicle was counted for treated and untreated plants. Female fertility was determined as follows:

Percent female fertility =  $\left(\frac{Sp-Sf}{Sc-Sf}\right) \times 100$ , Where,  $Sc$  = seeds per panicles in control plants  $Sf$ = seeds per panicles in bagged and treated plants  $Sp$  = seeds per panicles in pollinated plants.

Fertile pollen grains were considered to be well-filled pollen grains of normal size and shape that were wholly or partially stained while sterile pollen grains did not stain and were malformed and shriveled. Pollen sterility was quantified as follows (Amelework *et al.*, 2016).

Percent pollen sterility =  $\left(\frac{Ps}{Ps+Pf}\right) \times 100$ , Where,  $Ps$ =number of sterile pollens  $Pf$ = number of fertile pollens.

Cytoplasmic male-sterility in sorghum makes possible the commercial production of hybrid seed. Male-sterility results from an association of Milo cytoplasm with sterility genes found primarily among kafirs but also in some varieties of other races. The genetics involved is not completely clear, but two genes ( $msci_1$  and  $msci_2$ , when recessive in the presence of Milo cytoplasm) result in male-sterility. There are other factors that influence the sterility reaction, possibly having a modifying effect on the level of partial fertility. A technique for breeding against modifying factors is mentioned in the section on making new male-sterile seed parents. The dominant allele  $Ms$  restores male fertility in plants with S cytoplasm, while plants possessing the normal (N) cytoplasm are male fertile irrespective of the genotype of the restorer gene. For hybrid production, a male sterile inbred line -A line- (S *msms*) and a maintainer line to propagate the A line-B line- (N *msms*) are needed. The A line is pollinated by the pollinator inbred line for hybrid production. Although CGMS is widely applied in hybrid production, it presents some limitations which may restrict its adoption (Dhall, 2010).

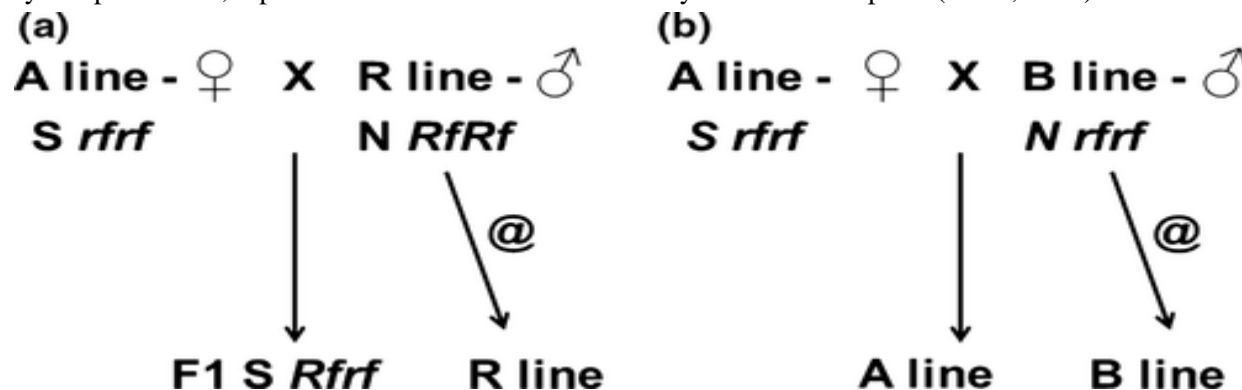


Figure 2: Hybrid seed production and multiplication of lines using CMS. (a) F<sub>1</sub> seed production and multiplication of R line; (b) multiplication of A and B lines

### 2.1.1.5 Self-incompatibility

Self-incompatibility (SI) is a genetically determined prezygotic mate-recognition system preventing self-pollination and is very common in angiosperms (Ferrer and Good, 2012). Self-incompatibility response is comprised of a self- and non-self-recognition process between pollen and pistil that is followed by selective inhibition of the self-pollen development. In most species, self-incompatibility is controlled by a single multi-allelic locus, the S-locus, which determines pollen inhibition when the same ‘S-allele’ specificity is expressed by both pollen and pistil. Current knowledge indicates that S-locus consists of at least two linked genes, each of them coding for the male and female determinants expressed in the pollen grain and pistil, respectively.

The variants of the gene complex are called S-haplotypes and the self-incompatibility response occurs when both determinants are issued by the same S-haplotypes (Takayama and Isogai, 2005). Self-incompatibility systems have been classified as gametophytic (GSI) and sporophytic (SSI). In GSI, the most widespread system, the incompatibility type of the pollen is controlled by its own haploid genotype, whereas in SSI, the pollen incompatibility type is controlled by the diploid (sporophyte) genotype of the parental anther in which it was produced (Hiscock and Tabah, 2003). The identities of female and male determinants have been determined and molecular models for different types of self-

incompatibility have been developed recently (Serrano *et al.*, 2015). A major advantage of using self-incompatibility for hybrid seed production is that only two self-incompatible lines carrying different S alleles are necessary (Kucera *et al.*, 2006).

### 2.2 Systems Not Determined By Genetic Control

#### 2.2.1 Manual Emasculation

Manual emasculation consists on manual removal of the stamens from hermaphrodite flowers or the elimination of complete male flowers when they are separated from the female ones. This method is labor intensive and requires highly skilful human resources to ensure complete emasculation without affecting female organs (Adhikari, 2018).

#### 2.2.2 Chemical-Hybridizing Agents (CHAs)

Since the middle of the 20th century, many attempts have been made to find substances effective to selectively disrupt pollen development without affecting the female functionality. These compounds, known as gametocides, pollen suppressors or chemical-hybridizing agents (CHAs) belong to different chemical groups: auxins and auxin inhibitors (NAA, IBA, 2, 4-D, TIBA, MH), gibberellins, ethylene (ethephon-ethrel), halogenated aliphatic acids (FW450; Dalapon), arsenicals and brassinosteroids. Cytokinins, auxin, gibberellins, ethylene, jasmonic acid and brassinosteroids play a role in another development, and male sterility has been associated with changes in many plant growth regulators, suggesting that normal male development is controlled in concert by multiple hormones (Ye *et al.*, 2010). The main advantages of this technology are as follows: ease of making and evaluating hybrid combinations, labor efficient seed production and no need for developing male sterile and restorer lines. The major drawbacks are as follows: toxicity effects on the female or F<sub>1</sub> seed, difficulties in field applications due to precise stage of plant development and environmental factors and less effectiveness due to interaction with genotypes and environment (Adhikari, 2018).

### 2.3 Development of New Hybrid Parents (A-, B- and R-lines)

The lines that produce fertile F<sub>1</sub>s 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.

**2.3.1 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 (Murthy *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, i. e., 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, i.e., 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.

**2.3.2 Development of New A- And R-Lines:** Three criteria are used in the selection of parents for this purpose: genetic diversity, the 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 (Murthy *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 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 out-crossing 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.

### 2.4 Hybrid Development of Sorghum

Hybrid is the progeny ( $F_1$ ) as a result of cross between two or more distinct parents or genotypes (Ghaderi *et al.*, 1984). Hybrid development requires complementarity between parents. This complementarity in sorghum is achieved by the use of male sterility to facilitate crossing, resulting in identification heterosis for many traits such as yield (Reddy *et al.*, 2008). The development of strategies to improve crop plants by the production of hybrid varieties is a major goal in plant breeding. Hybrid progeny often have a higher yield, increased resistance to disease and enhanced performance in the different environment compared with the parental lines. Hybrid seeds are used for stimulated crop production, as they harness heterosis. The achievement of complete male-sterility in the female-parent and the restored-fertility in  $F_1$ -hybrids are the major bottlenecks in the commercial hybrid seed production.

Hybrid crops have been contributing to the substantial global rise in agricultural output over the past few decades as they harness heterosis, a phenomenon of outperformance of  $F_1$  hybrid progeny compared with their parents in terms of yield, biotic and abiotic resistance (Schnable P. S. and Springer N. M., 2013). Their utilization offers a 20% to more than 50% yield increase (Tester M and Langridge P., 2010) and contributes to more than half of the production of the major crops. Precise control over pollen fertility in the female parent and the fertility restoration in  $F_1$  hybrids are the prerequisites in the commercial production of the  $F_1$  hybrid in self-pollinating crops (Kempe K *et al.*, 2014). Restoration of male fertility in hybrids is especially important in crops where the desired agricultural products are seeds, such as cereals, pulses and so on.

Among breeding strategies,  $F_1$  hybrids have several advantages over open-pollinated varieties.  $F_1$  hybrids are obtained by crossing parental inbred lines, and their special importance is due to the uniformity that is based on the resulting genetic homogeneity as well as on higher yields through heterosis, due to their heterozygous nature (Feistritzer and Kelly, 1987). The heterozygous nature of the hybrids requires seeds to be obtained from breeding companies. A further important application for male sterility systems, apart from hybrid breeding, is their use for gene confinement for the increasing number of genetically modified crop plants used in field trials and agricultural production (Daniell, 2002).

The CMS in sorghum genotypes was developed by backcrossing chromosomes of kafir into the cytoplasm of milo. Similarly, genetic male sterility (ms) has been discovered in sorghum male sterile plants (Msms) (Acquaah, 2015). The discovery of cytoplasmic male sterility in sorghum facilitates the



commercial utilization of hybrid vigor (Akata *et al.*, 2017). Stephens and Holland (1954) reported for the first time, the use of cytoplasmic genetic male sterility for developing hybrids to increase sorghum production. Different male-sterility inducing systems, such as A<sub>2</sub> and A<sub>3</sub> cytoplasm, have been discovered in the last few decades and hold promise for widening the genetic variability of elite lines.

The sorghum conversion program continues to serve as a major source of new germplasm for many breeding programs throughout the world (Smith and Frederiksen, 2000). There was a report as sorghum hybrids can provide a 20 to 60% grain yield advantage over the open pollinated parents (Pfeiffer *et al.*, 2010). Breeding for heterosis in sorghum accomplished by identification of stable cytoplasmic male sterile lines, maintainer and restorer lines having high GCA for desirable character. The genetic effects of various characters can be better understood through the application of biometrical principles. Biometrical models are available for getting information on the combining ability status of parental lines of which lines x tester approach (Kempthorne, 1957).

This approach provides information on relative magnitude of fixable and non-fixable genetic variation available in the material. The hybrid seed production involves a CMS line (A line), a maintainer line (B line) and a restorer line (R line). Scheme of hybrid seed production using the CGMS involves two main steps. First is the production of A line (A x B) and second involves production of hybrid Seed (A x R). The lines that produce fertile F<sub>1</sub>s 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. This is to need to develop alternative male sterility sources and fertility restoration systems. Research on sorghum hybrid development in Ethiopia began in the mid-seventies, with an objective of developing sorghum hybrids for the low altitude and moisture stress ecological zones. The practice of hybridization has greatly contributed to the increase in crop productivity. A major component that exploits heterosis in crops is the cytoplasmic male sterility (CMS)/nucleus-controlled fertility restoration (Rf) system. In general, the crop performance and yield can be increased by generating hybrid plants which out compete pure lines due to heterosis or hybrid vigor.

### 3. CONCLUSION

Genetic improvements in crop performance are crucial for increasing crop productivity, but current rates of improvement will not be sufficient to meet future food demands in an era of global climate change. As arable land is limited, increased crop productivity must come from yield increases achieved by innovation in plant breeding. Future advances in crop species to produce more feed and food contributing to a sustainable agriculture will require synergy among several research fields, including traditional breeding, crop management, physiology, genetics and biotechnology. Sorghum hybrid development involves development of parental lines based on a cytoplasmic male sterile system including the pollen parent (R-line) and seed parents (A- and B-lines). Discovery of genetic male sterility and cytoplasmic-nuclear male sterility facilitated the application of recurrent selection procedures and hybrid cultivar development methods in sorghum improvement respectively.

Cytoplasmic male sterility system has been widely been used for increasing sorghum yield through heterosis exploitation. To effectively use male-sterility inducing cytoplasm, it is necessary to identify restorers and lines that are suitable for conversion to male sterility. Increased productivity in sorghum has been achieved in the developed world using hybrids. Hybrid seeds are known to increase productivity of many crops including sorghum. Hybrid production utilizes the hybrid advantages common in biology, with selective breeding conducted on the hybrid offspring. Hybrids now make up 80% of world crops. Apart from developing countries, which still use conventional varieties, hybrid strains dominate the market in seeds. Hybrids in sorghum have demonstrated increased productivity and stability of performance in the developed world.

The A<sub>1</sub> cytoplasmic-nuclear male sterility system in sorghum is used almost exclusively for the production of commercial hybrid seed and thus, the dominant genes that restore male fertility in F<sub>1</sub> hybrids are of critical importance to commercial seed production. The genetics of fertility restoration in sorghum can appear complex, being controlled by at least two major genes with additional modifiers and additional gene-environment interaction. Globally, hybrids are the preferred type of varieties in sorghum as they yield 20 to 30% additional grain and Stover over a range of environments compared to open pollinated varieties and landraces. However, hybrid cultivar development requires

additional research investment over those needed for non-hybrid cultivars for developing suitable parental lines. Crop genetic improvement has led to enhanced productivity and yield gains globally.

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