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**Prabha Banjarey**
Guest Teacher, Department of Genetics and Plant Breeding, BTC CARS Bilaspur, Chhattisgarh, India

**Madhuri Singh**
Guest Teacher, Department of Genetics and Plant Breeding, College of Horticulture and Research Station, Ramanujgang, Chhattisgarh, India

**Bhavana Bhardwaj**
M.Sc. (Ag.), Department of Genetics and Plant Breeding, Berister Thakur Chhedilal College of Agriculture and Research station Bilaspur, IGKV Chhattisgarh, India

**Bhawna Netam**
M.Sc. (Ag.), Department of Genetics and Plant Breeding, Thakur Chhedilal College of Agriculture and Research station Bilaspur, IGKV, Chhattisgarh, India

**Corresponding Author:**
**Prabha Banjarey**
Guest Teacher, Department of Genetics and Plant Breeding, BTC CARS Bilaspur, Chhattisgarh, India

# Cytoplasmic male sterility in hybrid wheat: Mechanisms, genetic systems, applications and future prospects

Prabha Banjarey[1], Madhuri Singh[2], Bhavana Bhardwaj[3], Bhawna Netam[4]

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**Abstract**
Cytoplasmic male sterility (CMS) is a maternally inherited condition in which plants fail to produce functional pollen while retaining female reproductive capacity. It is an important biological tool for hybrid seed production because it can prevent self-pollination in the female parent and remove the need for manual emasculation. In bread wheat (*Triticum aestivum* L.), hybrid breeding has the potential to exploit heterosis for grain yield, adaptation and stress resilience, but its practical deployment is constrained by the crop's predominantly self-pollinating floral biology, the cost of hybrid seed production and the need for reliable fertility restoration. Among the investigated systems, T. timopheevii-type cytoplasmic male sterility (T-CMS) is particularly significant. Recent molecular work identified the mitochondrial gene orf279 as the causal CMS-associated factor and showed that the nuclear restorer genes $Rf_1$ and $Rf_3$ encode pentatricopeptide-repeat proteins that bind the orf279 transcript and promote its cleavage. This review summarises the genetic and molecular basis of CMS in wheat, the three-line A/B/R breeding system, fertility restoration, applications, limitations and future research priorities. Integration of molecular markers, genomic selection, mitochondrial genomics and improved pollination management may strengthen the feasibility of hybrid wheat breeding.

**Keywords:** Cytoplasmic male sterility, Hybrid wheat, *Triticum timopheevii*, Orf279, $Rf_1$, $Rf_3$, Fertility restoration, Heterosis

## 1. Introduction

Wheat is a major cereal crop and a central component of global food and nutritional security. Continued improvement in grain yield and stability is required to meet demand under changing climates, increasing production costs and pressure on natural resources. Conventional wheat breeding has delivered substantial genetic gain through selection, crossing and the development of improved inbred cultivars. Hybrid breeding offers an additional route by combining genetically complementary parents and exploiting heterosis, expressed as improved performance of an $F_1$ over its parents or an appropriate commercial check.

Unlike maize, wheat is predominantly self-pollinated and has floral characteristics that make controlled cross-pollination difficult. Manual emasculation is labour-intensive and costly at the scale required for commercial seed production. A reliable male-sterility system can prevent self-fertilisation of the female parent and facilitate hybrid seed production. CMS is especially attractive because the sterility-inducing factor is carried in the cytoplasm and is usually maternally inherited. However, a commercially useful CMS system must combine stable sterility, good female fertility, effective restoration in the $F_1$ and an economical method of transferring pollen between parents (Melonek *et al.*, 2021; Whitford *et al.*, 2013) [1, 2].

## 2. Concept and genetic basis of cytoplasmic male sterility

CMS is a form of reproductive dysfunction caused primarily by interactions between mitochondrial genetic factors and nuclear genes. In most well-characterised systems, rearrangements of the mitochondrial genome generate novel or chimeric open reading frames (ORFs). Expression of a CMS-associated ORF can disrupt mitochondrial processes, cellular energy balance or developmental events in the anther, resulting in defective microspore development or pollen abortion. The female reproductive organs may remain functional, allowing the sterile plant to set seed when pollinated by a fertile plant.

Because the mitochondria of most angiosperms are transmitted predominantly through the egg cell, CMS is generally inherited maternally. This contrasts with nuclear male sterility, where the causal allele is located in the nuclear genome and may segregate according to Mendelian inheritance. CMS expression and stability can be influenced by the nuclear background and environmental conditions; therefore, sterility must be evaluated across generations and representative production environments (Chen *et al.*, 2014; Kaul, 1988) [3, 4].



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**Figure 1.** Wheat (Triticum aestivum L.), the crop species targeted for heterosis-based hybrid improvement.

## 3. Cytoplasmic male sterility systems investigated in wheat

Several alien and wild-relative cytoplasms have been examined for their capacity to induce male sterility in wheat nuclear backgrounds. *T. timopheevii* cytoplasm is the best-characterised and most widely discussed CMS source for bread wheat. Transfer of this cytoplasm into suitable *T. aestivum* backgrounds can produce T-CMS lines. Other cytoplasmic sources, including those associated with *Aegilops* species, have been explored, but their practical value depends on the completeness and stability of sterility, the availability of restorers and agronomic compatibility. Not every cytoplasm that induces partial sterility is suitable for a commercial hybrid system [1, 5].

The T-CMS system is important not only as a breeding tool but also as a model for understanding mitochondrial–nuclear communication. Earlier work associated sterility with mitochondrial regions including orf256; however, the 2021 molecular study by Melonek *et al.* provided strong evidence that orf279 is the relevant CMS-associated transcript targeted by the identified restorers $Rf_1$ and $Rf_3$ (Chen *et al.*, 2014) [3].

## 4. Three-line system for hybrid wheat breeding

The conventional CMS-based hybrid seed system uses three lines. The A-line is the male-sterile female parent and carries the sterile cytoplasm. The B-line is a fertile maintainer with a similar nuclear background but normal cytoplasm and no effective restorer allele for the CMS system. The *R*-line is a pollen parent carrying one or more effective nuclear restorer genes. The A-line is multiplied by crossing it with the B-line, whereas commercial $F_1$ seed is produced by crossing the A-line with the *R*-line.

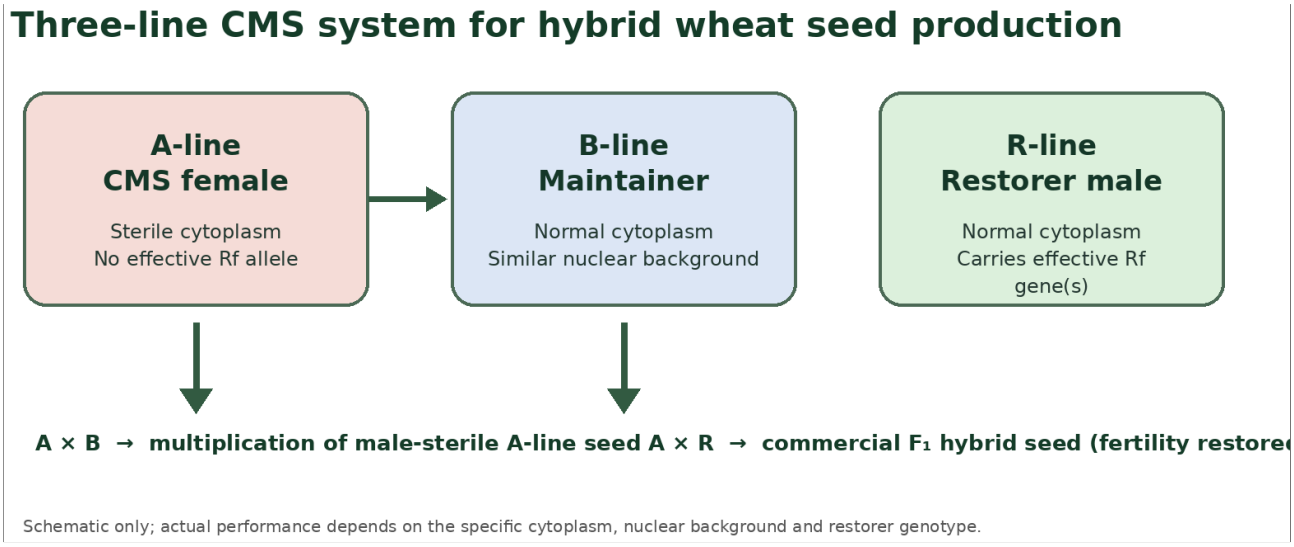



**Figure 2.** Simplified A/B/R three-line system. A × B is used for A-line maintenance; A × R produces commercial $F_1$ hybrid seed.

Successful operation requires synchronised flowering, adequate pollen production by the *R*-line, effective pollen movement to CMS plants, field isolation and rigorous genetic purity control. Wheat's floral biology and often limited natural outcrossing make pollen transfer a central production challenge. Consequently, the presence of CMS alone does not guarantee economical hybrid seed production (Whitford *et al.*, 2013) [2].



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| Line | Genetic constitution / key feature | Main function |
|---|---|---|
| A-line (CMS) | Sterile cytoplasm; lacks effective restorer allele | Female parent for hybrid seed; prevents self-pollination |
| B-line (maintainer) | Normal fertile cytoplasm; similar nuclear background to A-line | Maintains and multiplies the A-line |
| *R*-line (restorer) | Carries effective Rf gene(s) | Supplies pollen and restores fertility in $F_1$ progeny |

## 5. Molecular mechanism of T-CMS and fertility restoration

Plant mitochondria encode components of the respiratory machinery and contain their own transcriptional and translational systems. In CMS plants, novel mitochondrial ORFs may produce proteins that interfere with mitochondrial membrane function or energy metabolism, particularly in developing anthers with high metabolic demand. The resulting disturbance can impair tapetal function, microsporogenesis or pollen maturation.

Melonek *et al.* (2021) [1] identified orf279 as the key mitochondrial transcript associated with T-CMS in wheat. They demonstrated that $Rf_1$ and $Rf_3$ encode nuclear-encoded pentatricopeptide-repeat (PPR) proteins. These proteins recognise the orf279 RNA and promote its cleavage, reducing the accumulation of the CMS-associated transcript and permitting pollen fertility to recover. The study also showed that combining restorer activities can improve restoration, although complete and stable fertility remains a breeding objective (Chen *et al.*, 2014) [3].

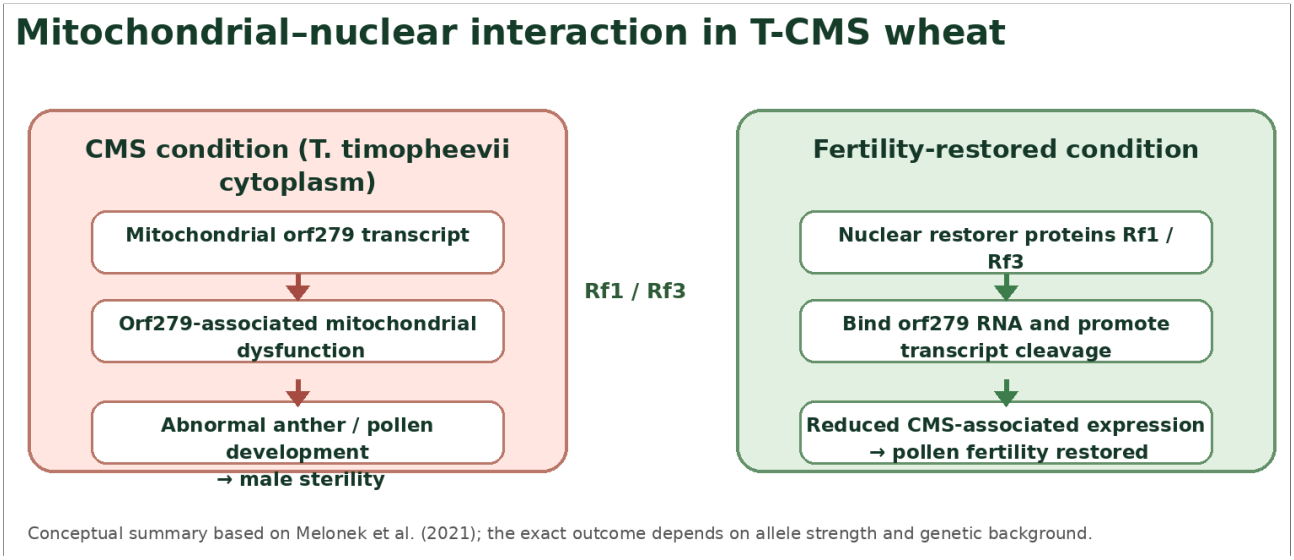



**Figure 3.** Conceptual representation of the T-CMS mitochondrial factor and nuclear restoration pathway, based on Melonek *et al.* (2021) [1].

Restorer genes are not interchangeable across all CMS systems: restoration is commonly specific to the cytoplasm and its associated mitochondrial factor. Therefore, an Rf allele must be validated in the target cytoplasm and nuclear background rather than assumed to function universally. In wheat, additional reported restorers and fertility-restoration loci have been investigated, including $Rf_8$, for which linked molecular markers have been evaluated in T. timopheevii-based material (Bakala *et al.*, 2020) [6].

## 6. Identification and deployment of restorer genes

Restorer identification begins with crossing CMS lines to candidate pollen parents and assessing fertility in the resulting $F_1$ and segregating generations. Phenotyping may include pollen staining or in-vitro germination, anther dehiscence, spikelet fertility, seed set and the proportion of fully fertile plants. Pollen staining alone should not be treated as a complete measure of field fertility; seed set under field conditions provides an important complementary assessment.

Conventional backcrossing can transfer restorer alleles into elite lines, while marker-assisted selection can accelerate selection when reliable markers are tightly linked to the target gene. For $Rf_8$, SSR markers including Xwmc503, Xwmc296 and Xwmc112 have been examined for marker–trait association in T-CMS wheat material (Bakala *et al.*, 2020) [6]. Marker utility must be tested in the intended breeding population because linkage phase and recombination can vary among genetic backgrounds.

## 7. Applications and potential benefits in wheat improvement

The primary application of CMS is the production of hybrid seed without emasculating the female parent. This can enable breeders to test and exploit heterotic combinations that may improve grain yield, biomass, early vigour, adaptation, lodging resistance, disease response or tolerance to heat and drought. The expression of these traits is dependent on the parental combination and environment; CMS itself does not directly confer high yield or stress tolerance.

Hybrid wheat programmes can also use the A/B/R system to organise parental-line development and evaluate combining ability systematically. A large pool of diverse female and restorer lines is desirable to avoid dependence on a narrow genetic base. The economic case for a hybrid depends on a consistent yield or quality advantage over competitive varieties, seed production efficiency, seed cost and the farmer's ability to obtain fresh hybrid seed for each sowing.

## 8. Limitations and challenges

- Predominantly self-pollinating floral biology and limited natural cross-pollination can restrict seed set on CMS female rows.
- Flowering synchrony, pollen production, wind conditions and field layout must be managed carefully.
- Sterility and restoration may vary with temperature, genetic background and environment; multi-location testing is essential.
- Effective restorer alleles may be absent or weak in otherwise desirable elite lines.



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- Dependence on a narrow cytoplasmic source can increase genetic vulnerability; cytoplasmic diversity is desirable.
- Hybrid seed production, quality control and distribution can cost more than production of conventional inbred seed.
- A commercial hybrid must deliver a sufficiently reliable yield or quality advantage to offset the additional seed cost.

## 9. Molecular breeding and emerging technologies

Genome-wide SNP genotyping, high-density linkage maps and genomic selection can improve the prediction of parental performance and hybrid combinations. Mitochondrial genome sequencing and transcriptome profiling can help identify CMS-associated ORFs, RNA-processing events and candidate restorer genes. Functional validation through transformation or gene editing can test whether a candidate gene is sufficient to restore fertility, as illustrated by the functional work on *Rf*$_1$ and *Rf*$_3$ (Chen *et al.*, 2014) [3].

CRISPR-based approaches may also be useful for studying nuclear genes involved in anther development or for engineering male-sterility systems. Such approaches should be distinguished from naturally occurring CMS: editing a nuclear fertility gene does not automatically create a maternally inherited CMS system. High-throughput phenotyping, controlled-environment testing and field-based pollen-transfer studies are equally important because the success of hybrid wheat depends on reproductive performance as well as molecular genotype.

## 10. Future prospects

Future research should prioritise (i) discovery and validation of additional stable cytoplasmic sources, (ii) stronger and more predictable fertility restoration, (iii) development of molecular markers for key Rf alleles, (iv) improvement of floral traits and pollen transfer, (v) selection of parents with high general and specific combining ability, and (vi) economic evaluation of hybrid seed production at commercial scale. Genomic selection and precise phenotyping may help shorten the cycle of parental improvement, while mitochondrial and nuclear genome analyses can deepen understanding of CMS/Rf interactions.

For wheat-growing regions such as South Asia, hybrid development should be assessed across contrasting sowing dates, temperature regimes and disease environments. A successful hybrid should combine high yield potential with stable grain quality, resistance to locally important diseases and reliable performance under farmers' management. The ultimate objective is not simply to produce fertile $F_1$ plants, but to establish a cost-effective seed system that delivers measurable and repeatable value to growers.

## 11. Conclusion

Cytoplasmic male sterility is a valuable genetic approach for facilitating hybrid wheat seed production and exploiting heterosis. The *T. timopheevii* cytoplasm and the molecular identification of orf279, *Rf*$_1$ and *Rf*$_3$ have advanced understanding of the T-CMS system. The three-line A/B/R system provides a practical framework, but its commercial success depends on stable sterility, effective restoration, flowering synchrony, efficient pollen transfer and competitive hybrid performance. Integrating classical breeding with molecular markers, genomic selection, mitochondrial genomics and reproductive phenotyping offers a promising route towards more reliable and economically viable hybrid wheat.

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