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Barnase-Barstar System: A New Era in The Development of Male Sterility in Self-Pollinated Crops

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Introduction

Heterosis is a phenomenon that describes how F1 hybrids outperform their parents in one or more characteristics. Many cross-pollinated crops, including bajra, sorghum, and maize, have been commercially exploited for this phenomenon. Although many self-pollinated crops exhibit heterosis as well, it is much less pronounced. It is, however, big enough to generate hybrid varieties, as is done with wheat and rice.

The usability of heterosis in a crop depends mainly on three factors: 1) the exploitable level of heterosis and combining ability (GCA - General Combining Ability and SCA - Specific Combining Ability); 2) the pollination control mechanism, which includes self-incompatibility and male sterility; and 3) the commercial viability of seed production, which consists of the benefit-cost ratio, the seed multiplication ratio, etc. The chief drawbacks in the use of hybrid varieties in self-pollinated crops are the small structure of hermaphrodite flowers, lower seed setting per pollination, a higher requirement of seeds for commercial cultivation as in wheat, polyploidy nature, complex genome, etc.

Many self-pollinated crop plants do not have self-incompatibility or any male sterility system. Moreover, to use male sterility, we need a fertility restorer system too. To address the problem, one of the potential solutions may be by using the barnase-barnstar technique to achieve male sterility and hence attain the desired parental cross.

Genetics of the Barnase-Barstar system

Mariana et al. (1990, 1992) was the first to achieve success in transforming rapeseed plants with this system. This Barnase-Barstar system is controlled by two genes, namely Barnase and Barstar. Let's first talk about the Barnase gene. It is a chimeric dominant gene (source: *Bacillus amyloliquefaciens*) that encodes for a potent ribonuclease driven by a promoter, TA29. Expression of this gene destroys the tapetal cells surrounding the pollen sac by hydrolysis. This results in abnormal pollen formation, which causes male sterility.

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As we know, to utilize male sterility in hybrid seed production, we need a fertility restorer gene too so that we obtain fertile F1 hybrids. In contrast to the Barnase gene, the barstar gene, also obtained from the same bacteria (*Bacillus amyloliquefaciens*), encodes for a barnase-specific ribonuclease inhibitor. This enzyme completely inactivates the activity of the barnase enzyme and restores male fertility by eliminating the cytotoxic effects of barnase.

The F1 hybrid will have both the Barnase and Barstar gene and show co-expression of both. In this system, fertility restoration is due to the formation of barnase/barstar protein complexes which acts specifically on tapetal cells, inactivating the barnase enzyme. To restore male fertility, the amount of Barstar in the hybrid must be equal to or greater than that of Barnase.

Maintenance of the male-sterility line (A-line)

To maintain the A-line, the male sterile line (A-line or female parent: hemizygous for the Barnase gene - barn-bar/0) is crossed with the male fertile line (B line, which is isogenic to the A-line except for any fertility or sterility system - 0/0). As a result of this cross, the female rows segregate in a ratio of 1:1 for male sterility and male fertility. Which means there will be two types of seeds formed in the flowers of male sterile plants - 1) barn-bar/0 (male sterile) and 2) 0/0 (male fertile). But herein lies a problem. In hybrid seed production, those male fertile plants obtained in female rows by this cross are undesirable as they will go for selfing, reducing the achievable heterosis in the farmer's field.

What's the solution? To counter this problem, the dominant barnase gene is linked to a dominant herbicide-resistant gene (bar), which confers resistance to the herbicide Basta (active ingredient: phosphinothricin). CaMV 35S acts as the promoter of this gene. This bar gene starts to express itself from the seedling stage. So, in the seedling stage, if we spray Basta, then only the male sterile plants will survive as they contain the barnase gene, which has been linked with the resistant gene bar. The use of the bar gene will eliminate the male fertile plants in the female line, solving the problem.

The question may arise: how to maintain the B line (male fertile)? The answer is simple. Since we are working with a self-pollinated crop, no additional labour is necessary. Assured self-pollination will be there in the B line and we will get its seed every season to maintain the A line.

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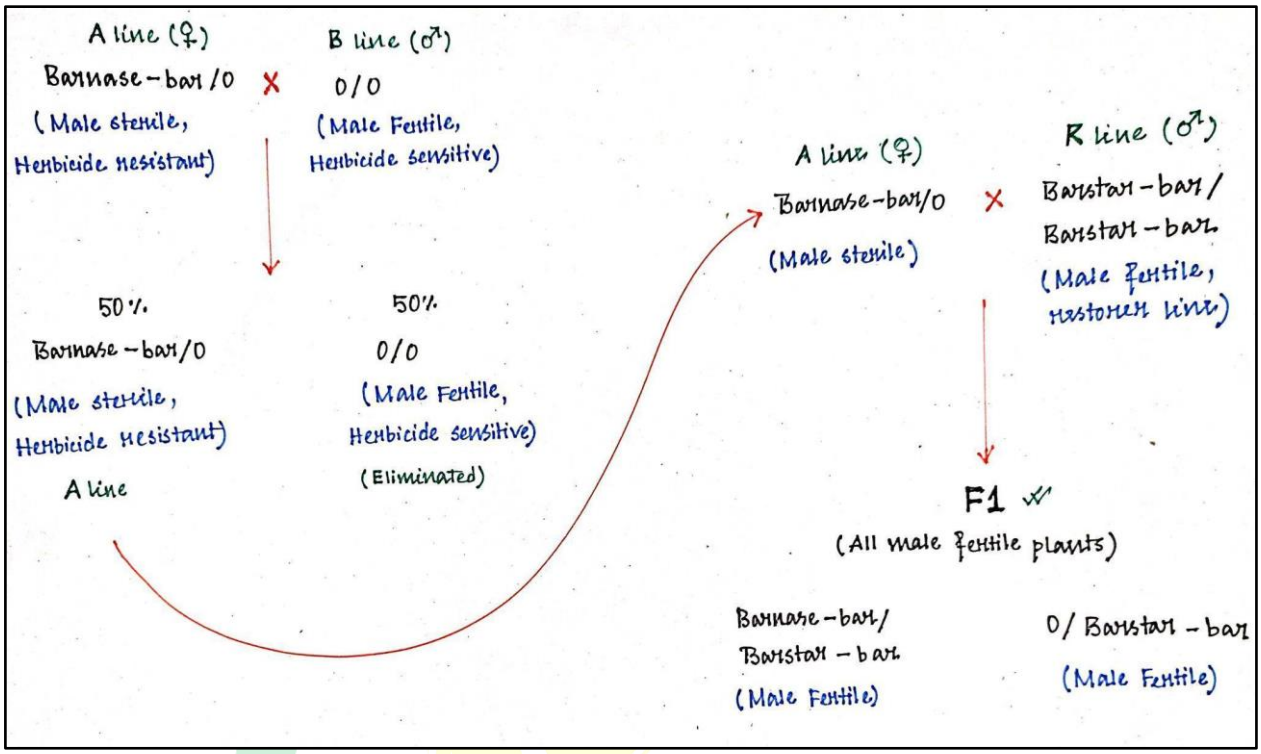

Figure 1. Illustration of barnase-barstar system

Hybrid seed production

For hybrid seed production, we now have to cross the A-line and the R-line. Here the A line is male sterile (barnase-bar/0) which is the female parent, and the R line is the fertility restorer line (barnstar-bar/barnstar-bar) which is the male parent. By this cross we will get two genetically diverse seeds - 1) barnase-bar/barstar-bar and 2) 0/barstar-bar. Hybrid seeds harvested from the male sterile plants will be fertile and resistant to Basta herbicide.

The restorer plant should be genetically different from the A-line so that we can expect a high level of heterosis in the hybrid. The barstar in the restorer plant (R line) is needed to restore the fertility in the hybrid for effective seed setting. This system was first time used in oilseed rape.

Advantages of the Barnase-Barstar system over the GMS

- In contrast to the GMS system, it does not depend on natural resources.
- The male fertile plants obtained in the female row during the maintenance of A line can be killed in the seedling stage by the use of the herbicide. This makes it cost-effective.
- No specific temperature or photoperiod requirement exists.
- Hybrid seeds are purer than those of GMS.

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Limitations

Transgenes are involved, for which there may arise ethical, social, and environmental issues. Particularly in Indian minds, there is still a confusion regarding the transgenics and subsequent doubts. Even there are different schools of thoughts for this technology among the scientists. This factor reduces the adaptability of this new mustard in the farming community.

DMH-11 hybrid

Based on the barnase-barstar gene system, DMH-11 (Dhara Mustard Hybrid - 11) is a newly created hybrid mustard variant. It is a result of a cross between two mustard varieties: 1) 'Varuna' and 2) East European 'Early Heera-2'. This cross has been done after introducing the barnase and barstar gene from the soil bacterium *Bacillus amyloliquefaciense*. The barnase gene in Varuna causes male sterility in it. Whereas, barstar in Early Heera-2 blocks the effect of barnase allowing seeds to be produced. DMH-11 is thus a transgenic crop. It has been developed at the Centre for Genetic Manipulation of Crop Plants, at the University of Delhi under the supervision of DR. Deepak Pental.

DMH-11 shows a 28% higher yield than its parent, Varuna, and is 37% better than zonal checks, or local varieties, which are considered to be the best in different agro-climatic zones. This marks the success of the barnase-barstar system-based development of the DMH-11 hybrid of mustard (*Brassica juncea*).

Recently, on October 18, 2022, the Government of India approved the environmental release of DMH-11 for its seed production as per existing ICAR guidelines and conditions imposed by GEAC (Genetic Engineering Appraisal Committee).

Conclusion

Sources of male sterility and fertility restoration are rare in nature. In such a scenario, the barnase-barstar gene system provides us with a way to develop male sterility in several self-pollinated crops using bacteria as a source for the genes. This system has been successfully deployed in countries like Canada, Australia, and the United States for many decades. In India, DMH-11 is a product of this system.

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