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MOLECULAR MECHANISMS OF MALE STERILITY AND FERTILITY RESTORATION IN CEREAL CROPS: RECENT ADVANCES, GENETIC REGULATION AND APPLICATIONS IN HYBRID SEED PRODUCTION – A REVIEW

Jagdeep Singh[1], Dr V Madan Mohan Rao[2*], Dr. Yaikhom Vivekananda[3], Dr Satyamaya Satapathy[4], Akhouri Ayush[5], Kruthika S J[6], Pallavi Puhan[7], Shorya Kapoor[8], Dharmendra Yadav[9] and Dhananjaya P[10]

[1]Assistant Professor, Department of Faculty of Agriculture, Maharishi Makandeshwar (deemed to be) University, Mullana
E-mail: jagdeep.gpb2011@gmail.com
[2]HOD, Department of Botany and Horticulture, Lecturer in Botany, Govt Degree College, Chintalapudi, Eluru Dist 534460, AP
Email id: vmm.bot74@gmail.com
[3]Associate Professor, Department of Genetics and Plant Breeding, College of Horticulture, Thenzawl, CAU (l), Mizoram- 796186
Email: yaikhom.v5@gmail.com
[4]Senior Scientist and Head, Department of Krishi Vigyan Kendra, Balangir, Odisha University of Agriculture and Technology (OUAT)
E-mail.: ssatapathy@ouat.ac.in
[5]PhD Scholar, Department of Genetics and Plant Breeding, GBPUAT, E-mail: akhouriayush@gmail.com
[6]Ph.D Scholor, Department of Genetics and Plant Breeding, University of Agricultural Sciences, GKVK, Bangalore
E-mail.: kruthikasj1999@gmail.com
[7]Ph.D. Scholar, Department of Seed Science and Technology, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar, E-mail.: pallavipuhan.rs22@ouat.ac.in
[8]Assistant Professor, Department of Horticulture (Vegetable Science), Teerthanker Mahaveer College of Agriculture Sciences, Teerthanker Mahaveer University, Email id: shoryak2@gmail.com
[9]Breeder (Wheat & Barley), Department of R&d, College/University: Star Agriseeds Private Limited, E-mail: dy22202@gmail.com
[10]Associate Professor (SST), Department of BCI, College of Horticulture, Kolar, E-mail: drpuhsb@gmail.com
***Corresponding email**: Dr V Madan Mohan Rao, vmm.bot74@gmail.com

ABSTRACT

Male sterility is an important biological phenomenon and a powerful genetic tool for the development of commercial hybrid varieties in cereal crops. It refers to the inability of plants to produce functional pollen grains while retaining female reproductive fertility, thereby facilitating controlled cross-pollination and large-scale hybrid seed production. In major cereal crops such as rice, wheat, maize, barley, sorghum and pearl millet, male sterility systems play a significant role in exploiting heterosis and improving crop productivity, yield stability and adaptability to changing environmental conditions. The development and regulation of male sterility involve complex molecular, genetic, cytological and physiological mechanisms associated with anther development, tapetum differentiation, microsporogenesis, pollen maturation and programmed cell death. Recent advances in molecular biology have revealed the involvement of nuclear genes, mitochondrial genes, transcription factors, non-coding RNAs, hormonal signalling pathways and epigenetic modifications in regulating male reproductive development. Three major genetic systems, namely cytoplasmic male sterility (CMS), genic male sterility (GMS) and environment-sensitive genic male sterility (EGMS), have been extensively utilized in hybrid breeding programmes. Among these, CMS-based three-line systems and EGMS-based two-line systems have considerably improved the efficiency of hybrid seed production. Fertility restoration is another essential component of hybrid breeding, primarily governed by nuclear restorer-of-fertility (Rf) genes that suppress the effects of sterility-inducing mitochondrial genes through molecular mechanisms involving RNA processing, transcript degradation, translation regulation and mitochondrial function. Advanced genomic approaches, including genome-wide association studies, quantitative trait locus mapping, marker-assisted selection, transcriptomics, proteomics, CRISPR/Cas-mediated genome editing and functional genomics, have expanded the understanding of male sterility and fertility restoration in cereal crops. Furthermore, the integration of molecular markers and high-throughput genomic technologies has accelerated the identification and utilization of fertility-restoration genes in hybrid breeding programmes. This review highlights recent advances in the molecular mechanisms of male sterility, genetic regulation of fertility restoration, interactions between nuclear and cytoplasmic genomes, and their applications in hybrid seed production. It also discusses existing challenges, including environmental instability, incomplete fertility restoration, genetic diversity and seed production costs.

KEYWORDS: Male sterility; Fertility restoration; Cereal crops; Cytoplasmic male sterility (CMS); Genic male sterility (GMS); Environment-sensitive genic male sterility (EGMS); Restorer-of-fertility genes; Nuclear–cytoplasmic interaction; Mitochondrial genes; Anther development; Pollen development; Heterosis; Hybrid seed production; Molecular genetics; Genome editing; CRISPR/Cas technology; Marker-assisted selection; Transcriptomics; Functional genomics; Sustainable hybrid breeding.

1. INTRODUCTION

Cereals are central to food security because they supply a large share of the calories and plant-derived protein consumed worldwide. Rice, wheat, maize, sorghum and barley are grown across contrasting production environments and support both subsistence and commercial agriculture (Hammani, K. and Giegé, 2014). Population growth, climate instability, pressure on land and water resources, and the need to raise productivity have increased interest in breeding approaches capable of delivering higher and more stable yields. Hybrid breeding is one such approach. It uses heterosis—the improved performance sometimes observed in the progeny of genetically distinct parents—to develop cultivars with favourable combinations of yield, vigour, adaptation and other agronomic traits (Allen *et al*., 2007).

A major practical difficulty in producing hybrid seed is preventing the female parent from fertilising itself. Manual removal of anthers can be labour-intensive, costly or difficult to perform on a commercial scale, particularly in crops with small flowers or large numbers of florets. Male sterility offers a biological means of controlling pollen production and promoting cross-pollination (Binder *et al*., 2016). A male-sterile plant does not produce functional pollen, or produces pollen that cannot fertilise, but can retain the ability to set seed after receiving pollen from a suitable male parent (Bohra *et al*., 2016).

The causes of male sterility are diverse. Some forms are associated with mitochondrial genome variation, others result from mutations in nuclear genes needed for anther or pollen development, and some depend on interactions between cytoplasmic and nuclear factors. In environment-sensitive systems, temperature or day length determines whether a plant expresses sterility or fertility. Chemical hybridising agents can also disrupt male reproductive development, although their performance depends on dose, crop stage and environmental conditions (Chase, 2007).

The molecular study of male sterility has moved beyond the description of abnormal anthers and non-viable pollen. Mitochondrial genome analysis has shown that recombination and structural rearrangements can produce novel gene configurations associated with CMS. Functional studies have connected some of these mitochondrial products with respiratory dysfunction, oxidative stress and abnormal tapetal development (Wilson *et al*., 2009). In parallel, the discovery of nuclear restorer genes—many of which encode organelle-targeted RNA-binding proteins—has explained how fertility can be recovered in particular cytoplasmic backgrounds Chen and Liu, 2014).

Male reproductive development is also governed by nuclear gene networks. These networks coordinate the formation of anther tissues, meiosis, microspore release, pollen-wall construction and the controlled degeneration of the tapetum (Ariizumi, T. and Toriyama, 2011). Transcription factors, hormone pathways, metabolic processes and epigenetic regulation contribute to this coordination (Parish, R.W. and Li, 2010). A fuller understanding of these processes is valuable not only for hybrid breeding but also for the study of plant organelle-nucleus communication and reproductive biology (Hanson and Bentolila, 2004).

2. Genetic categories of male sterility in cereals

2.1 Cytoplasmic male sterility

Cytoplasmic male sterility is generally transmitted through the maternal parent and is most commonly associated with changes in the mitochondrial genome. Plant mitochondrial DNA can undergo recombination and structural rearrangement. In some cases, these events create chimeric sequences or novel open reading frames that are expressed in reproductive tissues. The products of such sequences may disturb normal mitochondrial activity and interfere with pollen development (Ma, H. 2005).

Several CMS sources have been characterised in rice, including Wild Abortive (WA), Boro II (BT) and Honglian (HL) cytoplasms. The WA system is associated with the mitochondrial sequence *WA352* (Goldberg *et al*., 1993). In BT-CMS, the sterility-associated *orf79* sequence is transcribed in association with *atp6*. HL-CMS is linked to *orfH79*. These systems differ in their mitochondrial transcripts and in the nuclear genes capable of restoring fertility; a restorer effective for one cytoplasm should not be assumed to work in another (Kempken and Pring, 1999).

Maize provides another well-studied example. Its recognised CMS groups include T, C and S cytoplasms. The T-cytoplasm system is associated with *urf13*, a mitochondrial gene whose product is linked to susceptibility to the T-toxin produced by *Bipolaris maydis* race T. The severe southern corn leaf blight epidemic of 1970 exposed the risk of relying too heavily on a narrow cytoplasmic background (Zhang, D. and Wilson, 2009). This event remains an important lesson for hybrid breeding: cytoplasmic diversity and disease-response testing are essential components of parental-line management (Laser *et al*., 1972).

In wheat, T-type CMS derived from *Triticum timopheevii* cytoplasm has been investigated as a possible basis for hybrid production. The mitochondrial *orf279* sequence is associated with sterility in this system, while nuclear restorer loci influence the recovery of pollen fertility. Sorghum hybrid production commonly uses the A1, or milo, cytoplasm together with appropriate nuclear restorer backgrounds (Nonomura *et al*., 2003). The practical value of each CMS source depends on the stability of sterility, the availability of maintainers and restorers, and the performance of the resulting hybrids (Levings, 1993).

2.2 Genic male sterility

Genic male sterility is caused by changes in nuclear genes required for normal male reproductive development. Depending on the gene and allele involved, inheritance may be recessive or dominant (Jung *et al*., 2005). The affected processes can include the specification of anther tissues, meiotic progression, callose formation, tapetal

function, pollen-wall development and pollen maturation. Unlike CMS, this category does not depend on a particular sterility-inducing mitochondrial cytoplasm (Niu *et al*., 2013).

Rice research has identified several genes involved in anther development. For example, *UDT1* contributes to early anther differentiation, while *TDR* is required for normal tapetal development. Other regulators, including *EAT1*, *TIP2/bHLH142* and *GAMYB*, participate in developmental pathways associated with tapetal function and pollen formation (Ko *et al*., 2014). Mutations that disrupt these pathways can produce defective anthers or non-functional pollen. Comparable genetic studies in maize, wheat and barley have identified nuclear factors involved in meiosis, anther development and pollen maturation (Mackenzie and McIntosh, 1999, Aya *et al*., 2009).

Genic male sterility can be useful in breeding and genetic research because a defined nuclear mutation can be transferred or modified through crossing and molecular methods. Its practical deployment, however, requires a reliable way to multiply the sterile genotype. Recessive sterility may require a maintainer or a specialised propagation system, and the expression of sterility must be sufficiently uniform to protect hybrid seed purity (Schnable, P.S. and Wise, 1998).

2.3 Cytoplasmic-genic male sterility

Cytoplasmic-genic male sterility results from the interaction of a sterility-inducing cytoplasm with nuclear genes that either maintain sterility or restore pollen production. The system is widely used in three-line hybrid breeding. It involves three parental types: the A-line, which is male-sterile; the B-line, which maintains the A-line; and the R-line, which carries fertility-restorer genes (Zhang *et al*., 2010).

The A-line has the relevant sterile cytoplasm and lacks effective restorer alleles. It is multiplied by pollination from the B-line, which has a compatible nuclear background but normal fertile cytoplasm. For hybrid seed production, the A-line is crossed with the R-line. Where the restorer alleles are effective and inherited appropriately, the resulting F1 plants recover pollen fertility. This arrangement allows seed producers to maintain a female parent without relying on manual emasculation (Wise *et al*., 1999).

The success of CGMS depends on the compatibility of the cytoplasm and nuclear genome. Restorer genes often show specificity for a particular CMS source, and their effectiveness may be modified by genetic background or environment. Breeders therefore need to evaluate sterility maintenance and fertility restoration in the actual parental combinations intended for commercial use (Hu *et al*., 2012).

2.4 Environment-sensitive genic male sterility

Environment-sensitive genic male sterility is controlled by nuclear factors whose effects change in response to environmental conditions. Temperature and photoperiod are particularly important. Photoperiod-sensitive genic male sterility (PGMS), temperature-sensitive genic male sterility (TGMS), and combined photoperiod- and temperature-sensitive systems (PTGMS) are used or investigated in hybrid rice breeding (Kim *et al*., 2018).

In these systems, the same genotype may be sterile under one set of environmental conditions and fertile under another. This feature can remove the need for a separate maintainer line, as a sterile condition can be used for hybrid seed production and a fertile condition for multiplication of the parental line. Rice loci associated with these responses include *pms3*, *p/tms12-1* and *TMS5*. The *TMS5* gene encodes an RNase Z-like protein, and altered function of this gene is associated with temperature-dependent male sterility (Bhattacharya *et al*., 2024).

The main limitation is the need for a sufficiently wide and predictable environmental window. Unexpected temperature or day-length conditions may result in partial fertility when sterility is required, increasing the possibility of self-pollination. Reliable deployment therefore requires location-specific testing, careful sowing-date planning and monitoring of reproductive-stage weather (Ali *et al*., 2025).

2.5 Chemically induced male sterility

Chemical hybridising agents are compounds applied to plants to impair male reproductive development while retaining useful female fertility. Depending on the compound, treatment may affect meiosis, pollen mother cells, tapetal activity or later stages of pollen maturation. Chemical approaches can provide flexibility where a suitable genetic system is unavailable, but their performance is influenced by genotype, application rate, timing and growing conditions. Phytotoxicity, inconsistent sterility, application costs and environmental considerations can limit their use. For these reasons, chemical induction is generally treated as a complementary approach rather than a universal replacement for genetic male-sterility systems (Bhattacharya *et al*., 2024).

3. Cellular and molecular basis of cytoplasmic male sterility

3.1 Mitochondrial rearrangements and novel gene products

Plant mitochondria contain genes required for components of the respiratory machinery and other aspects of organelle function. Their genomes are structurally dynamic and may contain repeated sequences that facilitate recombination. Rearrangements can place previously separate DNA segments together, sometimes generating chimeric open reading frames. When such sequences are transcribed and translated, the resulting proteins may interfere with normal cellular processes (Wang *et al*., 2006).

The effect of a CMS-associated mitochondrial product is not necessarily identical in every crop. Some proteins affect membrane properties or respiratory-chain activity, while others influence interactions with nuclear-encoded mitochondrial proteins. Their effects may be especially apparent in developing anthers because pollen formation requires substantial energy and precise coordination among anther tissues. The rice examples *WA352*, *orf79* and *orfH79*, the maize-associated *urf13*, and wheat *orf279* illustrate the diversity of mitochondrial factors implicated in CMS (Toriyama, 2021).

3.2 Respiratory activity, energy supply and redox balance

Mitochondria support ATP production through oxidative phosphorylation and contribute to cellular redox regulation. Developing microspores and anther tissues require energy for cell division, biosynthesis, transport and pollen maturation. If a sterility-associated mitochondrial protein compromises respiratory activity, ATP supply may become inadequate for these processes (Kazama, T. and Toriyama, 2003).

Changes in mitochondrial electron transport can also influence the production of reactive oxygen species (ROS). ROS are normal signalling molecules at controlled concentrations, but excessive accumulation can damage lipids, proteins and nucleic acids. Male-sterile anthers may show an altered balance between ROS production and antioxidant protection. Enzymes such as superoxide dismutase, catalase and peroxidases are part of the antioxidant network that helps maintain cellular redox balance. The precise pattern varies among systems, and ROS changes should be interpreted in relation to tissue, developmental stage and the particular CMS source (Kazama, T. and Toriyama, 2014).

3.3 Tapetal development and microspore nutrition

The tapetum is the innermost layer of the anther wall and provides materials required for developing pollen. It contributes nutrients, enzymes, lipids and precursors used in pollen-wall formation. In particular, tapetal products are important for the development of the pollen exine, including the deposition of sporopollenin-related materials (. Tang *et al*., 2014).

Tapetal cells must develop and degenerate at the correct time. If they break down too early, microspores may lose nutritional and structural support before maturation. If degeneration is delayed, the release and transfer of required materials may be disrupted. Mitochondrial dysfunction can disturb this developmental timing through effects on energy metabolism, redox status and gene expression. In WA-CMS rice, WA352 has been linked to interaction with COX11, a protein associated with mitochondrial function and ROS regulation. This interaction has been connected with abnormal tapetal cell death and pollen abortion (Hu *et al*., 2012).

3.4 Programmed cell death and oxidative stress

Programmed cell death (PCD) is a normal part of anther development. At the appropriate stage, tapetal cells undergo controlled degeneration, allowing nutrients and wall precursors to become available to developing microspores. Male sterility can arise when this process occurs prematurely, excessively or at an unsuitable developmental stage (Huang *et al*., 2015).

Mitochondrial disturbances may alter ROS levels and activate stress-related pathways that influence PCD. When tapetal cells degenerate before microspores are ready, pollen development cannot proceed normally. The connection among mitochondrial activity, ROS balance and PCD is therefore an important part of the explanation for pollen abortion in several CMS systems. Nevertheless, the sequence of events and the relative contribution of each factor need to be established separately for each cytoplasm rather than assumed to be identical across cereals (Luo *et al*., 2013).

4. Nuclear control of fertility restoration

4.1 Fertility-restorer genes and nuclear–mitochondrial interaction

Fertility-restorer genes are nuclear genes that suppress the sterility phenotype associated with particular CMS cytoplasms. They are commonly designated *Rf* genes. Their effects are often cytoplasm-specific because the encoded proteins recognise particular mitochondrial RNA or protein targets. The presence of a restorer allele can therefore restore fertility in one CMS background but have little or no effect in another (Qin *et al*., 2012).

Many cloned *Rf* genes encode proteins targeted to mitochondria, where they regulate the expression of sterility-associated sequences. This relationship illustrates how the nuclear genome can control the expression of mitochondrial genes and maintain reproductive function. Restoration may involve reduced accumulation of a harmful mitochondrial protein rather than complete elimination of the corresponding mitochondrial DNA sequence (Melonek *et al*., 2021).

4.2 Pentatricopeptide repeat proteins

Pentatricopeptide repeat proteins form a large family of organelle-targeted RNA-binding proteins. Their structures contain repeated motifs, each contributing to the recognition of particular RNA sequences. By binding to specific mitochondrial transcripts, PPR proteins can influence RNA editing, cleavage, processing, stability and translation (Gupta *et al*., 2006).

Several rice fertility-restorer genes encode PPR proteins. In BT-CMS, RF1A and RF1B participate in regulating the *atp6-orf79* transcript. RF1A is associated with transcript processing, while RF1B contributes to the degradation of the processed RNA. In HL-CMS, RF5 and RF6 act in the regulation of the *atp6-orfH79* transcript through RNA-processing activities. In WA-CMS, RF4 has been associated with suppression of the WA352-related sterility pathway. Additional work on RF20 has drawn attention to protein interactions and ROS regulation in the same broad system (Xiao *et al*., 2020).

4.3 RNA cleavage, processing and degradation

One route to fertility restoration is the alteration of sterility-associated mitochondrial RNA. A restorer protein may recognise a sequence in the target transcript and promote cleavage or processing. The modified RNA may then be degraded more rapidly, reducing the amount available for translation. Other mitochondrial RNA-processing proteins can participate in the same pathway, so restoration may depend on a multi-protein complex rather than on a single restorer acting alone (Lin *et al*., 2024).

The biological consequence is a reduction in the expression of the mitochondrial factor responsible for disrupting pollen development. The degree of transcript reduction required for fertility may differ among CMS systems. It is therefore useful to measure both RNA abundance and the corresponding protein level when assessing a proposed restoration mechanism (Song *et al*., 2024).

4.4 Translational regulation

Fertility can also be recovered through regulation at the translation stage. In this case, the sterility-associated transcript may remain detectable, but its conversion into protein is restricted. Binding of a restorer protein to the RNA can interfere with ribosome activity or otherwise reduce translation. This distinction is important because the presence of a mitochondrial transcript does not necessarily indicate that the sterility-associated protein is being produced at a biologically effective level (Wang *et al*., 2021).

4.5 Other mitochondrial regulatory proteins

PPR proteins are prominent in the known fertility-restoration pathways, but they are not the only nuclear-encoded proteins relevant to mitochondrial gene expression. Mitochondrial transcription termination factors (mTERFs) and other RNA-associated proteins participate in organelle transcription and RNA metabolism. Their potential contribution to fertility restoration is an active area of investigation. Their specific roles should be distinguished from established functions of cloned *Rf* genes until direct genetic and biochemical evidence is available (Small, I.D. and Peeters, 2000).

5. Nuclear networks governing anther and pollen development

5.1 Tapetal differentiation and transcriptional control

Male reproductive development depends on the orderly formation of anther tissues, the production of microspores through meiosis, and the subsequent maturation of pollen. The tapetum is particularly important because it supports microspores and supplies compounds needed for pollen-wall construction. These developmental events are regulated by networks of transcription factors and signalling proteins (Lurin *et al*., 2004).

In rice, *UDT1*, *TDR*, *EAT1*, *TIP2/bHLH142* and *GAMYB* are among the genes associated with anther and pollen development. Their functions span early anther differentiation, tapetal development, regulation of cell death and pollen maturation. Disruption of these pathways can produce abnormalities in anther structure, tapetal timing or pollen viability. Other transcription-factor families, including MYB, bHLH, WRKY, NAC and MADS-box proteins, also contribute to reproductive development in plants (Barkan, A. and Small, 2014).

5.2 Hormonal signalling

Plant hormones influence several stages of male reproductive development. Gibberellins are involved in anther development and pollen maturation; auxin contributes to organ differentiation and cell division; and jasmonic acid has an important role in anther dehiscence and pollen release. Cytokinins, abscisic acid and ethylene also participate in developmental and stress-response processes that can affect reproductive performance (Schmitz-Linneweber, C. and Small, 2008).

Hormonal changes observed in male-sterile plants may be primary causes in some genetic systems or secondary consequences of mitochondrial dysfunction and developmental stress in others. It is therefore important to avoid treating a change in hormone concentration as proof of a direct causal role. Experiments that combine hormone measurements with gene-expression analysis and functional validation are needed to establish the direction of the relationship (Manna, 2015).

5.3 Epigenetic and RNA-based regulation

Gene expression during anther development is also influenced by epigenetic processes. DNA methylation, histone modifications and chromatin remodelling can alter the accessibility of regulatory regions and thereby influence transcription. Small RNAs, including microRNAs and small interfering RNAs, can regulate target transcripts through degradation or translational repression. RNA modifications such as N6-methyladenosine (m6A) are also being investigated for their roles in post-transcriptional control (Barkan *et al*., 2012).

6. Fertility-restoration systems in major cereal crops

6.1 Rice

Rice is one of the principal model crops for research on CMS and fertility restoration. Three-line hybrid rice systems make use of CMS female parents, maintainer lines and restorer lines (Fan *et al*., 2016, Zhang *et al*., 2013, Zhou *et al*., 2012). WA, BT and HL cytoplasms are among the major sources used in genetic and molecular investigations (Yin *et al*., 2013, Li *et al*., 2011, Virmani, 1994).

In BT-CMS, *orf79* is associated with pollen abortion. The nuclear genes *Rf1a* and *Rf1b* encode PPR proteins that regulate the mitochondrial *atp6-orf79* transcript through processing and degradation (Zhou *et al*., 2014, Virmani *et al*., 2003, Yuan, 1994). In HL-CMS, *Rf5* and *Rf6* participate in the regulation of the *orfH79*-associated pathway. In WA-CMS, *WA352* has been linked to COX11, altered ROS regulation and premature tapetal PCD (Ding *et al*., 2012). Restorer activity in this system involves nuclear factors including RF4, while studies of RF20 have further examined the role of protein interactions and oxidative balance (Fujii and Small, 2011, Ashikari, M. and Matsuoka, 2006).

The rice examples demonstrate that different CMS sources rely on distinct mitochondrial factors and restoration pathways. For breeding purposes, this means that the identity of the cytoplasm must be considered when selecting restorer alleles and interpreting molecular-marker results (Hammani, K. and Giegé 2014).

Title: Field View of Male-Sterile and Restorer Lines in Rice for Hybrid Seed Production



Sources: Muhammad Furqan Ashraf *et al*., 2020

6.2 Maize

Maize has a long history of CMS research and provides clear examples of the agronomic consequences of cytoplasmic uniformity. T, C and S cytoplasms are recognised sterility groups, each with its own mitochondrial features and restoration genetics (Rhoades, 1931). The *urf13* gene in T-cytoplasm is associated with sensitivity to T-toxin from *Bipolaris maydis* race T. The disease epidemic of 1970 highlighted the vulnerability that can arise when a large proportion of commercial hybrids share a susceptible cytoplasm (Laughnan, J.R. and Gabay-Laughnan, 1983).

The S-CMS system has been associated with the mitochondrial *orf355-orf77* region and nuclear restoration involving *Rf3*. C-CMS is linked to the mitochondrial *atp6c* region (Wise *et al*., 2002, Duvick, 1965, Fauron *et al*., 1990). Work on the maize restorer *ZmRf5* has implicated a P-type PPR protein and its interactions with mitochondrial RNA-processing components, including MORF8 and the splicing factor RS31A. These findings support a model in which restoration depends on coordinated RNA processing and control of mitochondrial protein accumulation. Functional studies, including targeted gene disruption, have helped test the role of candidate restorer genes (Yang *et al*., 2022, Żurek, 2024, Xiao *et al*., 2020 and Allen *et al*., 2007).

Title 2. Field Layout of Hybrid Maize Seed Production Showing Cytoplasmic Male Sterile (CMS) Female Rows and Pollen Parent Rows, with Harvested Hybrid Seeds.



Souces: Patil, A., *et al*. 2025.

6.3 Wheat

Hybrid wheat has attracted interest because heterosis may offer opportunities to improve grain yield and adaptation. Its commercial development is complicated by the predominantly self-pollinating floral biology of the crop, the need for reliable pollen transfer and the requirement for stable fertility restoration (Melonek *et al*., 2021, Gupta *et al*., 2026).

T-type CMS derived from *Triticum timopheevii* cytoplasm is an important system in wheat research. The mitochondrial *orf279* sequence is associated with sterility, while nuclear restorer genes *Rf1* and *Rf3* contribute to fertility recovery (Jordan *et al*., 2025, Wasudeo *et al*., 2026). Functional studies have shown that these restorer loci can act independently in the relevant genetic background, and combining them may improve restoration. Continued work is required to evaluate their effectiveness across diverse wheat germplasm and environments, and to identify additional restorer sources that can be used in breeding (Jordan *et al*., 2011).

6.4 Sorghum

Sorghum is well adapted to many dry and hot production environments and is an important crop in semi-arid regions. Commercial hybrid development commonly relies on the A1 or milo CMS system. The female A-line carries the sterile cytoplasm, while suitable nuclear restorer alleles in the pollen parent permit fertility recovery in the hybrid (Reddy *et al*., 2007).

Restoration is influenced by major nuclear loci and, in some backgrounds, additional genetic factors. Environmental conditions can also affect the expression of fertility. Partial restoration under high temperature is of particular concern because it may permit self-pollination in a line intended to remain sterile during hybrid seed production. Mapping and marker-development studies can support the identification of restorer and maintainer genotypes, but marker performance should be validated in the breeding populations where it will be used (Mace *et al*., 2013).

6.5 Barley

Barley is cultivated for food, animal feed and malting. Male-sterility mechanisms and possible hybrid-breeding systems have been investigated, but the development of widely usable CMS-based systems has been less straightforward than in some other cereals. Limitations include the availability of suitable sterile cytoplasms, dependable maintainers and effective restorer combinations, as well as the need for stable expression across environments.

Further work on nuclear control of anther development, cytoplasmic diversity and the identification of compatible restorer genes may help clarify the potential of hybrid barley. At present, claims about specific restoration pathways should be supported by crop-specific functional evidence (Jordan *et al*., 2018).

7. Comparative overview of sterility systems

The molecular basis of male sterility varies among cereals, although mitochondrial dysfunction, altered RNA metabolism and defective anther development recur across several systems. The following table summarises representative examples. Gene names and their reported functions are specific to the relevant cytoplasmic or genetic background; they should not be interpreted as interchangeable across crops.

Table 1. Representative male-sterility systems and associated restoration factors in cereal crops

Crop	Sterility source or system	Representative sterility-associated factor	Restorer or regulatory factor
Rice	WA-CMS	*WA352*	*Rf4* and other reported restorer factors
Rice	BT-CMS	*orf79*	*Rf1a*, *Rf1b*
Rice	HL-CMS	*orfH79*	*Rf5*, *Rf6*
Rice	TGMS	*TMS5* and temperature-sensitive regulation	Environmental conditions and nuclear background
Maize	T-CMS	*urf13*	Cytoplasm-specific nuclear restorers
Maize	C-CMS	*atp6c*-associated region	*ZmRf5* and other reported loci
Maize	S-CMS	*orf355*-*orf77* region	*Rf3*
Wheat	T-CMS	*orf279*	*Rf1*, *Rf3*
Sorghum	A1-CMS	A1 sterile cytoplasm	Major and modifier restorer loci, including *Rf* genes
Barley	Investigated genetic/CMS systems	System-dependent cytoplasmic or nuclear factors	Restorer mechanisms depend on the system

Note: This is a representative overview, not an exhaustive catalogue. The strength of functional evidence differs among the listed systems. Confirm gene designations and current nomenclature against the primary studies selected for the final bibliography.

8. Molecular and genomic tools for studying male sterility

8.1 Molecular markers and marker-assisted selection

Molecular markers enable breeders to track alleles associated with sterility maintenance and fertility restoration. SSR, SNP, insertion–deletion and KASP markers can be used to identify parental genotypes, follow restorer loci during crossing and verify the genetic identity of breeding materials. Marker-assisted selection is particularly useful when a fertility-restoration trait is difficult or costly to phenotype, or when selection must be carried out at an early growth stage (Varshney *et al*., 2005).

The value of a marker depends on its linkage with the targ*et al*lele and its reliability across genetic backgrounds. A marker developed in one population should therefore be tested before routine use in unrelated germplasm. Combining markers for major restorer loci with field assessment of pollen fertility can improve selection accuracy.

8.2 QTL mapping and genome-wide association studies

Quantitative trait locus (QTL) mapping and genome-wide association studies (GWAS) are used to identify genomic regions associated with pollen fertility, sterility stability and restoration ability. Linkage mapping examines segregation in defined populations, whereas GWAS uses historical recombination and marker variation in diverse panels. Both approaches can reveal loci that contribute to complex traits.

Fertility restoration may be controlled by major genes as well as modifier loci. For that reason, mapping studies should include appropriate cytoplasmic backgrounds, reliable fertility phenotyping and, where possible, evaluation across environments. High-density SNP data can then support fine mapping, candidate-gene identification and genomic prediction (Varshney *et al*., 2011).

8.3 Transcriptomics and functional genomics

RNA sequencing can compare gene expression in sterile and fertile anthers, identify changes in mitochondrial transcript abundance and reveal pathways that respond during pollen development. Analyses may identify expression differences involving respiration, ROS defence, carbohydrate metabolism, hormone signalling, tapetal differentiation and PCD. Sampling at defined anther-development stages is essential because gene expression changes rapidly during reproductive development.

Transcriptomic results identify candidates but do not, by themselves, prove gene function. Candidate genes can be examined using gene overexpression, RNA interference, gene silencing, targeted mutagenesis and complementation. Combining expression evidence with genetic segregation and biochemical assays provides a stronger basis for assigning a role in sterility or restoration (Jinek *et al*., 2012).

8.4 Genome editing

CRISPR/Cas systems allow targeted modification of nuclear genes involved in anther development, mitochondrial gene regulation and fertility restoration. Gene editing can be used to test whether a candidate gene is necessary for restoration, to generate new male-sterile lines or to modify regulatory sequences. In maize, targeted disruption of candidate restorer genes has been used as a functional approach to investigate CMS-C restoration.

Direct editing of plant mitochondrial genomes remains more technically difficult than editing nuclear genes. Consequently, many current applications focus on nuclear-encoded proteins that influence mitochondrial transcription, RNA processing or reproductive development. Edited lines must be evaluated for unintended effects, fertility stability, seed production performance and their behaviour in the intended genetic background (Cong *et al*., 2013).

8.5 Proteomics and metabolomics

Transcript measurements do not always predict protein abundance or metabolic activity. Proteomics can identify differences in mitochondrial respiratory proteins, antioxidant enzymes and factors associated with tapetal development. Metabolomics can detect changes in sugars, organic acids, amino acids, fatty acids and other compounds needed for pollen formation. These data can help explain how mitochondrial dysfunction affects the physiology of developing anthers.

The greatest value comes from integrating these approaches with developmental phenotyping and genetic evidence. For example, a change in a metabolite is more informative when it can be related to a defined developmental stage, a specific mitochondrial defect and a measurable pollen phenotype (Shan *et al*., 2013).

8.6 Small RNAs and epigenomic approaches

Small-RNA sequencing, DNA-methylation profiling, chromatin assays and analysis of histone modifications can be used to investigate regulatory processes during anther development. These approaches may identify regulatory RNAs or epigenetic changes associated with altered expression of reproductive genes. RNA modifications, including m6A, provide another potential layer of post-transcriptional control.

Although these methods have expanded the range of candidate mechanisms, the contribution of individual epigenetic pathways to CMS and fertility restoration remains to be established in many cereal systems. Functional experiments and independent validation are needed before such associations can be treated as causal mechanisms (Bortesi, L. and Fischer, 2015).

Table 2. Molecular approaches used in male-sterility research and their potential breeding value

Technology	Main purpose	Potential contribution to cereal breeding
SSR and SNP markers	Track sterility and restorer-associated alleles	Selection and verification of parental lines

Technology	Main purpose	Potential contribution to cereal breeding
KASP genotyping	Rapid allele-specific screening	Efficient selection of restorer genotypes
QTL mapping	Locate genomic regions affecting fertility	Dissection of major genes and modifier loci
GWAS	Associate genome-wide variation with fertility traits	Discovery of additional candidate loci
RNA sequencing	Measure gene and organelle-transcript expression	Identification of pathways active in sterile and fertile anthers
Proteomics	Compare protein abundance and interactions	Characterisation of mitochondrial and tapetal changes
Metabolomics	Profile reproductive-tissue metabolites	Assessment of energy and biochemical disturbances
CRISPR/Cas editing	Test or modify candidate genes	Functional validation and development of new sterility systems
Small-RNA and epigenomic profiling	Examine regulatory RNAs and chromatin states	Investigation of additional layers of reproductive control
Genomic selection	Predict breeding value from genome-wide data	Faster improvement of parental lines and hybrid combinations
Mitochondrial genome sequencing	Detect structural variation and novel ORFs	Characterisation of CMS sources and cytoplasmic diversity
Spatial or single-cell transcriptomics	Resolve expression by cell type or tissue location	More precise analysis of anther-development pathways

9. Use of male sterility in hybrid seed production

The main breeding advantage of male sterility is that it prevents self-pollination in the female parent and allows pollen from a selected male parent to produce hybrid seed. This can reduce the need for manual emasculation and make hybrid seed production more practical in crops where hand removal of anthers is difficult or uneconomic.

In a three-line system, the A-line is the sterile female parent, the B-line is used to maintain the A-line, and the R-line supplies pollen and fertility-restorer alleles for hybrid production. A-line seed is produced by crossing A × B, whereas commercial hybrid seed is produced by crossing A × R. The resulting hybrid should be assessed for pollen fertility, seed set and agronomic performance. The restorer parent must be compatible with the CMS cytoplasm and should provide adequate restoration in the target production environment (Chen *et al*., 2019).

Rice hybrid breeding has made substantial use of CMS and environment-sensitive genic male-sterility systems. In maize, CMS can reduce the need for mechanical detasseling, but the history of T-cytoplasm demonstrates why cytoplasmic diversity and disease resistance must be considered together. Sorghum hybrid programmes commonly use A1-CMS. In wheat, hybrid production remains technically demanding because of floral biology, pollen movement, flowering synchronisation, seed-production economics and the need for reliable fertility restoration (Hickey *et al*., 2019).

A successful hybrid seed system requires more than a sterility gene. It depends on stable expression of sterility in the female parent, adequate pollen production by the male parent, synchronised flowering, favourable weather during pollination, genetic purity and a high level of fertility restoration in the F1. Molecular markers can support parental-line selection, but field testing remains necessary to confirm that the system performs reliably under commercial conditions (Cobb *et al*., 2029).

10. Constraints and future research priorities

Despite progress in identifying mitochondrial sterility factors and nuclear restorer genes, several questions remain unresolved. In some systems, the exact sequence of molecular events connecting a mitochondrial ORF with tapetal dysfunction is not fully understood. The interactions among restorer proteins, mitochondrial RNA-processing complexes and respiratory components also require further biochemical and structural investigation. Understanding these relationships could help explain why particular restorer alleles are effective in one cytoplasm but not in another (Varshney *et al*., 2020).

Environmental stability is another major concern. Temperature and photoperiod can influence reproductive development and the expression of environment-sensitive male sterility. Unexpected weather during the sensitive developmental period may lead to incomplete sterility or partial restoration, threatening hybrid seed purity. Breeding programmes should therefore evaluate candidate lines across locations, sowing dates and seasons, with particular attention to temperature during anther development (Bailey-Serres *et al*., 2019).

The availability of effective restorers is uneven among crops and CMS sources. Rice has several well-characterised systems, while hybrid wheat and barley continue to face practical limitations associated with parental compatibility, restoration and seed-production logistics. In sorghum, the contribution of modifier loci and environmental effects needs to be incorporated into selection strategies. Across crops, breeders need diverse cytoplasms and restorer sources to avoid excessive genetic uniformity (Bhattacharya *et al*., 2024).

Future studies should combine long-read and short-read mitochondrial genome sequencing with transcriptomics, proteomics, metabolomics and precise reproductive phenotyping. Such integration can reveal structural variants,

identify candidate mitochondrial transcripts and establish how their expression affects anther development. Protein-interaction assays and structural approaches may clarify the way PPR proteins recognise target RNAs and recruit other components of the mitochondrial RNA-processing machinery.

Genome editing can strengthen functional analysis by testing candidate genes and modifying nuclear regulators of fertility. However, edited material should be evaluated for off-target changes, unintended effects on female fertility, environmental stability and hybrid seed performance. Multi-locus restoration may be worth investigating where fertility is controlled by more than one gene, but the value of gene pyramiding must be demonstrated in the relevant cytoplasmic and breeding backgrounds.

Genomic selection and machine-learning methods may also contribute to hybrid-parent improvement. By combining marker profiles, phenotypic observations, environmental information and hybrid performance, predictive models could help identify promising parental combinations. Their usefulness will depend on the quality and representativeness of training data, accurate phenotyping and independent validation. These tools should complement, rather than replace, biological understanding and field evaluation.

11. CONCLUSION

Male sterility is a valuable genetic mechanism for controlling pollination and exploiting heterosis in cereal crops. Its causes include mitochondrial genome rearrangements, mutations in nuclear genes, interactions between cytoplasmic and nuclear factors, environmental responses and chemical treatments. In many CMS systems, mitochondrial chimeric sequences are associated with altered organelle function, energy metabolism and redox regulation. These changes can disturb tapetal development and the normal progression of microspore maturation, resulting in pollen abortion.

Fertility restoration is largely governed by nuclear genes whose products regulate the expression of sterility-associated mitochondrial sequences. PPR proteins are prominent in several characterised systems because they recognise specific RNA targets and influence transcript processing, degradation or translation. At the same time, nuclear developmental networks involving transcription factors, hormones, ROS signalling and epigenetic regulation determine whether anthers and pollen develop normally. The specific genes and mechanisms differ among rice, maize, wheat, sorghum and barley, making crop- and cytoplasm-specific validation essential.

Molecular markers, mapping populations, GWAS, multi-omics and genome editing have improved the ability to discover and test genes involved in male sterility and fertility restoration. Their integration with careful field evaluation can support the development of stable parental lines and dependable hybrid seed-production systems. Future progress will depend on improving environmental stability, broadening cytoplasmic and restorer diversity, resolving the molecular basis of nuclear–mitochondrial interactions and ensuring that laboratory advances translate into reliable hybrid performance.

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