International Journal of Farm Sciences **8(2):** 109-113, 2018; doi: 10.5958/2250-0499.2018.00054.X

Review

Barnase-barstar system: an indelible technique to produce hybrid seeds in self-pollinated crops

SUBHASH CHAND, OM PRAKASH PATIDAR, VIJAY KAMAL MEENA and AALOK SHIV

Division of Genetics, Indian Agricultural Research Institute, Pusa 110012 New Delhi, India
Email for correspondence: subhashchand5415@gmail.com

 Received: 9.11.2017/Accepted: 13.2.2018

ABSTRACT

Self-pollinated crops are sensitive to exploit high level of heterosis. The improvement of crop plants through the production of hybrid varieties is a major goal of plant breeding. The genetically engineered techniques have a pivotal role in developing male sterility and restorer systems. Chimaeric ribonuclease gene (barnase) expression within the anther selectively destroys the tapetal cell layer that surrounds the pollen sac, prevents pollen formation and leads to male sterility. A chimaeric tapetal cell-specific ribonuclease inhibitor gene (barstar) was used in male fertile plants to restore male fertility in F_1 progeny. Cytotoxic ribonuclease activity in the anther was suppressed to restore male fertility in F_1 hybrid by the formation of cell-specific RNase/RNase inhibitor complexes.

Keywords: Heterosis; genetic engineering; male sterility; barnase; barstar

INTRODUCTION

In general cross-pollinated species show heterosis particularly when inbred lines are used as parents. Heterosis has been commercially exploited in many cross-pollinated crops such as maize, bajra, sorghum, alfalfa etc. Many crosses in self-pollinated crops also show heterosis but the magnitude of heterosis is generally smaller than that in case of cross-pollinated crops. But in some self-pollinated crops heterosis is large enough to be used for production of hybrid varieties eg rice, wheat etc (Singh 2016).

The exploitation of heterosis in any crop depends upon several factors such as exploitable level of heterosis or magnitude of heterosis, the pollination control mechanism (male sterility, self-incompatibility etc) and the commercial viability of seed production programme (benefit-cost ratio, seed multiplication ratio etc). The chief drawbacks in the use of hybrid varieties in self-pollinated crops are small structure of hermaphrodite flowers, lower seed setting per pollination, high requirement of seeds for commercial cultivation especially in wheat, polyploidy nature, complex genome etc. Many crop plants do not have self-incompatibility and/or male sterility genes and use of male sterility requires a fertility restorer system (Singhal 2013). These factors make a difficult situation for the production of large quantities of hybrid seed. Thus there is an urgent need to develop alternative ways to construct male sterility system which can act in different groups of crops. The genetic engineering-based nuclear genetic male sterility system may be pivotal in this journey.

In this article more focus is on the genetically engineered system of male sterility (barnase-barstar system) to control the pollination in self-pollinated crops to produce hybrid seeds without using mechanical process ie hand emasculation and pollination mechanism (which enhances the cost of hybrid seed production as compared to yield advantage over development of commercial varieties).

Male sterility is characterized by the production of non-functional unviable pollen grains (male gametes) while female gametes function normally (Albertsen and Phillips 1981). It occurs in nature sporadically due to spontaneous mutation and is also caused by environmental factors. In general nuclear genetic male sterility is governed by single recessive gene *ms* but in some cases it may be dominant in nature eg safflower etc. Barnase-barstar system is a type of nuclear genetic male sterility (Kaul 1988).

Genetics of barnase-barstar system

The first success in developing genetically engineered male sterility in crop plants was achieved by Mariani et al (1990) by transforming tobacco and rapeseed plants with a chimeric dominant gene barnase (bacterial RNase from *Bacillus amyloliquefaciense*) driven by a tapetum-specific promoter (TA29) from tobacco. TA29 promoter is responsible for the expression of the barnase gene specifically to anther tapetal cells which causes selective destruction of the tapetal cell layer that surrounds the pollen sac by hydrolysing the tapetal cells. It leads to abnormal pollen formation and causes male sterility without hampering female fertility. Male sterile flowers show reduction in length of stamen filament, petal size, bud diameter and tapetal cell content. Sterile anther carries empty exine.

Mariani et al (1992) demonstrated fertility restoration in TA29-barnase male sterile plants by gene encoding the barnase-specific RNase inhibitor called barstar which was isolated from same bacteria *B amyloliquefaciense*. When genetically engineered male sterile plant is crossed with plant carrying TA29-barstar gene the F_1 progeny shows co-expression of both genes in the anther of male fertile plants. In this system fertility restoration is due to the formation of tapetal cell-specific barnase/barstar protein complexes which completely inactivate the barnase enzyme. However to eliminate the cytotoxic effects of barnase in the tapetum cells the amount of barstar must be equal or greater than that of barnase.

This dominant nuclear genetic male sterility system faces same drawback as GMS system. During hybrid seed production the plants in female rows segregate in the ratio of 1:1 for male sterility and male fertility (Bedinger 1992). To counter this problem the barnase gene was linked to a dominant herbicide resistant gene (bar) which conferred resistance to broad-spectrum herbicide Basta (active ingredient is phosphinothricin or PPT). The bar gene has been put under control of the constitutive promoter CaMV 35S. The bar gene expression starts from the seedling stage itself. When seedlings are sprayed with Basta only the male sterile plants survive and the male fertile plants are killed as they lack bar gene. Phosphinothricin also known as glufosinolate is a glutamine synthetase inhibitor that binds to the glutamate site.

Phosphinothricin-treated plants die due to building up of ammonia in the thylakoid lumen leading to the uncoupling of photophosphorylation (Denis et al 1993). The uncoupling of photophosphorylation causes the production of reactive oxygen species, liquid peroxidation and membrane disruption. The use of bar gene allows elimination of male fertile segregants from female rows in the hybrid seed production plot thus assuring 100 per cent pure hybrid seed production.

Maintenance of male-sterile line (A-line)

Male-sterile plants (A-line, hemizygous for male sterility gene) are crossed with male fertile plants (B-line, isogenic line of A-line except for gene of fertility and resistance against phosphinothricin herbicide). Two genetically different types of seed (barn-bar/0 and 0/0) will be formed on the flower of male sterile plants after fertilization process in 1:1 ratio (male sterile:male fertile).

Male fertile plants are undesired in hybrid seed production programme because it leads to the production of spurious seeds due to selfing of male fertile plants which usher to reduce the level of achievable heterosis in the farmers' fields (Heslop-Harrison and Heslop-Harrison 1970). Thus male fertile plants must be removed from the hybrid seed production field by using herbicide at seedling stage (before initiation of flowering). The bar gene is attached with constituive promoter CaMV 35S. Thus bar gene will be expressed from the seedling stage itself. The Basta (trade name) herbicide is used at seedling stage of crop to rogue out male fertile plants from the field and all the remaining plants in the field will be male sterile (seed parent for production of hybrid) (Fig 1).

Hybrid seed production

Male sterile plants (barnase-bar/0) are crossed with male fertile plants (restorer line which is having male fertility gene in homozygous condition ie barstar-bar/barstar-bar). Pollens will move from male fertile plants to male sterile plants and pollinate the male sterile plants. The seeds from restorer lines must be harvested first compared to male sterile plants because selfing in restorer lines will adversely affect the quality of hybrid seed. After fertilization process two genetically diverse seeds (barstar-bar/barnase-bar and barstar-bar/0) will be formed.

Hybrid seeds harvested from male sterile plants will be male fertile and resistant to Basta herbicide. In earlier case restorer parent was having only barstar gene (not the bar gene) so 50 per cent of

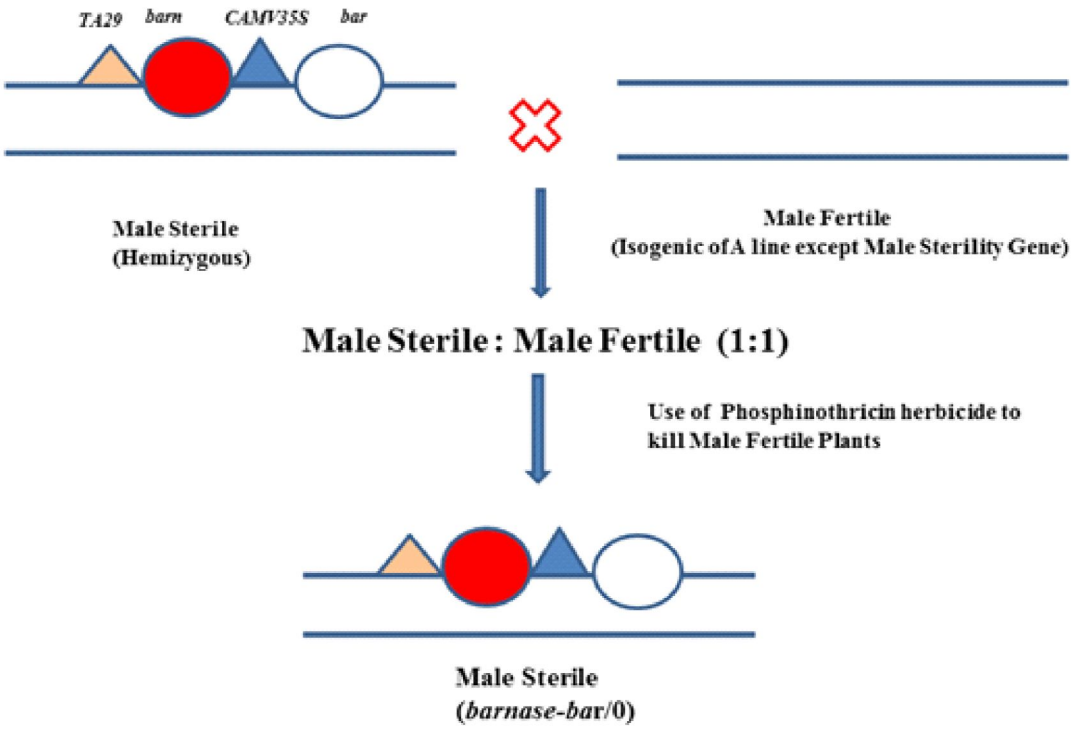

Fig 1. Maintenance of A line in barnase (barn)- barstar (bars) system of male sterility

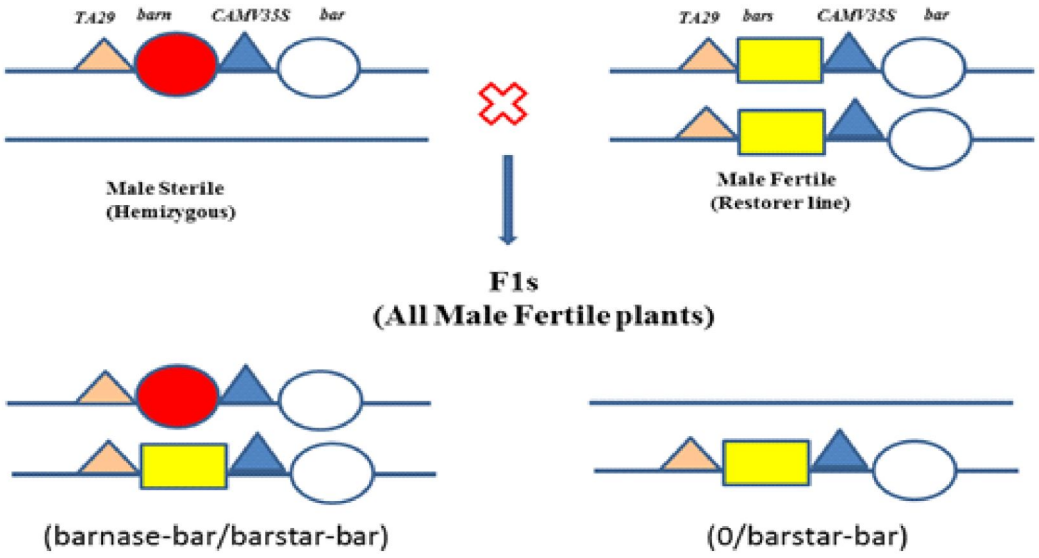

Fig 2. Hybrid seed production in barnase- barstar system of male sterility

hybrid seeds were susceptible to herbicide and 50 per cent were resistant against herbicide. So the farmers were advised that they should not use those herbicides which are having active ingredients as phosphinothricin. But in current situation farmers can use same herbicide because all hybrid plants will show resistance due to presence of bar gene in the genome.

The restorer parent should be genetically diverse and must show high specific combing ability with male sterile parent. As like B line it should not be isogenic to male sterile line. Otherwise high level of heterosis cannot be exploited in hybrid seed programme. This system was first time used in oilseed rape (*Brassica napus* cv Drakkar).

This strategy is used successfully for the commercial production of canola hybrid in Canada, lettuce and chicory hybrids. Earlier DMH-11 hybrid (*B juncea*) was also developed in India whose commercial release was stayed by the Supreme Court of India even though genetic engineering appraisal committee (GEAC) had approved its commercial production (Ray et al 2007) (Fig 2).

Advantages of barnase/barstar system over genetic male sterility (GMS)

Basically barnase/barstar technique is a genetically engineered nuclear genetic male sterility but has several advantages over GMS (Virmani et al 2003):

- GMS system in crop plants is dependent upon the availability of natural sources of male sterility and effective restoration system. But barnase/barstar technique does not depend on natural sources of male sterility.
- GMS system is crop- and genotype-dependent but any genotype can be converted into male sterile line in case of barnase/barstar system.
- In GMS system to remove male fertile plants from segregant plants there is need of morphological markers which must express before flowering and cost of rouging is high and tedious. Male fertile plants can be killed at seedling stage by using herbicide in case of barnase/barstar system.
- GMS system is depending upon specific requirement of temperature (thermo-sensitive genic male sterility) and/or photoperiod (photoperiod sensitive genic male sterility). But specific congenial condition is not required in case of barnase/barstar system.
- In GMS system produced hybrid seeds are less pure compared to barnase/barstar system.

Limitations of barnase/barstar system

- There is reversion to fertility from male sterile plants.
- Fifty per cent population is lost; so double the amount of female parent seeds is needed for hybrid seed production which limits the applicability of the system in crops with a low multiplication factor such as wheat.
- The seeds of hybrid produced are having transgene (barnase, barstar and bar genes). So it is a big challenge for their release in public domain due to ethical, social, environmental and biological issues.

CONCLUSION

In nature availability of male sterility and fertility restoration sources are limited. Thus barnase/barstar system provides a way to develop male sterility system in different self- and cross-pollinated crops by using genetic engineering. In crop plants where fruit is not of economic importance (eg lettuce, carrot and cabbage) male sterile plants can be crossed with any pollinator line to produce hybrid seeds. But it will be necessary to restore full male fertility in the progeny of those crops where fruit is harvested product such as tomato, wheat, rice, corn etc.

REFERENCES

Albertsen MC and Phillips RL 1981. Developmental cytology of 13 genetic male sterile loci in maize. Canadian Journal of Genetics and Cytology **23(2):** 195-208.

Bedinger P 1992.The remarkable biology of pollen. Plant Cell **4:** 879-887.

Denis M, Delourme R, Gourret JP, Mariani C and Renard M 1993. Expression of engineered nuclear male sterility in *Brassica napus*. Plant Physiology **101(4):** 1295-1304.

Heslop-Harrison J and Heslop-Harrison Y 1970. Evaluation of pollen viability by enzymatically induced fluorescence: intracellular hydrolysis of fluorescein diacetate. Stain Technology **45(3):** 115-120.

Kaul MLH 1988. Male sterility in higher plants. Monographs on Theoretical and Applied Genetics, Vol 10, Springer, Heidelberg, Berlin.

Mariani C, De Beuckeleer M, Truettner J, Leemans J and Goldberg RB 1990. Induction of male sterility in plants by a chimaeric ribonuclease gene. Nature **347:** 737-741.

Mariani C, Gossele V, De Beuckeleer M, De Block M, Goldberg RB, De Greef W and Leemans J 1992. A chimaeric ribonuclease-inhibitor gene restores fertility to male sterile plants. Nature **357:** 384-387.

Ray K, Bisht NC, Pental D and Burma PK 2007. Development of barnase/barstar transgenics for hybrid seed production in Indian oilseed mustard, *Brassica juncea* (L) Czaern and Coss using a mutant

acetolactate synthase gene conferring resistance to imidazolinone-based herbidice 'Pursuit'. Current Science **93:** 1390-1396.

Singh BD 2016. Plant breeding principles and methods. Kalyani Publishers, Ludhiana, Punjab, India.

Singhal NC 2013. Hybrid seed production in field crops: principle and practices. Kalyani Publishers, Ludhiana, Punjab, India.

Virmani SS, Sun ZX, Mou TM, Jauhar AA and Mau CX 2003. Two-line hybrid rice breeding manual. International Rice Research Institute, Los Banos, Philippines.