AgriCos e-Newsletter
ISSN: 2582-7049
AgriCos e-Newsletter
Open Access Multidisciplinary Monthly Online Magazine
Volume: 03 Issue: 05 May 2022 Article No: 06

Biotechnology Based Genic Male Sterility System in Maize

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SUMMARY

MAIZE is one of the most essential crops for heterosis that has been effectively utilised. Manual or mechanical detasseling has various drawbacks, including being time-consuming, labor-intensive, and costly, as well as being damaging to plant development, low yield of hybrid seed. Similarly, the CMS method has some inherent issues, such as the limited genetic resources for restorer lines, the low genetic diversity in CMS lines and restorer lines, and the possibility for increased disease susceptibility and inconsistent CMS line restoration in maize. By exploiting the GMS genes and their related mutations to maintain and propagate GMS lines as female parents for hybrid seed production, a lot of work has gone into developing biotechnology-based male-sterility systems. For instance, a system called seed production technology (SPT) was developed to produce recessive GMS lines using the *maize male sterility 45* (*ZmMs45*) gene and the multi-control sterility (MCS) systems by using maize GMS genes *ZmMs7*, *ZmMs30,* and *ZmMs3*. In addition, employing the dominant GMS gene ms44 in maize, a dominant male-sterility system was established to produce dominant GMS lines.

INTRODUCTION

The SPT transgenic maintainer line is made by transforming the target plant with an SPT construct containing three genes: (1) a wild-type male-fertility gene (Ms45) to restore fertility, (2) a pollen lethality gene (ZmAA) to disrupt normal pollen development, and (3) a fluorescent seed colour marker gene (DsRed2) for seed sorting. All pollen grains generated by the SPT maintainer line have the ms45 genotype, 50% of which are non-transgenic and 50% of which contain SPT transgenic elements. The expression of the ZmAA gene prevents the later grains from germinating. Seeds with the same genotype as the SPT maintainer line (ms45/ms45+SPT-T-DNA) and seeds with the male-sterile genotype (ms45/ms45) result from self-pollination of the SPT maintainer line. The two types of seeds can be effectively distinguish by mechanical colour sorting as the 50 percent of seeds that have the SPT components produce a red hue under green excitation light. When the SPT maintenance line pollinates the male-sterile line (ms45/ms45), almost all of the seeds produced have the ms45/ms45 genotype and may be used as male-sterile female lines for cross-breeding and hybrid seed production. Although the SPT system has numerous potential benefits, the rate of transgenic transfer through pollen has been observed to range from 0% to 0.518 percent (Wu et al., 2016). Even though the final seed-sorting step reduces the risk of transgenic seed in the hybrid production field, transgenic pollen flow during the male-sterile line propagation phase via the transgenic intermediary remains a possibility, which limits its application in countries and regions with strict biotechnology regulatory oversight.

Maize Multi-Control Sterility System

The Multi-Control Sterility (MCS) system was designed for transforming a single MCS construct into the maize ms7 or ms30 mutant to reduce transgene transmission through the pollen of SPT maintainer lines (Zhang et al., 2018). The MCS construct has five functional modules: (1) a male-fertility gene (ZmMs) to restore fertility, (2) two pollen-disruption genes (ZmAA and Dam) to disrupt transgenic pollen production, (3) a fluorescent colour marker gene (DsRed2 or mCherry) for seed colour sorting, and (4) a herbicide-resistant gene (Bar) to prevent contamination of seeds as it is essential for the propagation of high-purity MCS transgenic maintainer line seeds through herbicide spraying during specific stages of production. The transmission rate of transgenic pollen is considerably reduced. In addition, the *Bar*gene in MCS construct aids in the propagation of very pure transgenic maintenance line seeds. Compared to the SPT construct, the MCS constructis more likely to produce maintainer and male-sterile lines with higher purity, as well as reduce transgene transmission risk. A field test of the MCS system is now underway in China to encourage commercial applicability.

Dual-Component BMS Systems

Maize and other plants have dual-component BMS systems. Many dual-component BMS systems have been developed to date, including the Barnase/Barstar system, the Cysteine protein/Cystatin system, the MYB103 chimeric repressorrestorer system, and transcriptional silencing of heterologous anther promoters. The discovery of these genes has increased our knowledge of BMS processes in plants and opened up new possibilities for establishing novel hybrid seed production systems in crops.

Barnase/Barstar Systems

This system was the first dominant BMS system developed in rapeseed and tobacco (Mariani *et al*., 1990, 1992). The individual plants are transformed using barnase and barstar genes fused with the tapetum-specific TA29 promoter. The TA29-barnase transformed plants are completely male sterile and are crossed with TA29-barstar-expressing fertile plants, which results in the co-expression of barnase and barstar genes in the anther tapetal cell layer. The restoration of fertility in the hybrid F1 plants is due to inactivation of barnase by barstar.

Cysteine Protease/Cystatin Systems

Similarly, expression of the cysteine protease in tapetal layer leads to male sterility in the Cysteine protease/ Cystatin system, and the co-expression of cystatin with cysteine protease restores male fertility.

MYB103 Chimeric Repressor-Restorer System

MYB103, which encodes an R2R3 MYB TF, is essential for tapetum and pollen formation in Arabidopsis. The EAR (ERF-associated amphiphilic repression) motif, a dominant repression motif, can be used to disrupt the actions of these TFs. An AtMYB103-EAR chimeric repressor disrupts AtMYB103 function, resulting in complete male sterility. The restorer lineintroduced using AtMYB103 gene driven by the stronger anther specific promoter At39 can be used to pollinate the transgenic male-sterile plants, to restore male fertility in the F1 plants.

Transcriptional Silencing of Heterologous Anther Promoters

TGS has been shown to be an effective technique for knocking down gene activity in maize, with male-sterile plants being generated in high frequency by constitutively producing Ms45 promoter-inverted repeat RNAs (pIR). As a consequence, a BMS system based on transcriptional silencing of heterologous anther promoters was developed (Cigan et al., 2014). The Ms45 gene is coupled to two non-maize promoters (ProA and ProB) in this technique to produce paired sets of ms45 recessive inbred parents that can self-pollinate and sustain themselves. A cross of the two pairs brings the two pIR cassettes together, silences the Ms45 gene, and produces male-sterile offspring.The male-sterile line is maintained in the dual-component BMS system by crossing it with an isogenic fertile line.

Inducible Male-Sterility Systems

Roundup Hybridization System in Maize

Monsanto created the Roundup Hybridization System (RHS) for hybrid seed production, which is based on glyphosate-mediated male sterility. RHS and RR transgenic constructs made up the RHS system. The CP4-EPSPS gene is driven by the enhanced 35S promoter, which has been shown to be poorly expressed in tapetum cells and microspores, and the resulting RHS plant shows male sterility following glyphosate application with little or no injury to the rest of the plant.The RR transgene construct has a twofold expression cassette that produces high levels of constitutive CP4-EPSPS expression, resulting in glyphosate resistance. Rows of an RHS female line are interplanted with rows of an RR male line in hybrid seed production fields, and over-the-top glyphosate spraying cause male sterility in RHS female plants, which are then pollinated by RR male plants.The RHS plants remain completely viable and capable of self-pollination for female line propagation without the requirement for a maintenance line when glyphosate is not used. The RHS approach eliminates mechanical detasseling, considerably simplifying the hybrid seed corn manufacturing process. Using

endogenous maize male tissue-specific short interfering RNAs to stimulate cleavage of the CP4-EPSPS mRNA selectively in tassels, resulting in glyphosate-sensitive male cells, this technique has recently been enhanced.

Non-toxic Chemical Induction of Male Sterility Mediated by an Anther-Localized Conversion Gene

The tapetum-specific expression of the L-ornithinase (argE) gene, which can convert the non-toxic chemical N-acetylphosphinothricin (N-ac-PPT) into the herbicide phosphinothricin (PPT), was used to produce an inducible male-sterility system in tobacco. The administration of N-ac-PPT to argE plants can cause male sterility because the argE gene's tapetum expression causes PPT buildup in the tapetum, resulting in empty anthers. ArgE plants that have not been treated with N-ac-PPT are entirely male fertile and are utilised to reproduce the female line. The argE gene driven by the rice pollen allergen (OSIPA) promoter was recently created in rice.

Syngenta has developed another chemical male-sterility system based on the anther-localized conversion of the inactive D-glufosinate to the phytotoxic L-glufosinate, which is mediated by tapetum-specific expression of a modified D-amino acid oxidase (DAAO). Foliar application of D-glufosinatejust prior to sporogenesisis given to transgenic plants expressing an activating enzyme (TAP1-DMRtDAAO) in the anthers. This enzyme converts D-glufosinate to L-glufosinate, which causes local destruction of anther tissue and thus induces male sterility.

Molecular regulation of ZmMs7 for development of a dominant male-sterility system in multiple species.

A DMS system was demonstrated in the paper of Xueli, *et al*., (2018) molecular control by ZmMs7 needed for maize male fertility and development. ZmMs7 functions as a transcriptional activator, forming multiprotein complexes with ZmNF-Y subunits that can directly activate downstream genes. Premature expression of ZmMs7 in maize induced by p5126 resulted in dominant male sterility and pollen production that was entirely aborted. Unlike other male-sterility mutants, the p5126- ZmMs7M-01 line has essentially no exine structure on the microspore surface, instead showing only erratic lipid droplet deposition.Using the same gene ZmMs7 with distinct maize promoters and molecular processes underlying male sterility, the two different male-sterility systems (multi-control sterility and DMS) were established. In the multi-control sterility system, loss of function of ZmMs7, a transcriptional activator, inactivates its regulated downstream genes related to cutin biosynthesis and tapetal cell PCD, resulting in delayed tapetal degeneration, abnormal Ubisch body development, and pollen wall formation, and eventually complete male sterility of the ms7-6007 mutant.Premature expression of ZmMs7 induced by p5126, on the other hand, results in altered expression patterns (i.e., early and high expressions) of a wide range of genes potentially involved in tapetum development and pollen exine formation, resulting in complete male sterility of the p5126-ZmMs7M lines.Plants with the hemizygous genotype (p5126-ZmMs7M/-/ZmMs7/ ZmMs7) have full male-sterility traits but female fertility and normal vegetative development, and are hence called dominant male-sterility lines.

The red fluorescent transgenic dominant male-sterility seeds (DMS hybrid seeds) and normal colour nontransgenic male-fertility seeds are produced using the DMS method. The two varieties of F1 hybrid seeds may be employed flexibly for agricultural field production in different nations for cross-pollinated plants.Nontransgenic male-fertility hybrid seeds, for example, can be separated and planted in the field. Although 50% of DMS plants are male sterile, the other 50% of nontransgenic male-fertility F1 sister plants may pollinate the male sterile F1 plants, ensuring no influence on the field production. Self-pollinated plants such as rice, sorghum, and millet, on the other hand, can have the almost 50% nontransgenic male-fertility hybrid seeds selected out and cultivated in the field.

Advantages of Dominant Genic Male Sterility

- First, unlike the CMS method, the DMS lines exhibit remarkable male sterility stability across a variety of genetic backgrounds.
- Second, this system is not constrained by the paucity of GMS mutants and R genes, as is the case with seed production and multi-control sterility systems.

- Third, unlike the Barnase/Barstar approach, the DMS technique generates male sterile lines using a plant endogenous gene (ZmMs7) and promoter (p5126), which has no ethical issues.

CONCLUSIONS

Given the importance of male sterility in hybrid seed production, more than ten BMS systems have been developed over the last two decades. However, with the exception of the SPT and RHS systems in maize and the Barnase/Barstar system in rapeseed, most BMS strategies have not been successfully applied in commercial hybrid seed production due to a lack of a cost-effective, environmentally friendly, and genetically stable strategy for large-scale hybrid seed production. Furthermore, all BMS systems are subject to the regulatory policy of "zero tolerance," which prevents transgenic planting in many countries.Therefore, there are several factors that should be considered when developing the ideal BMS system in the future.

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