REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 1s (2024)
http://www.veterinaria.org
Article Received: 20/06/2024 Revised: 02/07/2024 Accepted: 18/07/2024

Cytoplasmic Male Sterility (CMS) in Maize: A Review Highlighting Its Role in Hybrid Fodder Production and Livestock/Veterinary Feeding Programs

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Abstract

Cytoplasmic male sterility (CMS) is a maternally inherited condition that suppresses pollen formation and serves as a cornerstone for hybrid seed production in maize (Zea mays L.). Maize remains the most widely studied CMS model due to its diverse T-, C-, and S-cytoplasms, each associated with chimeric mitochondrial ORFs and restored by nuclear restorer-of-fertility (Rf) genes encoding PPR proteins. While CMS has transformed hybrid seed production for grain and silage maize, its relevance has expanded significantly into the fodder and veterinary nutrition sector, where uniform, high-biomass and highly digestible fodder is essential for livestock productivity.

CMS-derived maize hybrids produce superior green fodder and silage because of high soluble carbohydrate content, uniform flowering, improved biomass accumulation, and ideal forage quality traits. These attributes directly enhance rumen fermentation, dry matter intake, milk yield, and metabolic efficiency in dairy cattle, buffalo, sheep, and goats. Knowledge generated from maize CMS also provides a reference framework for CMS applications in related fodder crops such as sorghum (A1/A2/A3 CMS) and pearl millet (A1 CMS), which further support veterinary feeding programs in arid regions.

This review highlights the molecular basis of CMS in maize, compares CMS systems across other cereals, and emphasizes the increasing significance of CMS-based hybrids in fodder production and veterinary animal nutrition.

1.0 Introduction

Maize (*Zea mays L.)* stands among the most globally significant cereals, ranking after wheat and rice in production. Its extensive adaptability, genetic diversity, and use in food, feed, and industry have made it an indispensable crop worldwide. One of the greatest technological breakthroughs in maize improvement is the exploitation of heterosis (hybrid vigor), which has dramatically increased yields across continents. Successful hybrid-seed production depends on controlled cross-pollination; however, manual detasseling is labor-intensive and expensive. Cytoplasmic male sterility (CMS) provides a biological alternative, ensuring reliable cross-pollination without mechanical intervention (Rhoades, 1931).

CMS was first identified in maize when Rhoades (1931) observed that certain cytoplasmic backgrounds caused complete pollen sterility, which could be reversed by specific nuclear genes. Since then, maize has served as a model system for studying mitochondrial-nuclear interactions leading to sterility and restoration (Levings, 1990; Chase, 2007). CMS systems have subsequently been discovered in many crops, but maize remains the most extensively researched due to its diverse CMS cytotypes and their impact on modern agriculture.

The integration of CMS into hybrid-breeding programs revolutionized seed production efficiency in the mid-20th century. However, large-scale reliance on the Texas (T) cytoplasm in the 1960s led to vulnerability to Helminthosporium maydis race T, causing the devastating southern corn-leaf-blight epidemic in 1970 (Levings, 1990). This crisis prompted breeders to diversify cytoplasmic sources and deepen molecular understanding of CMS mechanisms.

In the post-genomic era, the use of next-generation sequencing, transcriptomics, and proteomics has illuminated the intricate cross-talk between mitochondrial CMS genes and nuclear Rf genes (Bentolila et al., 2002; Chen & Liu, 2014). Recent advances, including functional genomics and CRISPR-based editing, have enabled more precise manipulation of sterility and fertility restoration (Yang et al., 2022; Huynh et al., 2023).

This review aims to provide an updated synthesis of the cytological, genetic, and molecular basis of CMS in maize, its utilization in hybrid breeding, limitations, and future research directions, with reference to comparative insights from rice, wheat, and sorghum CMS systems.

2.0 Classification and Types of Cytoplasmic Male Sterility in Maize

Cytoplasmic male sterility (CMS) in maize has been classified primarily into three types—Texas (T-), Charrua (C-), and USDA (S-) cytoplasms—each distinguished by its unique mitochondrial genome organization, gene content, and response to specific nuclear restorer-of-fertility genes. These cytoplasmic variants originated through spontaneous or induced rearrangements in mitochondrial DNA that resulted in chimeric open reading frames (ORFs) responsible for sterility (Levings, 1990; Dewey et al., 1986).

2.1 T-Cytoplasm (Texas Cytoplasm)

The T-cytoplasm was the first CMS type widely exploited in commercial hybrid-seed production. Male sterility in this system results from a unique mitochondrial gene, T-urf13, which encodes a 13-kDa protein that integrates into the inner mitochondrial membrane. The protein forms a toxin-sensitive pore complex that causes mitochondrial dysfunction in the presence of *Helminthosporium maydis* race T toxin (Levings, 1990).

Fertility restoration is achieved by two nuclear genes—Rf1 and Rf2—that suppress the accumulation of urf13 transcripts and restore normal anther development (Wise & Pring, 2002). Despite its agronomic efficiency, extensive use of T-cytoplasm led to the catastrophic U.S. southern corn leaf-blight epidemic in 1970. As a result, T-cytoplasm was largely abandoned and replaced with safer CMS systems.

Recent molecular studies (Yang et al., 2022) revealed that sterility in T-cytoplasm involves the impaired assembly of the ATP synthase complex due to chimeric mitochondrial transcripts. These discoveries provide a mechanistic explanation for toxin sensitivity and have opened possibilities for genome-editing-based corrections in the mitochondrial genome.

2.2 C-Cytoplasm (Charrua Cytoplasm)

C-cytoplasm originated from South American germplasm known as “Charrua.” Male sterility in CMS-C is associated with rearranged mitochondrial sequences encoding orf355/orf77, which are co-transcribed with atp9 (Dewey et al., 1986). The aberrant proteins interfere with mitochondrial respiration, leading to defective pollen-grain development.

Fertility is restored by two major nuclear loci, Rf4 and Rf5, which code for PPR proteins that bind the CMS-related RNA, facilitating cleavage and degradation of orf355/orf77 transcripts (Liu et al., 2022). Compared with CMS-T, the CMS-C system exhibits higher disease resistance and environmental stability but occasionally suffers from partial fertility restoration under stress conditions.

Advances in marker-assisted selection have enabled efficient identification of restorer lines carrying Rf4, reducing dependency on phenotype-based screening (Cheng et al., 2023).

2.3 S-Cytoplasm (USDA Cytoplasm)

The S-type (USDA) cytoplasm is another well-characterized CMS system in maize. Male sterility here results from a chimeric region of the mitochondrial genome containing orf355 and orf77, which encode aberrant peptides that block anther dehiscence (Allen et al., 2007).

Restoration is primarily controlled by Rf3 and Rf8 (Qin et al., 2021). These Rf genes encode PPR proteins that recognize and destabilize CMS-associated transcripts, thereby re-establishing normal mitochondrial function. CMS-S is relatively stable, less disease-prone, and considered suitable for hybrid-seed production in tropical environments. However, the complex inheritance of its restorer genes and occasional instability still limit its broad commercial use.

2.4 Minor and Novel CMS Systems

Beyond the three major types, researchers have reported additional minor or recombinant cytoplasmic systems showing male sterility with varying stability. Some of these are derived from exotic maize accessions and inter-specific crosses (Chase, 2007). These alternative cytoplasms are currently under molecular investigation to enhance genetic diversity and to reduce dependence on a narrow cytoplasmic base.

2.5 Comparative Stability and Agronomic Importance

Among all CMS types, the C- and S-cytoplasms are now preferred in modern breeding programs due to their disease resistance and environmental stability. The introduction of molecular markers tightly linked to Rf genes has accelerated breeding of restorer lines. Furthermore, transcriptomic studies demonstrate that Rf genes differ in their regulatory mode: Rf1 and Rf4 primarily induce RNA cleavage, while Rf2 encodes an aldehyde dehydrogenase essential for mitochondrial redox balance (Bentolila et al., 2002; Huynh et al., 2023).

Such findings have strengthened our understanding of the mitochondrial–nuclear dialog governing fertility and have provided new molecular targets for maintaining hybrid vigor through CMS technology.

3.0 Molecular and Genetic Mechanisms of CMS and Fertility Restoration in Maize

Cytoplasmic male sterility (CMS) is a result of disrupted mitochondrial gene expression, leading to abnormal energy metabolism and failure of pollen development. The sterility is maternally inherited because mitochondria are transmitted through the egg cytoplasm, while nuclear restorer genes (Rf) provide biparental inheritance that can suppress or compensate for the defective mitochondrial function (Chase, 2007; Wise & Pring, 2002).

3.1 Mitochondrial Genome Rearrangement and Chimeric Genes

The mitochondrial genome of maize is highly dynamic, exhibiting frequent recombination and rearrangements. These rearrangements often lead to the creation of chimeric open reading frames (ORFs) that fuse coding sequences of essential genes with unknown fragments (Bentolila et al., 2002). Such ORFs, including T-urf13 (CMS-T), orf355/orf77

(CMS-C and CMS-S), and atp6c (novel CMS variant), produce proteins that interfere with mitochondrial membrane integrity and disrupt ATP synthesis (Yang et al., 2022).

These proteins cause a reduction in mitochondrial efficiency during anther development, leading to aborted pollen formation. The specific interaction of these aberrant proteins with the mitochondrial inner membrane can trigger programmed cell death in microspores (Levings, 1990). The molecular consequence is defective tapetum degeneration, which halts pollen maturation.

3.2 Role of Restorer-of-Fertility (Rf) Genes

Fertility restoration in maize depends on Rf genes, which are mostly dominant nuclear loci encoding pentatricopeptide repeat (PPR) proteins. PPR proteins are sequence-specific RNA-binding proteins that regulate post-transcriptional events such as RNA cleavage, degradation, and editing within mitochondria (Bentolila et al., 2002).
For example:

1. Rf1 and Rf2 restore fertility in CMS-T by suppressing urf13 transcript accumulation.
2. Rf4 and Rf5 are responsible for fertility restoration in CMS-C, promoting degradation of orf355/orf77 mRNA (Liu et al., 2022).
3. Rf3 and Rf8 control restoration in CMS-S by selectively destabilizing orf355 RNA (Qin et al., 2021).
4. Recent research by Huynh et al. (2023) discovered that unique C-terminal domains in some Rf-related PPR proteins contribute to specificity and RNA-cleavage efficiency, enhancing our understanding of Rf gene evolution and function.

3.3 Transcriptomic and Proteomic Insights

Modern transcriptomic studies have revealed complex gene expression patterns between sterile and fertile lines. Differentially expressed genes (DEGs) in CMS plants are mainly involved in oxidative phosphorylation, carbohydrate metabolism, and cell-wall modification (Farinati et al., 2023). Proteomic analyses confirm the down regulation of ATP synthase subunits and cytochrome oxidase components in sterile anthers, reflecting energy deficiency.

Additionally, RNA-sequencing data suggest that mitochondrial retrograde signaling affects nuclear gene expression in CMS plants (Wang et al., 2022). This bidirectional communication indicates that fertility restoration involves coordinated changes in both organellar and nuclear transcriptomes.

3.4 Functional Genomics and CRISPR-Cas Advances

The advent of functional genomics and gene-editing technologies has accelerated CMS research in maize. CRISPR-Cas9-mediated editing of mitochondrial-associated nuclear genes has shown potential to modulate sterility without affecting plant viability (Yang et al., 2022). Although direct mitochondrial transformation remains challenging, targeted editing of nuclear Rf genes can enhance restoration efficiency and stability.

Comparative genomics between maize and other crops, such as rice and wheat, has identified conserved PPR gene families that play universal roles in CMS restoration (Melonek et al., 2021). This cross-species analysis aids in predicting new restorer candidates and developing molecular markers for breeding.

3.5 RNA Editing and Mitochondrial Biogenesis

Another important aspect of CMS regulation is RNA editing, which converts specific cytidine residues to uridine in mitochondrial transcripts, affecting protein-coding potential. Defects in RNA editing due to dysfunctional PPR proteins often result in male sterility. In maize, editing of genes like atp6, cox2, and nad9 is critical for normal pollen development (Wang et al., 2022).

Recent studies suggest that restorer proteins assist in restoring proper RNA editing patterns, reinstating mitochondrial function and fertility (Cheng et al., 2023). This highlights RNA metabolism as a central mechanism underlying CMS expression and restoration.

3.6 Mitochondrial-Nuclear Crosstalk and Energy Metabolism

Fertility restoration not only corrects mitochondrial transcript processing but also reestablishes energy homeostasis. Restored hybrids show enhanced ATP synthesis and ROS (reactive oxygen species) detoxification compared with sterile lines (He et al., 2020). This implies that CMS disrupts energy flow, while Rf gene expression reprograms energy metabolism, ensuring proper tapetum function and viable pollen formation.

Together, these molecular interactions illustrate CMS as a dynamic system of organellar dysfunction and nuclear compensation—a prime model for studying plant developmental biology.

4.0 Role of Cytoplasmic Male Sterility (CMS) in Hybrid Seed Production

Cytoplasmic male sterility has become an indispensable tool in commercial maize hybrid seed production. By eliminating the need for manual detasseling, CMS enables efficient, large-scale hybrid development with uniformity and genetic purity. The CMS system forms the foundation of the three-line hybrid breeding scheme, which involves:

1. A-line (male-sterile line) – possesses CMS cytoplasm and recessive nuclear genes responsible for sterility.
2. B-line (maintainer line) – carries normal cytoplasm but is genetically identical to the A-line; used to maintain male sterility during seed multiplication.
3. R-line (restorer line) – contains dominant Rf genes that restore fertility in the hybrid progeny.

Crossing an A-line (female) with an R-line (male) results in fertile hybrid offspring that combine high-yield potential with stable fertility restoration (He et al., 2020).

The efficiency of CMS-based hybrid breeding in maize has significantly reduced production costs and human labor. Moreover, CMS lines are useful for genetic studies on pollen development, mitochondrial gene regulation, and fertility restoration.

4.1 Advantages of Using CMS in Hybrid Breeding

The advantages of CMS systems are both biological and economic:

1. Elimination of manual detasseling: Reduces labor and time while maintaining genetic purity.
2. Consistent hybrid production: Ensures stable hybrid seed yield in large-scale operations.
3. Exploration of heterosis: Allows more effective use of heterotic groups and parental combinations.
4. Enhanced genetic research: Provides a biological model to study mitochondrial–nuclear interactions.
5. The use of CMS-based hybrids has resulted in significant yield gains and uniformity in grain and silage maize varieties (Chase, 2007; Chen & Liu, 2014).

4.2 Limitations and Challenges

Despite its advantages, CMS faces certain limitations that must be addressed for sustainable hybrid breeding:

1. Disease susceptibility: The Texas cytoplasm disaster in 1970 highlighted the danger of relying on a single cytoplasmic source (Levings, 1990).
2. Incomplete fertility restoration: CMS-C and CMS-S sometimes exhibit partial fertility restoration under adverse environmental conditions (Dewey et al., 1986).
3. Cytoplasmic–nuclear incompatibility: Certain combinations may reduce yield or vigor due to genetic imbalance.
4. Restorer gene scarcity: The limited availability of strong Rf genes restricts hybrid development in diverse genetic backgrounds.

Modern breeding programs thus emphasize cytoplasmic diversification and molecular marker-assisted selection (MAS) to ensure the stability of CMS-based hybrids. Integration of genomics and transcriptomics tools has improved the efficiency of restorer gene discovery and hybrid line development (Liu et al., 2022; Cheng et al., 2023).

5.0 Comparative Analysis: CMS in Other Major Crops

While maize remains the classic model for studying CMS, similar systems have been discovered and exploited in other cereals and crops. A brief comparison with CMS in rice, wheat, and sorghum reveals both shared and unique mechanisms.

5.1 CMS in Rice (*Oryza sativa L.)*

In rice, CMS was discovered in the 1970s and has since been extensively utilized for hybrid seed production. The most widely used CMS systems include Wild Abortive (WA), Honglian (HL), and BT (Boro II) cytoplasms (Chen & Liu, 2014).

Rice CMS is typically associated with chimeric mitochondrial genes such as WA352 and orf79, which disrupt tapetum function. Fertility restoration is governed by nuclear Rf genes like Rf3 and Rf4, encoding PPR proteins that degrade the CMS-associated mRNAs (Sun et al., 2022). Advances in genomic breeding and CRISPR-Cas tools have allowed manipulation of these loci to enhance fertility restoration efficiency and reduce environmental sensitivity.

5.2 CMS in Wheat (*Triticum aestivum L.)*

CMS in wheat was first developed through interspecific crosses with *Triticum timopheevii.* The Timopheevii cytoplasm (T-CMS) remains the most studied, used for hybrid seed production and as a model to understand mitochondrial–nuclear interactions (Melonek et al., 2021).

In this system, the mitochondrial orf256 and orf279 genes are associated with sterility, while restoration involves Rf1 and Rf3 genes. Wheat CMS has faced challenges in achieving complete fertility restoration, but recent genomic analyses have identified new Rf candidates across the wheat genome, improving hybrid performance (Melonek et al., 2021).

5.3 CMS in Sorghum (*Sorghum bicolor L.*)

Sorghum was among the first crops to successfully adopt CMS technology for commercial hybrid seed production. The major CMS types are A1 (milo cytoplasm), A2, and A3, each differing in mitochondrial genome structure. The A1 cytoplasm is widely used due to its stability and effective restoration by Rf1 and Rf2 genes (Chen & Liu, 2014).

Sorghum CMS involves chimeric mitochondrial genes such as orf79 and atp6, similar to those found in maize and rice. Its success demonstrates the versatility of CMS systems in different plant species and underscores the potential of cross-species studies to identify conserved fertility restoration mechanisms.

5.4 Cross-Species Insights

Comparative analysis of CMS systems across crops highlights several universal features:

1. CMS is always linked to mitochondrial genome rearrangements.
2. Restorer genes often encode PPR proteins with RNA-binding capacity.
3. RNA editing and transcript degradation are the key restoration mechanisms.
4. Diversification of cytoplasmic sources is crucial for disease resistance and hybrid sustainability.

The study of CMS in maize has greatly influenced breeding strategies in rice, wheat, and sorghum, demonstrating the evolutionary conservation of mitochondrial–nuclear communication in plants.

6.0 Recent Advances and Future Prospects

The study of cytoplasmic male sterility (CMS) in maize has evolved significantly with the advent of molecular biology, genomics, and bioinformatics. Recent years (2019–2023) have witnessed a surge In the discovery of new CMS-associated mitochondrial genes and fertility restorer loci, aided by next-generation sequencing (NGS), transcriptomics, and proteomics.

Genomic Sequencing and Transcriptome Profiling:

The use of whole-genome and mitochondrial sequencing has helped identify novel chimeric genes such as atp6c and orf355/orf77, clarifying their role in mitochondrial dysfunction and male sterility (Yang et al., 2022; Cheng et al., 2023). Transcriptome studies comparing sterile and fertile lines have revealed that sterility is often accompanied by down regulation of genes involved in oxidative phosphorylation, carbohydrate metabolism, and tapetal cell differentiation (Farinati et al., 2023).

Molecular Breeding and Marker Development:

The development of molecular markers linked to Rf1, Rf3, Rf4, and Rf5 has greatly facilitated restorer line identification and hybrid purity assessment. Marker-assisted selection (MAS) has reduced the reliance on phenotypic screening and enabled pyramiding of multiple Rf genes to achieve stable fertility restoration (Liu et al., 2022).

Functional Genomics and Gene Editing:

The integration of CRISPR-Cas technologies is revolutionizing CMS research. Genome editing can target nuclear genes involved in mitochondrial regulation, offering the potential to engineer novel CMS or restorer systems (Yang et al., 2022). However, direct mitochondrial transformation remains a challenge due to the organelle's double-membrane barrier and limited DNA import mechanisms.

Comparative and Evolutionary Insights:

Comparative genomics across crops such as rice, wheat, and sorghum has revealed that the interaction between mitochondrial CMS genes and nuclear PPR-based restorer genes is evolutionarily conserved (Melonek et al., 2021). This opens new opportunities to transfer or engineer CMS systems across species.

Future Prospects:

1. Development of multi-restorer lines to ensure fertility under variable environments.
2. Exploration of novel cytoplasmic sources to enhance disease resistance.
3. Application of bioinformatics and machine learning to predict CMS–Rf interactions.
4. Integration of CMS systems with hybridization-promoting traits such as self-incompatibility for superior hybrid breeding efficiency.
5. Together, these advancements ensure that CMS will continue to play a pivotal role in sustainable maize improvement and global food security.

7.0 Conclusion

Cytoplasmic male sterility (CMS) in maize is one of the most well-explored examples of mitochondrial–nuclear interaction in plant systems. Although challenges such as incomplete fertility restoration and disease susceptibility persist, the expansion of molecular and genomic tools promises innovative solutions.

Beyond hybrid grain production, CMS-based hybrids have become indispensable in fodder and veterinary nutrition, providing uniform, high-biomass, and highly digestible feed crucial for dairy and livestock productivity. The CMS principles established in maize form the scientific foundation for CMS exploitation in related fodder crops such as sorghum and bajra, strengthening fodder security across diverse agro-climatic regions. As molecular tools, genomics, and restorer-gene research advance, CMS will continue to support sustainable crop improvement and livestock feeding programs essential for veterinary science.

8.0 CMS Diversity in Maize and Related Fodder Crops: Veterinary & Fodder Science Relevance

Although maize is the primary crop for CMS research, the biological principles and hybridization efficiency observed in maize have guided CMS utilization in sorghum, pearl millet (bajra), and napier–bajra hybrids, which together form the backbone of green fodder production for India's livestock sector. Maize CMS mechanisms—mitochondrial ORF formation, PPR-mediated restoration, and stable hybrid expression—serve as a template for improving fodder crop hybrids.

8.1 Why Maize CMS Is the Central Model for Veterinary Fodder Systems

Most diverse CMS types (T, C, S)
Well-characterized restorer genes (Rf1, Rf2, Rf3, Rf4)
Stable hybrid production → high biomass fodder
Ideal for silage, a critical veterinary feed for dairy animals
Uniform plants → uniform nutrient supply
CMS maize hybrids improve:

1. Dry Matter (DM) yield
2. TDN, ME, and digestibility
3. Rumen microbial efficiency
4. Milk yield and fat percentage

8.2 CMS in Sorghum & Bajra – Extension of the Maize Model

Although less diverse than maize, CMS systems in sorghum (A1, A2, A3) and bajra (A1 CMS) follow similar principles:

1. Mitochondrial DNA rearrangements
2. Chimeric genes causing pollen sterility
3. Nuclear Rf genes restoring fertility

These CMS-based hybrids:

1. Provide drought-tolerant green fodder
2. Have high leaf:stem ratio
3. Produce soft, digestible stover
4. Support body weight gain and milk performance

8.3 Veterinary Application of CMS-Based Fodder Hybrids

CMS hybrids directly contribute to:

1. Better rumen fermentation
2. Lower feeding cost
3. Consistent fodder availability
4. Higher growth rate in calves, kids, lambs
5. Increased milk production and reproductive efficiency

8.4 Role of CMS Diversity in Fodder Seed Industry

CMS ensures:

1. Cheap & large-scale hybrid seed production
2. Availability of uniform hybrid fodder
3. Better planning for dairy farms, gaushalas, and veterinary institutes.
4. Thus, CMS diversity in maize and related crops is not only a plant breeding tool but a veterinary necessity for fodder security.

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