Male Sterility Systems in Major Field Crops and Their Potential Role in Crop Improvement

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K.B. Saxena and Anupama J. Hingane

Abstract

Male sterility is a phenomenon where the male reproductive parts of the plants do not develop normally and fail to participate in sexual reproduction. The male sterility is of different kinds and can arise through a number of biological abnormalities. Among these, cytoplasmic nuclear male sterility has been extensively used by plant breeders to achieve breakthrough in the productivity of various field and horticultural crops through the development of hybrid cultivars. The impact of this technology is visible in crops like maize, rice, sorghum, pearl millet, etc., and this has helped in encountering the challenges of global food security. Among high-protein legumes, the world's first hybrid was released recently with record 3–4 t/ha of grain yield. This chapter briefly discusses the types of male sterility systems available in different crop species and their potential uses. Besides this, various methods of creating different male sterility systems are also described.

Keywords

Male sterility • Fertility restoration • Microsporogenesis • Genetic male sterility • Cytoplasmic nuclear male sterility • Mitochondria

25.1 Introduction

Male sterility is a unique gift of nature to mankind. The contribution of this system in combating global hunger through its use in developing high-yielding hybrids in various food crops has been immense. Male sterility is a situation where the male reproductive parts of a plant are either absent, aborted, or nonfunctional, and hence they fail to participate in the process of

K.B. Saxena (✉)
Pigeonpea Breeding, Grain Legumes, ICRISAT, 2, ICRISAT Colony (Phase I), Bowenpally, Secunderabad 502324, Telangana, India
e-mail: kbsaxena1949@gmail.com

A.J. Hingane
Pigeonpea Breeding, Grain Legumes, ICRISAT, Building No-305, Room No 124, ICRISAT Campus, Patancheru 502324, India

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natural sexual reproduction. This situation can arise due to any developmental defect at any stage of microsporogenesis or release of pollen grains. Kolreuter (1763) was the first to record the existence of plants in nature with impaired anthers in some natural populations. Since then numerous reports of such abnormal events have been published in a number of crop species. Darwin (1877) recognized the importance of this phenomenon and hypothesized that the loss of reproducing ability of plant helps evolutionary processes in enhancing adaptation through gene transfer from various related and unrelated individuals through cross-pollination.

With respect to utilization of male sterility in crop breeding, it is essential that the individuals with altered male fertility keep their female fertility intact. The fertilization of such plants with the pollen grains from other plants that may be transferred with the help of external agencies such as wind, insects, or human beings produces viable seeds. Historically, the male-sterile mutants had appeared naturally in the populations of cultivars and germplasm, but at that time their economic value was not recognized, and hence these were lost over a period of few generations. However, with the evolution of the concept of heterosis by Shull (1908) and subsequently by others, the potential benefits of male sterility in enhancing productivity of crops were realized. In this context it should be mentioned that Stephens (1937) for the first time utilized male sterility in hybrid seed production in sorghum. At the same time Jones and Emsweller (1937) also demonstrated its use in hybrid seed production of onion. The male-sterile trait may arise in nature through mutations, or it can be bred through induced mutagenesis or hybridization and selection. Considering the economic importance of this unique natural phenomenon, vast scientific information has been generated on its genetics, physiology, and genomics. This information has been very elegantly compiled by Kaul (1988) in his monograph *Male Sterility in Higher Plants*. In the present chapter, the author has not made any hard attempt to compile available literature on various aspects of male sterility, and rather issues of general importance have been briefly explained with focus on its origin and utilization in 12 major field crops involving cereals, legumes, and oilseeds.

25.2 Fundamentals of Different Male Sterility Systems

As mentioned earlier the male sterility is an abnormality that is observed occasionally in higher plants, where the male reproductive system of an individual fails to participate in producing its progenies. Such plant defects can arise due to a number of reasons such as inability of anther tissues to grow and differentiate normally, failure of normal microsporogenesis, failure to release the mature pollen grains, and/or inability of mature pollen grains to germinate on the stigmatic surface. These abnormalities, however, do not impair the female reproductive system, and if such plants are pollinated through manual or natural means, they produce fertile seeds. Since male sterility is a manifestation of abnormal growth and development, the action of genes controlling male sterility may also be variable and inconsistent across the crops and genotypes. Based on the type of malfunctioning of the androecium, the male sterility systems have been classified as structural (absence or deformity of anthers), sporogenous (defective microsporogenesis), and functional (failure of mature pollen to germinate). In addition, on the basis of genetic control mechanisms, it has also been classified as genetic, cytoplasmic, and cytoplasmic nuclear (or genetic) male sterility.

25.2.1 Genetic Male Sterility

This is the most common form of male sterility found in a number of plant species in both monocots as well as dicots (Kaul 1988). In this system the male sterility is controlled by nuclear genetic factors, and it is independent of cytoplasmic influences. In most cases its expression is controlled by one or two pairs of recessive alleles, which segregate independently. However, a few exceptions are also found where the male sterility is

reported to be controlled by one or two dominant genes. Also in certain cases, the male sterility is linked to some easily identifiable morphological traits such as pigmentation of the stem, translucent anthers, sparse podding and delayed flowering etc. (Kaul 1988; Verulkar and Singh 1997).

The mutant male-sterile plants may arise spontaneously carrying homozygous alleles (*msms*), and these will be lost if not maintained as heterozygotes (*Msms*). For this to happen, the male-sterile mutants need to be pollinated with fertile homozygous (*MsMs*) or heterozygote (*Msms*) counterparts. In cases where male sterility is controlled by dominant alleles, its maintenance through reproductive means is very difficult.

25.2.2 Cytoplasmic Male Sterility

This type of male sterility is governed by cytoplasm which contains defective mitochondrial DNA. This happens due to deleterious interactions of mitochondrial genes with those present in the nucleus. This type of cytoplasm is designated as "sterile" (S), and it can originate spontaneously or through wide hybridization. Such plants do not produce fertile pollen grains because its nucleus also contains a pair of recessive non-restoring (*msms*) alleles. The cytoplasmic male sterility is maintained by the genotypes which carry "fertile" (F) or "normal" (N) cytoplasm (Fig. 25.1) and non-restoring recessive nuclear alleles. According to Kaul (1988), about 150 plant species have been reported to carry this type of male sterility.

In the cytoplasmic male sterility system, the diversification of hybrid parents may be difficult due to incorporation of recessive non-restorer nuclear alleles. At genotypic level the male hybrid parent should resemble its maintainer but with diverse nuclear genome that is capable of producing heterotic hybrid progenies. This type of male sterility cannot be used for field crops due to absence of fertility-restoring genes and difficulties in producing large quantities of hybrid seed. Alternatively, this system has been used in horticultural crops where fruits are consumed or the seeds are noncommercial entity.

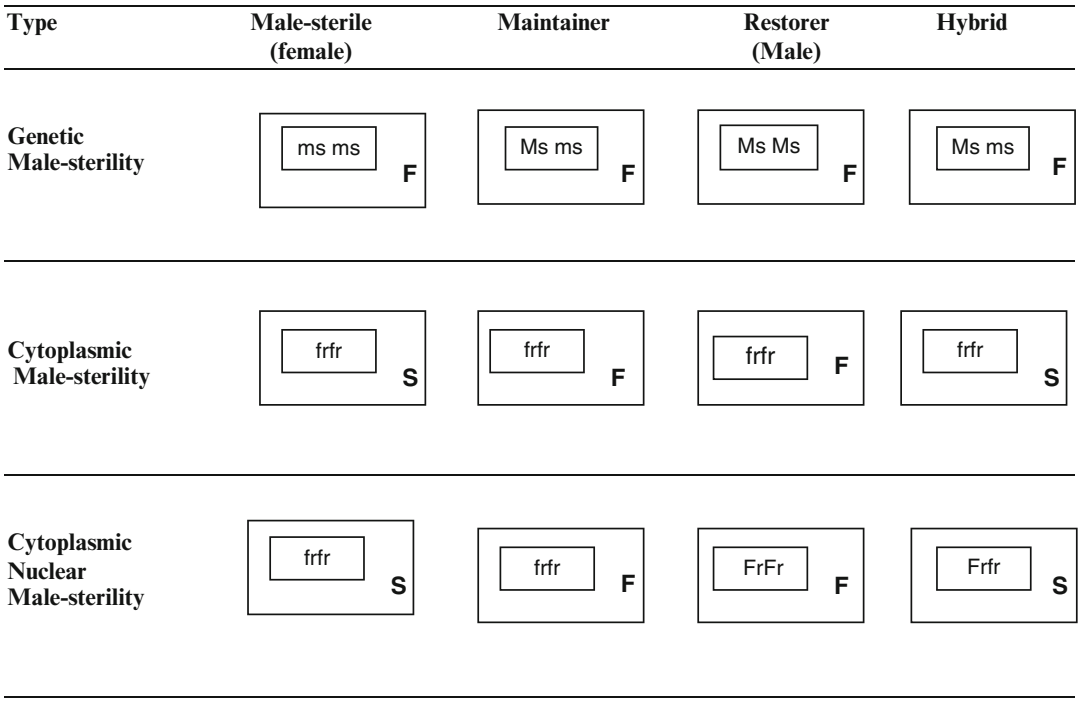

Fig. 25.1 Generalized hereditary constitution of the nucleus and cytoplasm of the three male sterility systems

25.2.3 Cytoplasmic Nuclear Male Sterility

Like that of cytoplasmic male sterility, in cytoplasmic nuclear male sterility also, the manifestation of male sterility is a consequence of interaction between cytoplasmic and nuclear genomes. The difference between the two types is in their fertility restoration mechanisms. In the former male fertility is controlled by its "N" cytoplasm of the maintainer lines, while in the latter type dominant fertility-restoring genes are located in the nucleus of restorer line (Fig. 25.1). Hence, it is termed as cytoplasmic nuclear/genetic male sterility. Further depending on the type of fertility-restoring gene, the expression of male fertility/sterility could be total or partial. Sometimes such expressions are also affected by prevailing environmental conditions such as photoperiod, temperature, or both. This form of male sterility has been used most extensively in hybrid breeding programs in a number of field crops. The complete hybrid system involves three distinct genotypes:

- "A" line is the male-sterile female line with "S" cytoplasm and recessive fertility nuclear alleles (*frfr*).
- "B" line is a maintainer of the female line, and it has fertile "F" cytoplasm and recessive nuclear alleles (*frfr*). When this line is crossed with "A" line, the entire progeny is male sterile.
- Third parent is designated as "R" line and it contains dominant fertility-restoring gene (*FrFr*). This line has the ability to restore the male fertility of the hybrid plants produced by crossing with "A" line.

Molecular studies on this male sterility system revealed that the expression of male sterility is associated with mitochondrial genome rearrangements that results in the production of toxic proteins and reduction in respiration. In fact various theories have been proposed, but still the molecular basis of this male sterility is not well understood in most crops. Recent studies have shown that the male sterility is associated with chimeric mitochondrial ORFs (open reading frames). Wang et al. (2006) demonstrated that in rice the ORF encodes a cytotoxin peptide which determines the expression of male sterility. Iwabuchi et al. (1993) showed that an abnormal copy of a mitochondrial gene produced aberrant mRNA transcripts containing an additional ORF. Hanson and Bentolila (2004) reported that male sterility may also be associated with alterations in promoter regions and portions of coding regions of mitochondrial ATP synthase. This may impair the activity of ATP synthase. The genomic studies on mitochondrial genome of pigeon pea A_4 cytoplasm recognized 13 ORFs which can trigger male sterility (Tuteja et al. 2013). Further, Sinha et al. (unpublished) reported involvement of 10 bp deletion in *nad7a* gene which was responsible for producing male-sterile plants in pigeon pea.

25.2.4 Environment-Sensitive Male Sterility

This is a unique male sterility system where the expression of male sterility and fertility of the plants is controlled by environmental factors. Under this system the male sterility gene expresses only under specific environment such as low or high temperature, short or long photoperiod, variable light intensity, different soilborne stresses, or their specific combinations (Kaul 1988). This situation can arise both in genetic as well as cytoplasmic nuclear male sterility systems. According to Levings et al. (1980), the reversion of sterility is influenced by cytoplasmic rather than nuclear genetic factors, and loss of such factors is correlated with reversion of male sterility to male fertility. Escote et al. (1985) and Small et al. (1988) showed that no DNA loss was associated with the reversion of male sterility. The conversion of male sterility to fertility and its reversal is a complex genetic phenomenon, and more research is required at genomics and physiological levels to understand it better.

The environment-sensitive male-sterile line was first reported by Shi (1981) in rice, and later Yuan (1987) proposed its use in hybrid breeding program. Since it eliminates the requirement of maintainer "B" line, this hybrid system is

popularly called as "two-parent hybrid" breeding. At present this male sterility system is being used in China commercially, and in 1994 the hybrids based on this system of male sterility covered over 30,000 ha areas with yields as high as 8–9 t/ha (Lu et al. 1994).

25.2.5 Fertility Restoration of Male-Sterile Germplasm

Perfect male fertility restoration of cytoplasmic nuclear male sterility-based hybrids is an integral part of any hybrid breeding technology. Once an "R" line is crossed with "A" line, the dominant *Fr* nuclear gene of "R" line overcomes the ill effects of defective mitochondrial genome. The *Fr* gene produces certain proteins which repair the damage and make the plant male fertile. In most crops one or two dominant fertility restorer genes have been reported to control the pollen fertility (Kaul 1988). Saxena et al. (2011) reported that in pigeon pea two dominant genes were responsible for fertility restoration of A_4 cytoplasm. However, the hybrids with a single dominant gene were also fertile, but they produced less quantity of pollen and also showed a lot of instability with respect to fertility restoration in diverse environments. Similarly in maize also, four fertility-restoring genes were reported, and of these, two were major genes while the remaining two only resulted in partial restoration of male sterility (Wise et al. 1999). Further, it was also reported that one of the major fertility-restoring genes reduced sterility-causing protein by 80 % (Kennel et al. 1987). The fertility restoration has also been associated with genes encoding pentatricopeptide repeat proteins (Hanson and Bentolila 2004).

25.3 Origin of Male Sterility Systems

25.3.1 Selection from Natural Variation

Nature has provided unlimited variability and in the past it has yielded a number of economic traits in different crops. There are numerous examples of it, and among these, various male sterility systems are unique, and these have benefitted millions through the cultivation of high-yielding hybrids. Over the period different types of male sterility systems have emerged, and in the future also we can expect some unexpected genetic variation which can be tapped by plant breeders.

25.3.1.1 Genetic Male Sterility

It arises due to mutation of the male fertility nuclear gene (*MsMs*) to its recessive form to produce heterozygote individuals. Self-pollination of such plants reveals male-sterile segregants. Under natural conditions in the self-pollinated crops, the male-sterile mutants are generally lost, but in cross-pollinated or partially cross-pollinated crops, such mutants are preserved by natural hybridization. According to Kaul (1988), genetic male sterility arising due to spontaneous variation has been reported in over 175 plant species.

25.3.1.2 Cytoplasmic Male Sterility

The frequency of this form of male sterility in nature is relatively less, because it requires natural mutation in mitochondrial genome to make its cytoplasm male sterile.

25.3.1.3 Cytoplasmic Nuclear Male Sterility

The natural occurrence of this form of male sterility system is also low since it requires simultaneously double occurrence of mutation; one in the mitochondria and the other in the nucleus. According to Kaul (1988), so far only 46 plant species are credited to have produced this form of male sterility under natural conditions.

25.3.2 Integration of Cultivated Genome into Alien Cytoplasm

This technology has been successfully used to develop cytoplasmic nuclear male sterility systems in cereals, oilseeds, legumes, and various other groups of crops. It is based on the concept

of bringing cytoplasmic and nuclear genomes of diverse origins within a single genotype. This is achieved by crossing a wild relative of a crop as female parent with a cultivated line as male parent. This combination integrates the cultivated nucleus into the cytoplasm of wild species and brings together the two diverse entities in a new genotype.

25.3.2.1 Intergeneric Hybridization

In this approach the wild relatives from different genera are crossed as female parent with cultivated types. Sometimes due to large diversity between the two parents, the crosses may not be successful, and it may require rescuing the young hybrid embryo. The resultant hybrid plants may be both male and female sterile due to severe abnormalities occurring during meiosis. Also, there may be problems with plant type itself with abnormal growth of vegetative and reproductive parts. It has also been observed at ICRISAT that in pigeon pea such hybrid plants failed to produce seeds. In many cases the male-sterile plants found through this approach failed to survive in the absence of any maintainer.

25.3.2.2 Interspecific Hybridization

This is the most common and successful approach used in breeding cytoplasmic nuclear male-sterile genotypes in various species of cereals, legumes, and other field crops. It also involves hybridizing the wild relatives as female parent. In this group of materials, the success from crossing is generally high because of relatively closer relationship between the two species. The process used in developing the male sterility in pigeon pea has been outlined in Fig. 25.2. Initially, due to strong linkage drag, there will be a lot of unwanted traits in the hybrid and in segregating generations, and breeders must be careful in selecting individual genotypes for backcrossing. The selection of an appropriate maintainer is also important, and it should be done with care while maintaining its genetic purity. In some cases the fertility restorers can be identified from cultivated germplasm, but in case it is not available, then the breeders need to select fertile segregants originating from the same cross.

25.3.3 Selection from Recombinant Populations

Large breeding populations derived from various intervarietal crosses sometimes reveal new genetic variation, and it may arise due to rare recombination of recessive alleles or transgressive segregation. In crops like pearl millet, soybean, and cotton, cytoplasmic nuclear male sterility has been derived from recombinant populations in the past (Kaul 1988). The frequency of such useful recombination is, however, very low.

25.3.4 Induced Mutations

Various known chemical and physical mutagenic agents have been used by breeders in different crops to create new variability in important economic traits. Scientists have also succeeded in developing male sterility systems in a number of crops. According to Kaul (1988), over 35 plant species have been tried successfully to breed male sterility systems. Among the mutagenic agents tried, gamma rays were the most effective. This, however, cannot be generalized due to differential crop x mutagen x application rate interactions. In some crops the success rate of EB has also been found to be very high. In most cases these mutagens have yielded genetic male sterility systems. In soybean and pearl millet, cytoplasmic nuclear male-sterile lines have also been developed through mutagenesis (Burton and Hanna 1976; Kaul 1988).

25.3.5 Chemical Hybridizing Agents

Some of the chemicals are known to have gametocide properties; these as a group are called as “chemical hybridizing agents” (CHA). Moore (1950) and Naylor and Davis (1950) were the first to induce male sterility by spraying a chemical called MH (maleic hydrazide) in maize. Soon other chemicals (alpha naphthalene acetic acid and beta indole acetic acid) were reported to have induced female flowers in cucumber

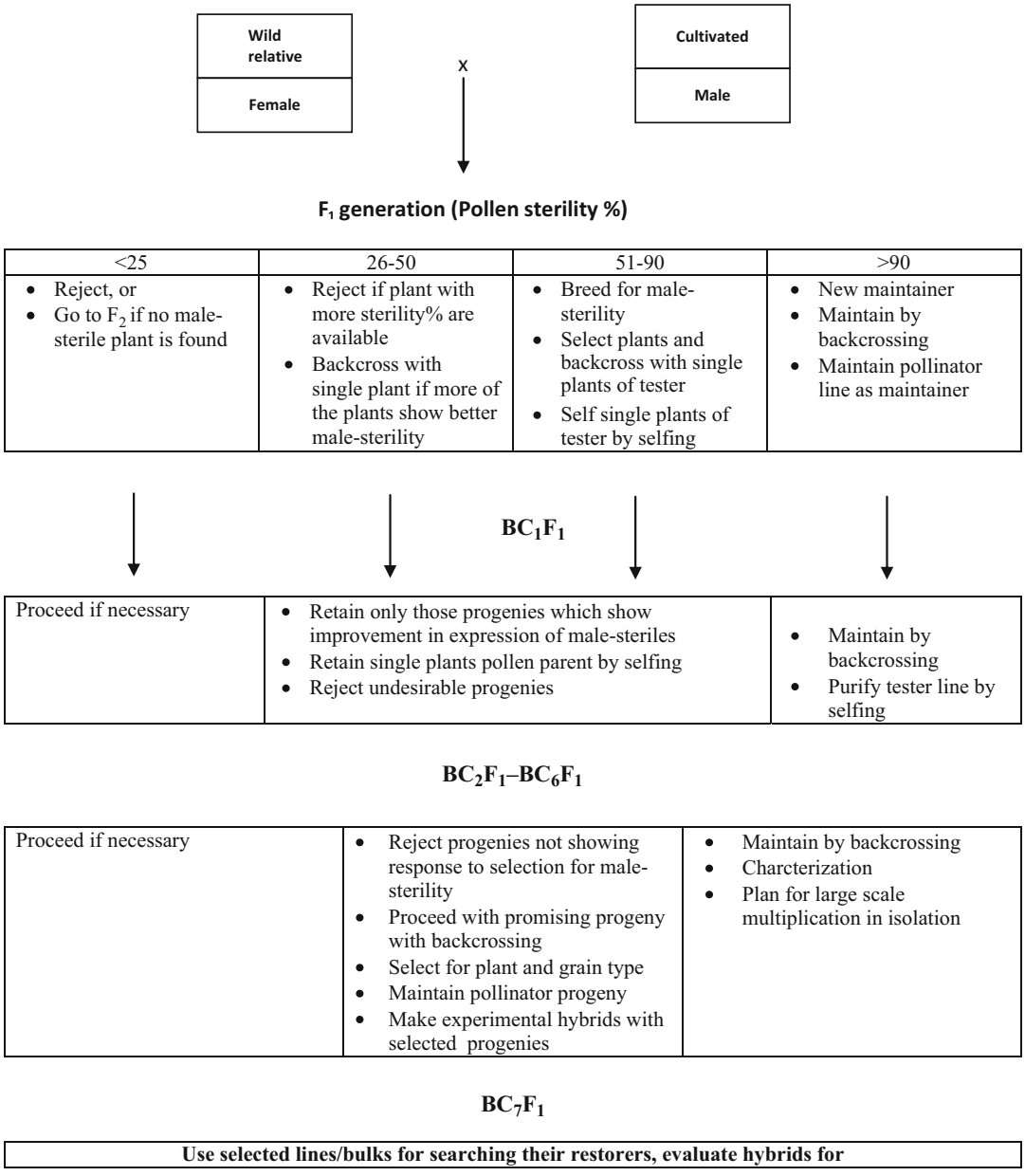

Fig. 25.2 Flow diagram followed in breeding alloplasmic CGMS line in pigeon pea

(Laibach and Kriben 1951). Besides this, some chemicals were also found accidently which altered the reproductive parts in crops such as rice. Subsequently, a large number of chemicals were screened for their potential use in the development of hybrids in different crops. According to Colhoun and Steer (1982), the ideal chemical hybridizing agents must be very specific and should not affect other parts of the plants and at the same time should not be transmitted to the progeny in any form. Besides this, these should be environment friendly and economical in use. Good CHAs should be specific in action with respect to crop, time of application, and doses.

In this system of hybrid production, the fertile (normal) crop is sprayed with CHA as per

the recommendations, and it results in the production of male-sterile female-fertile flowers in large proportions. Such induced male-sterile flowers can be pollinated with selected male parents to produce heterotic hybrids. The major advantage of this system is that there is no need of any maintainer line and it also allows the production and testing of a large number of experimental hybrids without many resources. The heterotic hybrid combinations can be selected in less time for promotion. It is not advisable to use this system of hybrid breeding in the field crops which produce more nonsynchronous tillers for relatively more time or have a perennial growth habit. In such cases the effect of chemical in producing male-sterile flowers will not be uniform, and there is a danger of late-emerging tillers or branches producing fertile or partial fertile flowers. This will adversely affect hybrid seed quality and the expected hybrid vigor may not be realized. Tu and Banga (1998) reviewed this subject and reported that chemicals like "dalapon," a known herbicide, can cause male sterility in cotton, pearl millet, wheat, linseed, sesame, capsicum, and some other crops. "Ethrel" is effective in barley, mustard, oat, pearl millet, rice, and wheat. Similarly, "gibberellic acid" has been found effective in inducing male sterility in crops like rice, maize, barley, oats, sunflower, and onion. "Maleic hydrazide" is another CHA which has been found effective on capsicum, cotton, oats, sorghum, and onion.

25.3.6 Genetically Engineered Male-Sterile Plants

Recent advances in DNA recombination technology have made it possible to synthesize male-sterile lines and their restorers. Mariani et al. (1990) were the first to develop such a genotype. This was achieved by transferring tobacco and rapeseed plants with a chimeric dominant gene from *Bacillus amyloliquefaciens*. This gene disrupts the normal process of pollen formation and causes male sterility. Besides this, some other technologies such as induction of modified glucanase gene (Worrall et al. 1992) and hormone engineering (Schmulling et al. 1988) have been explored in the past. Considering the scope of this paper, the details of these technologies are not discussed here. The cytoplasmic nuclear male sterility can also be produced through asexual recombination. The asexual method of somatic hybridization and transformation offers a positive alternative. Their use, however, has not found favor with plant breeders in any commercial hybrid crop.

25.3.7 Summary of Recorded Male Sterility Systems in Different Crops

In an attempt to enhance productivity, plant breeders have tried to exploit the well-known phenomenon of hybrid vigor in various field crops. In order to achieve this goal, it was essential that the ways for an effective seed production were developed, and hence, attempts were made to breed stable male sterility systems using different approaches. As a first step screening of germplasm was undertaken and natural mutants were selected. In addition, attempts were also made to breed for a reliable male sterility system using different mutagens, wide crosses, and chemical hybridizing agents. The review of literature on the 12 crops (Table 25.1) showed spontaneous occurrence of genetic male sterility in all the crops. A similar observation was also recorded in identifying environment-sensitive genetic or cytoplasmic nuclear male sterility. The use of different chemical or physical mutagens yielded genetic male sterility. The exceptions were sorghum and pearl millet where cytoplasmic nuclear male sterility systems were developed through mutagenesis. In some cereals, certain intervarietal crosses also yielded the male-sterile lines, while in rice and wheat, chemical hybridizing agents were successfully used (Table 25.1) to create temporary nonheritable male sterility for the purpose of hybrid seed production.

Table 25.1 Brief summary about the origin of different male sterility systems in some important field crops

Crop	Spontaneous	Spontaneous	Spontaneous	Mutagen	Mutagen	Intervariety	Interspecific	Intergeneric	CHA
	GMS	CMS	ESMS	GMS	CMS	CMS	CMS	CMS	
Rice	*	*	*	*		*		*	*
Maize	*			*		*	*	*	*
Wheat	*		*	*		*	*	*	*
Sorghum	*	*	*	*	*	*	*		*
Pearl millet	*	*	*	*	*				*
Cotton	*	*	*				*	*	*
Mustard	*	*	*	*			*	*	*
Sunflower	*	*	*			*	*	*	*
Pigeon pea	*		*	*			*		
Fava bean	*	*	*	*					
Soybean	*		*	*					
Safflower	*								

Source: Kaul (1988), Tu and Banga (1998), and various other papers
*Denotes presence of particular male sterility system in the crop

25.3.8 Microsporogenesis and Male Sterility

The development of seedling into an adult plant is characterized by a number of morphological changes. The plants are genetically programmed to enter into reproductive phase to pass on its genetic information to the progeny. This in many cases is controlled by photoperiod and/or temperature. The differentiation of meristem cells to pollen mother cells and finally pollen production is the result of many complicated biochemical events that are controlled by a network of genes. Any abnormality in a gene or two disturbs the natural process of microsporogenesis and that leads to male sterility. Such events could be spontaneous or induced. Since male sterility is of economic importance, a lot of research is being undertaken in a number of laboratories to unlock the mystery of this complex phenomenon.

The process of microsporogenesis can be impaired at premeiotic, meiotic, or postmeiotic stages to produce structural, sporogenous, or functional male sterility. It is a well-known fact that the most internal layer of meiotic cell wall, called tapetum, plays an important role in the development of pollen grains. The tapetum cells are hyperactive and constantly feed the meiotic cells with vital nutrients. Once the normal functioning of tapetum is disrupted, the food supply chain breaks and it triggers abnormalities. The anther and pollen morphology of such plants are generally determined by the time of breakdown of microsporogenesis.

Kaul (1988) while reviewing the subject concluded that in genetic male sterility the male-sterile gene acts at either early or late stage of meiosis, and only a few cases of premeiotic abortion of microsporogenesis are reported. In a large number of cases, the male-sterile gene has been reported to act toward the end of meiosis when tetrads are ready to be released. In certain cases the pollen mother cells develop normally, but they start degenerating even before meiosis starts; this may happen due to protein starvation of pollen mother cells. According to Vasal (1967), any abnormality in nutrient supply generally leads to aberrant outputs such as fusion of cells or degeneration of tapetum, and ultimately leading to abnormal development of pollen mother cells. Worrall et al. (1992) found that in case of male sterility, callose is synthesized due to the presence of high concentrations of cellular calcium. Katti et al. (1994) reported that a gradual reduction in concentration of sugars and proteins in the tetrads was responsible for disorientation of cytoplasm leading to malnutrition and poor tetrad growth. In contrast to many reports, Frankel and Galum (1997) suggested that early impairment of microsporogenesis was also associated with sterility of female gametes. Saxena et al. (1983) reported that in the male-sterile plants degeneration of pollen mother cells occurred at young tetrad stage, and it was accompanied with vacuolation and subsequent rupturing of nuclear membrane. In an interesting experiment, Saxena and Kumar (2001) demonstrated that if two male sterility genes *ms1* and *ms2* were incorporated into a single genotype, then the *ms2* gene, which acts first during early prophase, dominated the proceedings of microsporogenesis, and all the plants had phenotype of *ms2*, and none of the individual showed any sign of the presence of *ms1* gene, which acts at late tetrad stage. In the segregating generation (F_2), both the genotypes were present. These observations showed that the action sites of the two male sterility genes were different and independent of each other. Kuranouchi et al. (2000) demonstrated that male reproductive machinery is more sensitive than the female counterparts, and it results in high frequency of male sterility cases in nature as compared to female sterility.

In the last two decades, a lot of investigations have been carried out in the areas of physiology, genomics, and embryology to understand the phenomenon of male sterility, but the basic question, such as how the mitochondrial genomic abnormalities control the events of microsporogenesis in the anthers, still remains unanswered. However, some of the important observations recorded in the recent publications are:

- Some anther-specific substances might interact with *urf 13* proteins to cause sterile phenotypes. These proteins have also been

found to be detrimental to cell viability (Flavell 1974).
- The *urf 13* gene may be overexpressed in tapetal cells (Levings 1993).
- The tapetal cells exhibited characteristic features of prolonged cell death (Balk and Leaver 2001).
- Cytotoxic gene products were associated with male sterility (Nakai et al. 1995).
- Mitochondrial open reading frames played an important role in the expression of male sterility (Wang et al. 2006).
- The proposed "genomic conflict theory" explains the results of interaction of cytoplasmic determinant that prevent pollen production and nuclear restorers that restore fertility (Cosmides and Tooby 1981).
- The *urf 13* gene caused male sterility by programmed cell death (Wu and Cheung 2000).
- Polyhydroxybutyrate causes abnormal development of the epidermis and endothecium with a broken tapetal layer (Poirier et al. 1992).
- PhaA (a-ketothiolase) gene caused 100 % male sterility, and light illumination reverted the male-sterile lines to male fertility (Ruiz and Daniell 2005).

25.4 Utilization of Male Sterility in Crop Breeding

25.4.1 Population Breeding

Most breeding programs suffer from limited genetic variability, and it may arise due to non-availability of diverse germplasm or lack of recombination to allow breeders to select new genotypes. Since recombination breeding is primarily based on human resources, both in partially or completely cross-pollinated species, breeders have attempted to accumulate favorable alleles from diverse sources in one population. Besides serving as gene pool for deriving useful variability from time to time, these heterogeneous populations can also be released for cultivation, especially for stress environments. The exercise of breeding populations is cumbersome as it requires large-scale hybridization and selection of genotypes with required genetic constitution. To facilitate random mating and enhance gene frequency of favorable alleles, several breeding populations using genetic male sterility were developed in the past. This also helped in maintaining genetic diversity within and across the populations. This, when achieved, would yield populations with high yield, wide adaptation, and greater stability against various biotic and abiotic stresses (Doggett 1972; Eberhardt 1972). Utilization of genetic male sterility in reciprocal recurrent selection allows breeders to exploit additive, additive x additive, and epistatic genetic variations for crop improvement (Comstock and Robinson 1952). Lukhele and Obilana (1980) and Obilana and El-Rouby (1980) reported about 40 % gain in productivity after three cycles of recurrent mass selection. The genetic male sterility-based populations can also be bred to create vast gene pools for specific traits to encounter different production constraints for specific ecosystem with locally preferred market traits. Besides releasing elite populations for cultivation, some breeders have successfully derived high-yielding pure line cultivars from such genetic pools (Murthy and Rao 1997). In population breeding schemes, breeders should always ensure that the male sterility gene is not lost while advancing the generations. This will restrict recombination among genotypes in the population.

25.4.2 Hybrid Breeding

Enhancement of productivity has been an important goal for most breeding programs. Although the hybrid breeding was known as a potential way of yield increases, but the constraint of large-scale hybrid seed production prevented the commercialization of this technology in many crops. In maize the hybrid technology grew with the aid of detasseling (removing the male part from female rows) and wind-supported cross-pollination. In dioecious plants where male and female reproductive parts are in the same flower, the exploitation of hybrid vigor remained a challenge.

Stephens (1937) was the first to demonstrate the use of genetic male sterility in producing quality hybrid seed in sorghum. In the genetic male sterility system, where the male-sterile genotype is multiplied only by crossing heterozygotes with male-sterile plants, and in the resultant population, the male-fertile and male-sterile plants will be equal proportion. This means that it will be a difficult task to produce quality seed of female line and its hybrids. The development of cytoplasmic nuclear male sterility system has changed the seed production scenario, and its application can be seen in a large number of crops (Singhal 2013). Thus the problems faced by seed producers in using genetic male sterility were overcome, and hybrid seed production became easy. With the advent of this technology, the hybrid seed tonnage of all kinds of crops has increased by many folds, and now commercial hybrid breeding has become a well-established industry across the world.

25.5 Diversification of Male-Sterile Lines

25.5.1 Genetic Male Sterility

The genetic diversity of genetic male-sterile lines is accomplished by following a standard backcrossing procedure, and since the male sterility is controlled by recessive alleles, each cycle of backcross should be followed by selfing. This will expose the male-sterile segregants, and these should be used for pollinations for the next cycle of backcross. Theoretically, six backcrosses are recommended to transfer the male-sterile trait in the desired genetic background, and it will consume about 12 crop seasons.

25.5.2 Nuclear Diversity of Cytoplasmic Nuclear Male-Sterile Lines

Genetic diversity with respect to various traits of economic importance is the key in any breeding program. It has generally been observed that most hybrid breeding programs are based on one or two cytoplasm, and to develop hybrids for different production niches, it is essential that sufficient nuclear diversity is available among the male-sterile lines. This can be achieved by selecting a fair number of unrelated testers, selected on the basis of their phenotypic, genotypic, and geographic diversity. These testers should be crossed with selected "A" lines with good agronomy traits and high general combining ability in a line x tester design. In F_1 generation all the hybrid plants of each cross should be tested for the expression of male fertility and sterility. The fertile combinations should be transferred to hybrid breeding program for the assessment of their productivity and other traits, while the hybrids showing male sterility should be examined very carefully. The testers of such hybrid combinations should be maintained, keeping their genetic purity intact. After reconfirming their pollen sterility, the hybrids and their testers should be selected for backcrossing (Fig. 25.2). In each generation the breeder needs to double-check the male sterility of the plants before going for the next cycle of backcrossing. If need arises some selection pressure can also be imposed for one or two important agronomy traits. This may enhance the chances of getting a good "A" line. If required, then some promising "B" lines can also be converted to male sterility by simple backcrossing, using "A" line as female parent and "B" line as recurrent parent.

25.5.3 Cytoplasmic Diversity of "A" Lines

For a sustainable hybrid breeding program based on cytoplasmic nuclear male sterility system, it is essential that a fair amount of cytoplasmic diversity is also maintained. This will protect the breeding program from any potential threat of single cytoplasmic susceptibility against certain specific biotic or abiotic stresses.

Before launching such an expensive diversification breeding program, it is necessary that the candidate cytoplasm should be tested for their genetic diversity. This can be done in two ways,

the first being the traditional way of making experimental hybrids with a few male-sterile lines of a specific cytoplasm and a set of common testers. These hybrids are evaluated for their genetic variation with respect to some important traits and prepare an inventory of diverse traits. This method, however, does not give accurate information about the real genetic diversity among different sources of cytoplasm due to various interactions with known and unknown environmental factors. The other method is based on genomics technologies. An RFLP analysis of mitochondrial DNA based on specific male sterility enzyme probe combinations can be adopted, and this will provide information on the similarity or dissimilarity of the cytoplasm sources. The differences in the RFLP patterns will suggest mitochondrial genomic differences. It seems that from practical breeding points of view, both the traditional restoration patterns and molecular studies would provide more or less conclusive evidences on the diversity of the available cytoplasm sources. In rice, Virmani and Shinjyo (1988) listed several CMS sources; however, Brar et al. (1998) reported that 95 % of the hybrid rice in China represents only a single WA cytoplasm. It is because this cytoplasm has high frequency of fertility restorers and it provides an opportunity to breed high-yielding hybrid combinations. In pigeon pea also, eight cytoplasmic male sterility sources have been reported (Saxena et al. 2010; Saxena 2013), but only one (A_4) cytoplasm derived from a wild relative of pigeon pea is being used for commercial hybrid breeding program. In summary, diversification of cytoplasm is an essential activity of a dynamic breeding program, and plant breeders should take a serious view of it. A breeding program to introduce a new cytoplasmic male sterility that was adopted in pigeon pea at ICRISAT is outlined in Fig. 25.3.

25.6 Seed Production of Male-Sterile Lines

For any technology to become popular among users, it is important that quality of the product is maintained, and it should be economically viable. In seed business also, these two factors are of prime importance. Since the hybrid seed is a product of two parents, it is essential that the seed of the parental lines is of highest quality. To achieve this, care should be taken at every step of seed production, its storage, and its distribution. The hybrids involving different type of male sterility would require specialized approach.

25.6.1 Genetic Male Sterility

Since genetic male sterility is controlled by a pair of recessive genes, it needs to be maintained in heterozygote form. For the seed production of male-sterile line, the heterozygote (*Msms*) seeds are planted in isolation, and these segregate in the Mendelian fashion and produce about 50 % fertile and 50 % sterile plants. In this population these two types of the plants should be tagged with different identity. At maturity the seed should be harvested only from the male-sterile plants, which get pollinated by heterozygote fertile segregants.

The production of hybrid seed with genetic male sterility lines is very tricky and requires greater attention and resources. The seed of male and female parents is planted in isolation. As mentioned earlier the female rows will segregate for fertility/sterility. It is very important that each and every plant is examined, and the fertile plants should to be removed from the field. To maintain the quality of the hybrid seed, it is essential that the fertile segregants should be removed before they start shedding pollen. This will permit crossing of the male-sterile plants with designated male parent only. In case the male sterility gene is linked to any morphological trait, then rouging becomes very easy. In the field crops, there are only a few cases, such as safflower, where male sterility is linked to dwarf phenotype (Vijender Singh, personal communication).

25.6.2 Cytoplasmic Nuclear Male Sterility

In both cytoplasmic and cytoplasmic nuclear male sterility systems, the male-sterile ("A"

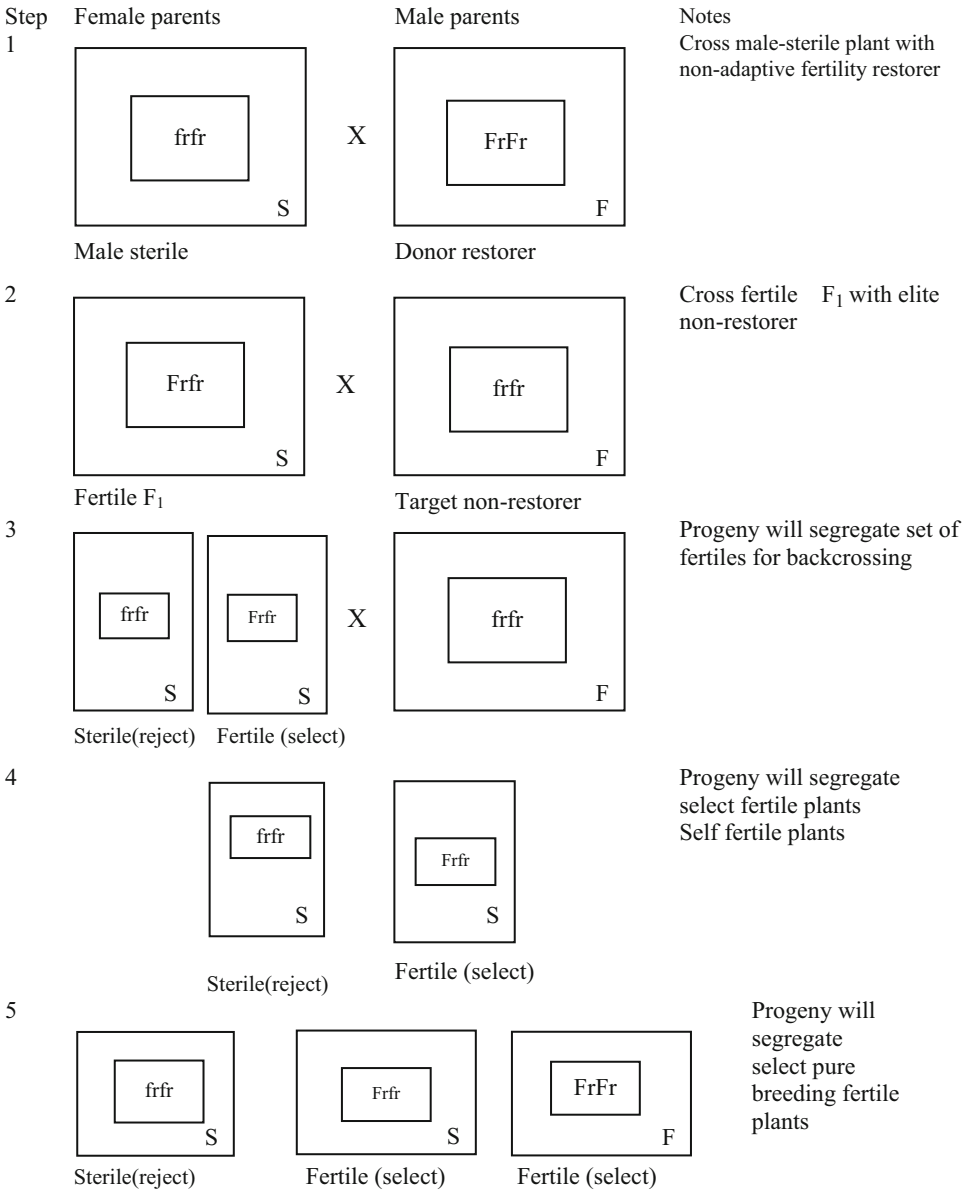

Fig. 25.3 Conversion of elite B-line or other non-restorers into fertility restorers with CGMS system

line) genotypes with sterile cytoplasm and recessive nuclear fertility genes (Fig. 25.1) are crossed with lines having fertile cytoplasm and recessive nuclear fertility genes ("B" line). The seed of "A" and "B" lines is planted in isolation in rows in certain proportions, determined by the availability of pollen and pollinators. Since all the plants in the female rows are expected to be male sterile, examination of each plant for male sterility is not required and crossed seeds are harvested from "A" line only. The production of hybrid seed in the cytoplasmic nuclear system is easy, and "A" and restorer line "R" are planted in isolation in rows, and the seeds set on the "A" lines through cross-pollination are harvested.

25.6.3 Environment-Sensitive Male Sterility

The seed production system involving environment-sensitive line is interesting, and selection of production sites is the most critical. In this system two different sites with distinct and stable temperature requirements during crop growth are essential. These sites should also satisfy the requirement of the length of photoperiod. Site # 1 should have the temperatures under which the male sterility of the line will be maintained. In this site all the plants will be male sterile and these are used for hybrid seed production. For this, the male and female lines are grown in specific ratio, and the cross-pollinated seed is harvested from the male-sterile rows.

For the maintenance of male-sterile line, its seed is planted in site # 2. This site should have the temperatures under which the male fertility is induced and all the plants should be male fertile. Thus, the seed produced from this site will be the self-seed. The next season can be used for the second cycle of seed production.

25.7 General Discussion

Providing quality seed to masses has been an important goal for most national and international organizations dealing with global food security issues. A gradual reduction in arable land and ground water has created challenges for meeting food needs in many countries. With little or no chance of horizontal expansion, scientists from both public and private sectors are aiming for vertical production growth. This can only be achieved through introduction of new technologies. Among these, the most economic and stable technology is hybrid breeding.

The pivoting role of hybrids in enhancing global food availability is well established. The concept of hybrid breeding was developed by Shull (1908) in maize and involved crossing of two diverse pure breeding lines. For large-scale seed production of high-yielding combinations, detasseling (manual removal of male parts) of female parents was adopted. Subsequently, this activity was replaced by introducing cytoplasmic nuclear male-sterile lines. Since 1930, there has been a sixfold increase in the productivity of maize in the USA by the turn of the century (Fig. 25.4; Trayer 1991). This has been possible due to remarkable progress made in breeding high-yielding hybrids and the role of male sterility in easing the seed production technology that allowed access of hybrid seed to most crop growers. Rice is another staple food crop where a tremendous progress has been made in their productivity. This endeavor began in China in 1976, and since then over 55 % rice area has come under hybrid crop production, and it

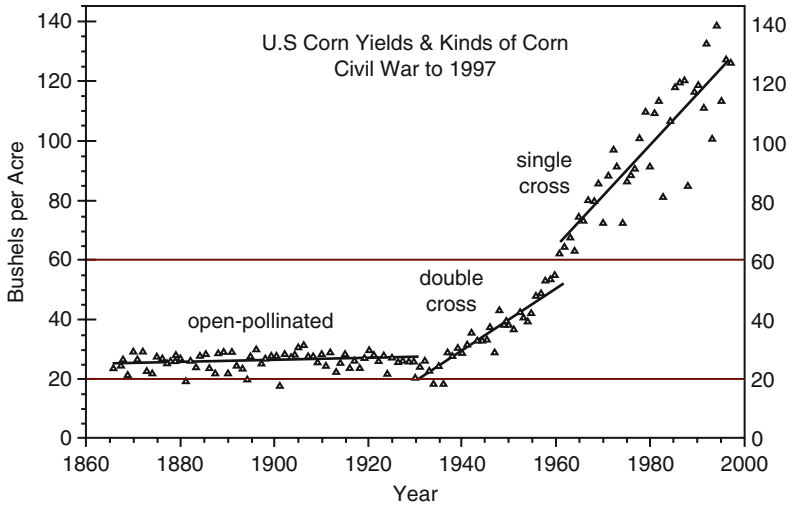

Fig. 25.4 Trends of maize production in America (Source: Trayer 1991)

contributes to 65 % of total production of the country. The adoption of hybrid technology in China has also increased the crop productivity from 2 to over 6 t/ha. This has released over three million ha of rice lands to other crops (Brar et al. 1998). Almost similar achievements have been recorded in cereals like sorghum and pearl millet in India. The other crops which have directly benefitted from hybrid technology are cotton, sunflower, safflower, caster, and a number of vegetable and fruit crops.

Among legumes, although heterotic, male sterility systems have been reported but these could not be utilized for enhancing production and productivity. The exception, however, is pigeon pea where breeders have exploited hybrid vigor commercially very recently. The productivity of pigeon pea had been stagnant at about 700–800 kg/ha for the past six decades, and breeders did not succeed in spite of using different breeding approaches. The breakthrough in the productivity was achieved recently in 2010, when the first high-yielding hybrid was released in India (Saxena et al. 2013). This hybrid is based on cytoplasmic nuclear male sterility and partial natural outcrossing. It has demonstrated 30–40 % yield advantage over 3 years of testing in farmers' fields. Also, under high-input conditions and good management yields, up to 4,000–5,000 kg/ha have been recorded by farmers (Kumar RV, personal communication).

The examples of rice, wheat, and pigeon pea have shown that with concerted research efforts it is also possible to breed hybrids in the non-cross-pollinated crops. This fact demonstrates that heterosis can be exploited in both self- as well as cross-pollinated crops, provided their seed production technology is good enough for adoption by both large- and small-scale seed producers. It can be facilitated by the use of suitable male sterility system. The genetic and cytoplasmic diversifications of male-sterile lines with high combining ability are the key for the future, and it can break low-yield barrier in other food crops also. The better understanding of the phenomenon of heterosis at genetic, physiological, agronomic, and molecular levels can pave the way for obtaining record high yields. Besides seed yield, the hybrid technology can also take care of delicate issues like nutrition, drought, stability, and response to inputs. In this context, maize, rice, pearl millet, and sorghum are good examples where breeding of new hybrids involves a balance between productivity and nutrition. In essence, the success of hybrid technology in continuously enhancing yield levels, as has been demonstrated in maize in the USA, will require robust breeding programs to develop elite hybrid parents with sufficient nuclear and cytoplasmic diversity and accurate tools to predict the productivity of potential hybrid combinations for specific and wide adaptation.

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