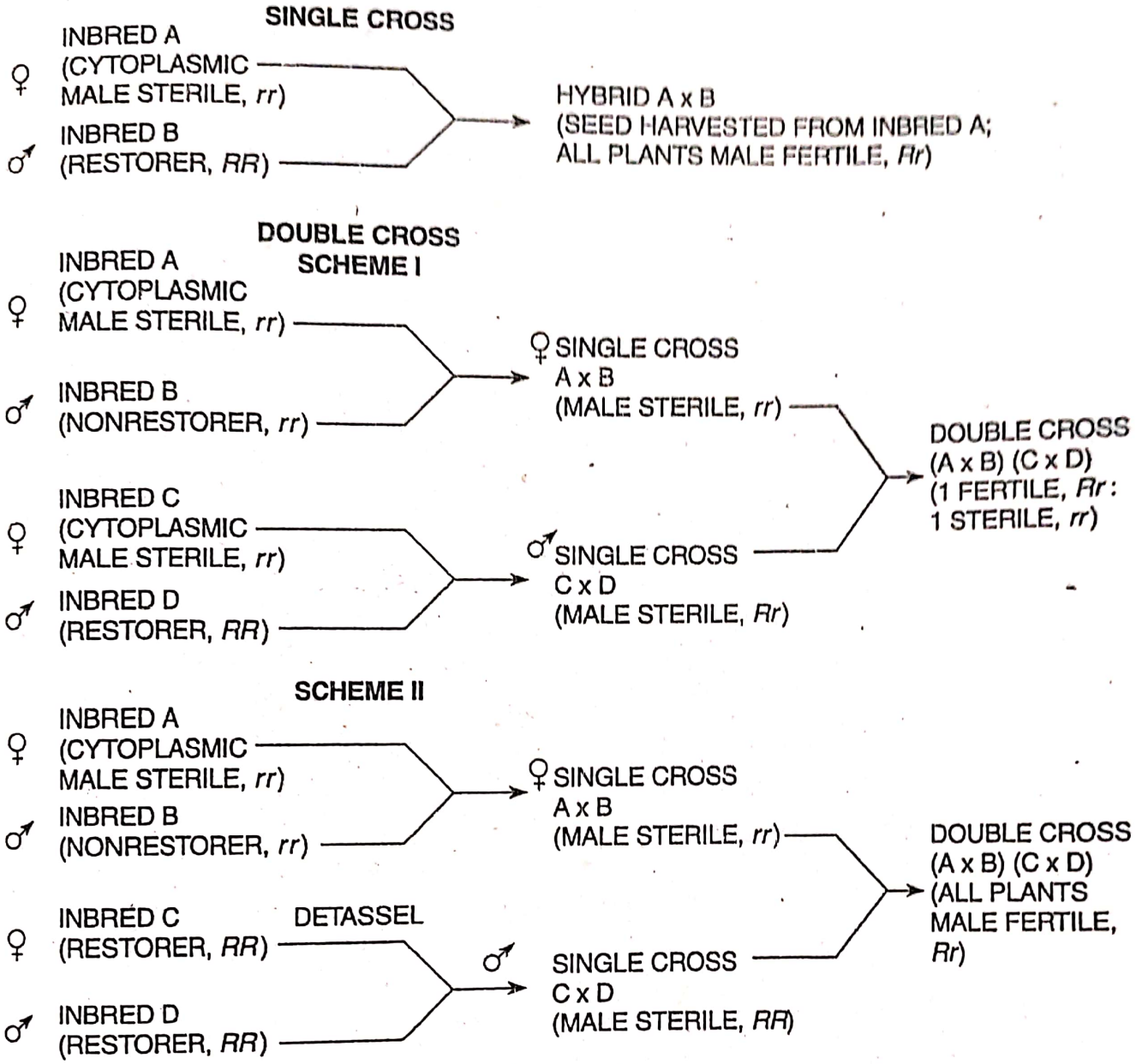

Fig. 22.2. The use of cytoplasmic-genetic male sterility for the production of hybrid seed.

22.6.2. Cytoplasmic Male Sterility

The scheme for hybrid seed production is the same as that with cytoplasmic-genetic male sterility, except that the male fertile line is nonrestorer (by the definition of cytoplasmic male sterility system). The hybrid, therefore, is male sterile. It may be useful in crops where grain or seed is not the commercial product. It has not been exploited commercially to any appreciable extent.

22.6.3. Genetic Male Sterility

The male sterile line (*ms ms*) is allowed to be cross-pollinated with a male fertile line (*Ms Ms*) to yield a male fertile hybrid (*Ms ms*); the seed produced on male sterile line will be the hybrid seed. The development and maintenance of male sterile lines has been discussed in some detail in Chapter 6. It has been commercially exploited in castor (U.S.A.) and pigeonpea (India; on a small scale). Genetic male sterility presents a serious problem. In the

female parent (the GMS line), 50% of the plants are male fertile (*Ms ms*). These plants must be identified and eliminated before they shed pollen. This problem has limited the application of GMS in hybrid seed production. This defect of GMS is overcome entirely when photoperiod sensitive (PGMS) or temperature sensitive genetic male sterility (TGMS) is used. For example, a TGMS line of rice, *viz*, line 5460S, is fully male fertile at temperatures below 28°C, while it is completely male sterile at temperatures above 30°C. This TGMS is *maintained and multiplied* in a region where temperature during the critical period of growth is below 28°C. In this environment, the TGMS plants will be male fertile, and will produce all TGMS progeny. For *hybrid seed production*, the TGMS line is grown in an area where the temperatures during the critical growth period remains above 30°C; as a result, all the plants in this TGMS line will be male sterile. The TGMS line is pollinated by any male fertile line, which combines well with this line.

This system is the most efficient system of hybrid seed production (Fig. 22.3). (1) The TGMS line does not contain male fertile plants (which is the case in normal GMS) and all the plants are male sterile. (2) TGMS is maintained by selfing since TGMS plants are fully fertile in specific areas. In addition, (3) any male fertile line combining well with the TGMS line can be used as the male parent in hybrid seed production. In contrast, the CGMS system required a maintainer line (B line) for maintaining the CMS line (A line), and a suitable restorer line (R line) has to be developed and used as the male parent of the hybrid. Thus the use of CGMS requires 3 lines [A, B and R lines (***three-line hybrid seed production***)]. Since, TGMS (or PGMS) requires only two lines (TGMS and male fertile lines) and any male fertile line can be used as the male parent of the hybrid (***two-line hybrid seed production***). Further, (4) the negative effects associated with male sterity-inducing cytoplasms are not encountered in TGMS/PGMS. Finally, (5) seed production programme is simpler and more efficient; in rice, the ratio among areas used for the multiplication of female parent, hybrid seed production and commercial cultivation of the hybrid seed thus produced is 1 : 100 : 10,000 for TGMS/PGMS as against only 1 : 50 : 5,000 in the three-line system (CGMS).

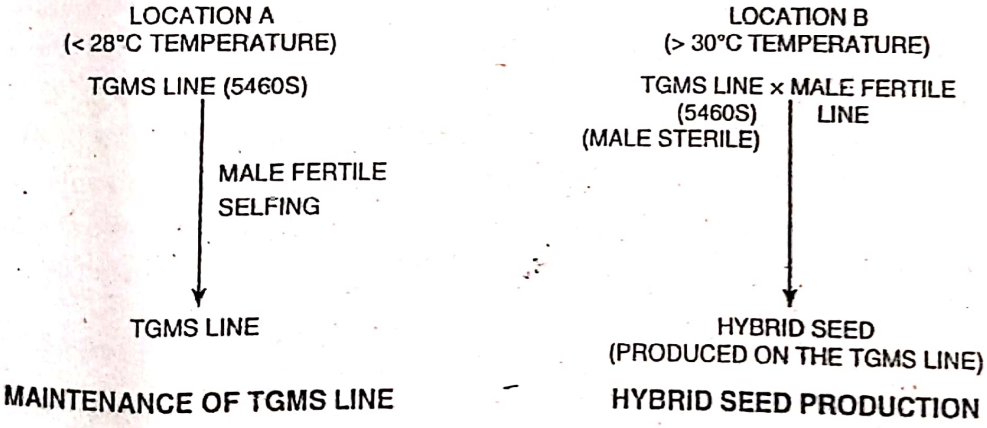

Fig. 22.3. Hybrid seed production using TGMS. TGMS line is multiplied at location A, where this line becomes male fertile due to the prevailing temperature. Hybrid seed is produced at location B where TGMS line is completely male sterile, again due to the prevailing temperature during the critical period of growth.

22.6.4. Self-Incompatibility

Two self-incompatible but cross-compatible lines are planted in alternate rows; the seed produced by both the lines would be hybrid seed. Alternatively, a self-compatible line may be

interplanted with a self-incompatible line. In this case, the seed from self-i ompatible line will be the hybrid seed, while that from the self-compatible line will be a m ture of hybrid and selfed seed. Therefore, the seed from self-incompatible line only is us l as the hybrid variety. This system is being commercially used for hybrid seed production n almost all the *Brassica* vegetable crops in Europe and Japan, *e.g.*, Brussel's sprouts (*B oleracea*) and cabbage (*B. oleracea*). The first hybrid varieties produced using self-incompatibility appeared in Japan in 1950, while such hybrids were produced in U.S.A. in 1954.

22.6.5. Pistillate Lines

Pistillate lines (Chapter 6) are being used for hybrid seed production in castor in India; all castor hybrids are based in such lines. Pistillate lines are of N, S or NES type. N type pistillate condition is akin to genetic male sterility and presents the same opportunities and suffers from the same limitations as the latter. This type is the most difficult to use and is not favoured. In contrast, NES is comparable to TGMS or PGMS and is the easiest to use in hybrid seed production, the scheme being the same as that for TGMS/PGMS. It is being used for castor hybrid seed production, *e.g.*, of GCH6. The S type, particularly, the improved S type pistillate lines are also used for seed production, but they are relatively more difficult to use than are NES type pistillate lines. In case of improved S type pistillate lines roguing of monoecious plants and of revertant inflorescences is necessary.

22.6.6. Manual Emasculation and/or Pollination

This method relies on manual emasculation and, in many cases, on manual pollination. Early hybrid maize production was based on manual emasculation, *i.e.*, detasselling. The Scheme of double cross production (Fig. 22.2) also involves detasselling for producing the single cross (C × D). Manual emasculation and pollination has been successfully used for hybrid seed production of tomatoes in Europe, and of cotton in India. The hybrid seed produced by manual operations is very costly. Consequently, this system of hybrid seed production is limited only to those crops where the returns are very high making the production of hybrid seed economically feasible.

22.6.7. Chemically-Induced Male Sterility

Several chemicals induce male sterility when applied during specific developmental stages of plants; such male sterility is known as ***chemically-induced male sterility*** (Chapter 6), and the chemical compounds are called ***chemical hybridizing agents (CHAs).*** The use of CHAs for rendering the pollen grains nonfunctional so that the treated line is used as the female parent in hybrid seed production is termed as ***chemical emasculation.*** Some of the CHAs are highly effective and are being used for commercial hybrid seed production in rice and wheat.

CHA-based hybrid seed production is being practised in China in case of rice. Two arsenical CHAs, named MG1 (based on zinc methyl arsenate) and MG2 (based on sodium methyl arsenate), have been developed for this purpose; of these MG2 is popularly used. Some of the hybrids based on CHAs are Qing-Hua-Fu-Gwi, Gang-Hua-Qing-Lan, Gang-Hua 2, You-Za I, Ya-You 2, etc. CHA-based hybrid seed production of wheat is being done in

U.S.A. The hybrid wheat s ...l so produced is marketed as HYBREX; the CHA employed for the purpose is RH0007 or h ...rex.

22.7. IMPROVING TH CHARACTERISTICS OF INBRED LINES

The direct isolation of in ...eds from source populations and their evaluation is a time consuming and expensive ...ocess. Further, the frequency of outstanding inbreds in direct isolations is very low. As ... result, various schemes have been suggested to improve the existing inbreds in respect ... (1) the productivity of inbreds to make them suitable for use in hybrid seed production, (2) ...isease and insect resistance or some other characteristics of the inbreds so that the characte...stics of the hybrid are improved, or (3) the combining ability of inbreds to increase the yielding ability of their hybrids. One or more of these objectives are fulfilled by the following methods: (1) pedigree selection, (2) backcross method (3) convergent improvement, (4) gamete selection, (5) somatic hybridization, (6) somaclonal variation and (7) genetic engineering.

The importance of inbred improvement in a hybrid programme can be illustrated by data pertaining to maize inbreds in U.S.A.-In 1936, only 2% of the inbreds were second cycle inbreds, while by 1960s, ~50% of the inbreds were second or higher cycle inbreds. Most of the new inbred lines produced since then are second or higher cycle inbreds.

22.7.1. Pedigree Selection

Pedigree selection consists of the isolation of inbreds from an outstanding single, or even a double cross; in U.S.A. single crosses are the most commonly used. *The pedigree method is the most commonly used method of inbred improvement.* Two inbreds that complement each other for disease resistan... and for other desirable attributes are crossed and in the segregating generations des...able recombinants are selected. The segregating generations are produced by selfing or clos... inbreeding. Most breeders prefer to handle a relatively smaller population size per cross fr... a larger number of different crosses; in U.S.A., most breeders consider 500 plants per cr... as adequate. In general, selection is initiated in F_2 itself, but random mating in F_2 befo... initiating selfing may be more useful. The method is closely similar to the pedigree meth... used in self-pollinated crops, as is suggested by its name. The inbreds produced in this ma...er are known as ***second cycle*** inbreds. Second cycle inbreds are considerably superior to the ...rst cycle ones in their *per se* performance. The performance of their hybrid may also be im...oved, but generally the improvement is not substantial.

22.7.2. Backcross Meth...

This is essentially the s...ne method as that applicable to self-pollinated crops (Chapter 18), and has the same appl...ations. An otherwise desirable inbred is used as the recurrent parent in a backcross progra...me. The donor parent is an inbred that has the desired character in a high intensity. Backcr...s method can be used to transfer any character that has high heritability. It has been used ... improve disease resistance, insect resistance, *e.g.*, resistance to stem border in maize, lod...g resistance and several other characteristics of inbreds. A specialised application of th... backcross method is the transfer of cytoplasmic male sterility

and of restorer genes into new inbreds. An example of the improvement of inbreds through the backcross method is furnished by bajra. The cytoplasmic male sterile line Tift23A, introduced from U.S.A., was highly susceptible to downy mildew leading to the high susceptibility of hybrids produced by using it as the female parent. Downy mildew resistant male sterile lines have been produced by backcrossing Tift23A to some downy mildew resistant African and Indian lines. *Backcross method is the second most popular approach of inbred improvement.* It has generally been used for improving oligogenic traits, and sometimes for quantitive traits as well. Generally, 3-5 backcrosses are made, depending on recovery of features of the recurrent parent and other factors.

22.7.3. Convergent Improvement

The concept of convergent improvement was put forth by Richey in 1927. *It is a special case of backcross, where a single cross is backcrossed separately to the two parental inbreds.* Selection is made on the basis of phenotype during the backcrossing. It is hoped that the two parental inbreds, *e.g.*, A and B, would be improved by retaining some of the favourable genes contributed by the other inbred. Thus A is expected to be improved by the genes from B, and B is expected to be improved by the genes from A. There is some evidence that this method improves, to some extent, the performance of inbreds as well as that of the hybrids produced from them. But generally the improvement is not substantial, and the method has not been widely used.

22.7.4. Gamete Selection

Gamete selection was proposed by Stadler in 1944. The basis for this scheme is the consideration that the frequency of superior gametes (p) in a random mating population is appreciably higher than that of the superior zygotes (p^2). This is particularly so when $p < 0.5$. In ***gamete selection,*** a good inbred line is crossed with a random sample of gametes from an open-pollinated variety. The resulting F_1 plants are selfed as well as crossed to a suitable tester. The tester may be an inbred or a population with a wide genetic base, depending upon the objective of the programme. The testcross progeny are evaluated in a replicated trial. The differences in the performance of the testcross progenies would be entirely due to the gametes from the open-pollinated variety, since the other parent is an inbred, which would be homozygous for all practical purposes. The selfed seeds from those F_1 plants that produced superior testcross progenies are planted in progeny rows (Fig. 22.4). Selfing and selection is continued to develop inbred lines, which are expected to be superior to the inbred used as one of the parents of the initial cross.

This scheme has not been used on a large scale for the development of inbred lines. It is argued that the scheme has considerable potential and should be widely used for producing superior inbreds. But a serious limitation of the scheme is that the superior gamete can not be isolated as a homozygote. In order to overcome this disadvantage, Hallauer (1970) suggested zygote selection, *i.e.*, simultaneous selfing of those plants of the open-pollinated variety that are crossed with the tester. Inbreds are then developed from the selfed seeds of the superior plants of the open-pollinated variety. The merits of this proposal, however, are yet to be demonstrated (see, Banga and Banga, 1998).

22.7.5. Somatic Hybridization

Somatic hybridization, particularly production of cybrids and asymmetric hybrids, offers unique opportunities for the improvement of inbreds, especially the CMS lines (see, Section 22.8).

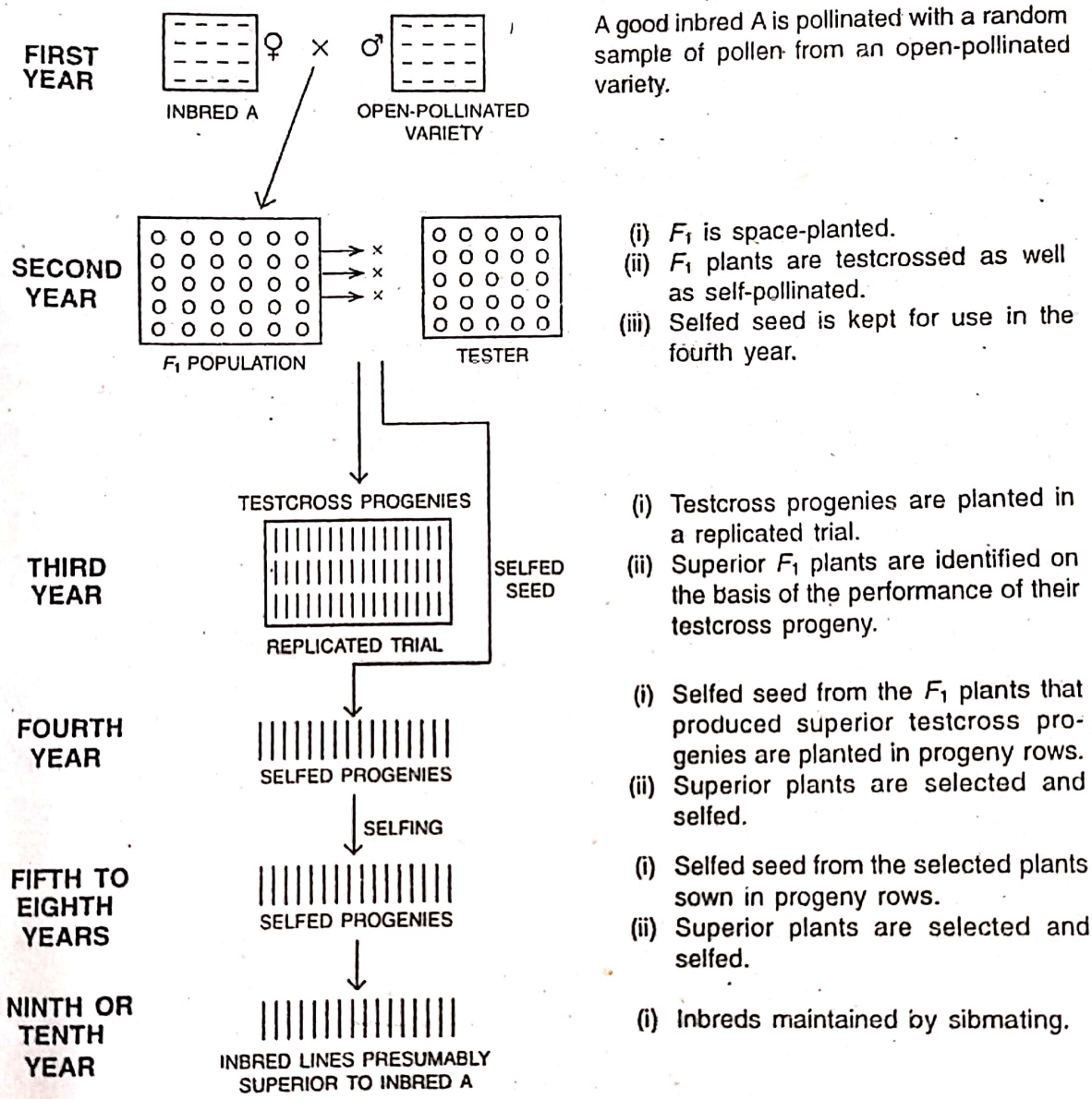

Fig. 22.4. Gamete selection in maize for the improvement of inbred lines.

22.7.6. Somaclonal Variation

In vitro culture of plant cells generates genetic variation, which is often expressed in the plants regenerated from them. This variation can be used to improve both nuclear as well as cytoplasmic genotypes of inbreds. For example, cell selection has been used to isolate a herbicide-tolerant somaclonal variant from cell cultures initiated from immature embryos of a maize inbred. However, so far there is no commercial example of useful exploitation of this potential approach for inbred improvement.

22.7.7. Genetic Engineering

Genetic engineering offers an exciting tool to improve specific traits of inbreds provided a

transgene is available for the purpose. For example, suitable versions of the *cry* gene of *Bacillus thuringiensis* have been transferred in maize inbreds and insect resistant commercial hybrids have been produced using such inbreds. Attempts are being made to use various plant genes, *e.g.*, protease inhibitors, lectins, etc., to engineer insect resistance in plants. Similarly, many transgenes have been shown to generate resistance to fungal diseases (*e.g.*, vacuolar chitinase, β-1,3-glucanase, groundnut stilbene synthase, tobacco pathogenesis-related protein PR-19 genes, etc.), while many others lead to resistance to bacterial diseases. Similarly, a variety of strategies can be used to generate resistance to viral diseases; some of the approaches being the use of virus coat protein gene, satellite RNA, nucleoprotein gene, etc. It may be expected that in future inbreds will be increasingly subjected to genetic engineering to rectify their specific defects, and even to introduce into them some novel useful features.

22.8. DIVERSIFICATION/IMPROVEMENT OF CMS LINES

In the past, one or a few outstanding CMS lines were extensively or even exclusively used in hybrid variety development programmes. For example, the early hybrid bajra programme in India was virtually exclusively based on the CMS line Tift23A, which had the Tifton cytoplasm for male sterility. Such a situation is highly undesirable for the following reasons.

1. The use of a single source of CMS results in all the hybrids of the crop having the same cytoplasm. This can be disastrous if the cytoplasm specifies susceptibility to a disease or insect pest. The Texas male sterility cytoplasm (CMS-T) provides an excellent example. CMS-T was virtually exclusively used for hybrid seed production in U.S.A. This cytoplasm specifies susceptibility to Southern leaf blight, a minor disease of maize. As a result, in 1970, maize crop suffered a colossal loss due to a severe Southern leaf blight epidemic.
2. When a single CMS line is used in hybrid programme, the nuclear genotype of this line may specify a deleterious feature, *e.g.*, susceptibility to a disease. In such a case, all the hybrid varieties of the crop will have the weakness specified by the nuclear genotype of this female parent. This could be disastrous as was exemplified by the severe downy mildew and ergot epidemics in India in the hybrid bajra varieties based on Tift23A CMS line (during the early seventies).
3. Use of one or two CMS lines puts a ceiling on the hybrid performance as well as limits the choice of the male parent of hybrids. This is because the genotype of female parent remains a fixed entity, and pollinators have to be developed that combine well with this genotype. Thus the potential of the hybrids is predetermined by the genotype of the female parent.
4. The CMS lines available in a crop may have a narrow range of adaptation. In such a case, use of these CMS lines in hybrid programmes would be limited to the areas of adaptation of the CMS lines.

In view of the above, it is highly desirable to deliberately generate diversity in both the cytoplasmic as well as the nuclear genotypes of CMS lines. The various approaches to achieve these ends are briefly outlined below (Table 22.4).

TABLE 22.4
Diversification of CMS lines, *i.e.*, female parents of hybrids

Type of modification	Breeding approach	Objective	Remarks
Cytoplasmic Genotype or Plasmatype*			
A. Mitochondrial genome			
1. Different sources of CMS			
(*i*) Mutant cytoplasms	Spontaneous or induced mutations	To avoid genetic vulnerability due to plasmon* uniformity	Many of MS cytoplasms can not be used in hybrid seed production
(*ii*) Alien cytoplasms	Transfer from a related species using backcross or protoplast/cytoplast fusion		
2. Defect correction			
(*i*) Somaclonal variation	Screening or cell selection	Correction of a defect specified by mtDNA either through mutation or recombination in mtDNA	—
(*ii*) Somatic hybridization	Production of cybrids or asymmetric hybrids		
B. Chloroplast genome (defect correction)			
Somatic hybridization	Asymmetric hybrids or cybrids	To replace the defective chloroplast with normal ones	Successful in *B. napus*** and *B. juncea*
Nuclear Genotype			
A. Diverse CMS lines			
Transfer of male sterile cytoplasm to new inbred lines	Backcross programme using the new inbred lines as recurrent male parents	To maximise the possibility of exploit-ing nuclear gene combinations and nuclear × cytoplasmic interactions	An important component of any vibrant hybrid development programme
	Production of asym-metric hybrids/cybrids		
B. Defect correction			
(*i*) Gene transfer	Backcross (two-steps): first using the gene donor line, then the A line as nonrecurrent parent	To correct defects specified by the nuclear genotype of existing CMS lines	In case of bajra Tift23A
(*ii*) Mutagenesis	Mutation induction using physical or chemical mutagens in the B line, followed by transfer into the A line		In case of bajra Tift 23A
(*iii*) Somaclonal variation	Screening or cell selection		—
(*iv*) Genetic engineering	Gene transfer through genetic transformation		For *cry* and other genes in the case of maize inbreds, etc.

* ***Plasmon*** denotes the complete set of genes present in the cytoplasm, *i.e.*, in mtDNA and cpDNA. ***Plasmatype*** denotes the cytoplasmic genotype, while ***plasmagene*** is a gene present in the cytoplasm.

** The *Ogura* cytoplasm of *B. napus*; mitochondria from wild radish (*Ogura* cytoplasm) combined with *B. napus* chloroplasts.

22.8.1. Diversification of Cytoplasmic Genotype

The ***cytoplasmic genotype*** consists of mitochondrial and chloroplast genomes, including any plasmid-like elements present in these organelles. The mtDNA is involved in the control of CMS; therefore, a diversification in the mitochondrial genome would mean (1) search for new sources of CMS and/or (2) a correction of the defects specified by mtDNA. Both these approaches are applicable to (3) the diversification of chloroplast genome as well.

22.8.1.1. Mitochondrial Genome. Diversification in mitochondrial genome may be achieved by (1) a search for new sources of CMS and by (2) correction of defects of an existing CMS source through (2a) somaclonal variation and (2b) recombination of mitochondrial genome.

1. ***Search for New Cytoplasmic Sources.*** It is highly desirable that more than one distinct CMS sources of commercial value should be available in a crop. In many crops, two or more sources of CMS are known, but unfortunately one of them has achieved commercial success. For example in case of bajra, five distinct CMS cytoplasms are available (Table 22.5) but only A_1 cytoplasm is in commercial use. Cytoplasm A_4 is extremely stable for male sterility, restorer genes are found in bajra (in low frequency) and wild millet (high frequency), and hybrids based on A_4 cytoplasm yielded more than those based on A_1 cytoplasm. It is likely that A_4 cytoplasm would be successful commercially.

2. ***Defect Correction.*** In case a weakness of the CMS line is known to be specified by mitochondrial genome, efforts may be made to correct this defect through (2a) mutagenesis, (2b) somaclonal variation or (2c) recombination in the mitochondrial genome.

2a. ***Somaclonal Variation.*** Plants regenerated from tissue culture and their progeny show genetic variation (***somaclonal variation***), which may arise due to changes in nuclear, mitochondrial and/or chloroplast genomes. This approach can be used to isolate somaclonal variants of a CMS line that is free from the weakness specified by mtDNA. Attempts were made to isolate *Helminthosporium* blight resistant somaclonal variants of Texas male sterility cytoplasm of maize. Cell cultures of CMS-T lines were selected for resistance to the toxin produced by the pathogen, and plants were regenerated from the toxin resistant cell lines thus isolated. The regenerated plants were resistant to the Southern leaf blight pathogen but, unfortunately, they were also male fertile.

2b. ***Recombination in Mitochondrial Genome.*** Mitochondria of the CMS line showing the defect may be brought together in the same cell with the mitochondria of another line, say, the maintainer line, free from this mitochondria-specified defect. This can be easily achieved by a fusion of protoplasts of the two lines. In somatic hybrids, mitochondrial genome undergoes extensive recombination. Ultimately, hybrid plants are regenerated from somatic hybrid cells; a given somatic hybrid plant usually contains the mitochondria of one or the other parent or a recombinant type of mitochondria. So long as the CMS and the concerned defect are specified by two separate regions of the mitochondrial genome, it should be possible to isolate plants expressing CMS but lacking the defect in question.