ZIBELINE INTERNATIONAL™ PUBLISHING

ISSN: 2521-5051 (Print)
ISSN: 2521-506X (Online)
CODEN: ASMCCQ

Acta Scientifica Malaysia (ASM)

DOI: http://doi.org/10.26480/asm.01.2024.21.27

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RESEARCH ARTICLE

A COMPREHENSIVE REVIEW ON-APPLICATION AND FUTURE PROSPECT OF CYTOPLASMIC MALE STERILITY

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ARTICLE DETAILS

Article History:

Received 09 February 2024
Revised 12 March 2024
Accepted 16 April 2024
Available online 17 May 2024

ABSTRACT

Cytoplasmic male sterility (CMS) is a plant's inability to reproduce fertile pollen due to nuclear and mitochondrial genomic incompatibility. In CMS plants, pollen production is disrupted, but this can be restored by nuclear fertility (Rf) genes, while the function of the female organ is typically unaffected. CMS has been intensively used for F1 seed production in self-pollinating crops and practical applications in plant breeding. This comprehensive review explores the intricate mechanisms, applications, limitations, advancements, and prospects of CMS in an agricultural context. Despite its notable advantages, CMS does present limitations, such as instability and genetic vulnerabilities, which are discussed alongside strategies for mitigation. Moreover, the review elucidates on the recent advancements in biotechnology, omics technologies, and breeding approaches, which promise to further harness the potential of CMS in addressing global food security challenges. From the identification of diverse CMS sources to the utilization of advanced genetic engineering techniques like CRISPR-Cas gene editing, the future of CMS in crop breeding appears promising. Thus, the development of diverse sources of CMS and its practical application in breeding are expected in the future.

KEYWORDS

Cytoplasmic male sterility (CMS), Nuclear fertility (Rf) genes, Hybrid seed, Genetic incompatibility, pollen disfunction

1. INTRODUCTION

Cytoplasmic male sterility (CMS) is a prevalent condition found in higher plants, leading to the impairment of pollen production, thus hindering their reproductive capabilities. Over 150 plant species are known to have stated to possess CMS, either naturally or artificially through mutations, hybridization, protoplasmic fusion, and genetic engineering (Laser and Lersten, 1972; Hanson and Bentolila, 2004; Yamagishi and Bhat, 2014).

When there is an imbalance in the recombination processes in the mitochondrial genome (mtDNA), chimeric open reading frames (ORFs) are produced, which leads to the failure of pollen development in CMS (Linke and Börner, 2005). At the molecular level, the development of CMS can be categorized into mitochondrial DNA recombination and interactions between mitochondrial and nuclear genomes; aberrant RNA editing; and accumulation of toxic protein products (Chen et al., 2017).

There are two types of CMS based on male gamete development viz. gametophytic CMS (Ga-CMS) and sporophytic CMS (Sp-CMS).BT-CMS, LD-CMS in rice, and S-CMS in corn are examples of gametophytic CMS and Fujian cytoplasmic male sterility (CMS-FA) is an example of sporophytic CMS (Toriyama, 2021; Jiang et al., 2022). CMS is maternally inherited in most of the plant species. CMS follows non-mendelian inheritance because it is inherited only through the female parent's cytoplasm and not determined by either parent's gene. (Toriyama, 2021). However, the mitochondrial gene responsible for causing CMS can be suppressed by the nuclear fertility restorer gene (Chase and Gabay-Laughnan, 2004).

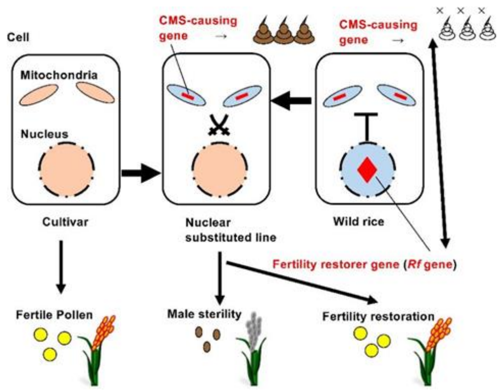

Figure 1: The schematic model illustrating cytoplasmic male sterility (CMS) and fertility restoration depicts the interaction between the CMS-causing gene in the mitochondria and the fertility restorer gene in the nucleus (Toriyama, 2021)

Quick Response Code	Access this article online	
	Website: www.actascientificamalaysia.com	**DOI:** 10.26480/asm.01.2024.21.27

Cite The Article: Sujan Chapagain (2024). A Comprehensive Review On-Application and Future Prospect of Cytoplasmic Male Sterility. *Acta Scientifica Malaysia, 8(1): 21-27.*

CMS has been widely used by plant breeders to produce hybrid seeds in self-pollinating crop species like cotton, cotton, rice, wheat, maize, sorghum, radish, and cross-pollinating species like onion, sunflower, and sugar beet (Havey, 2004). CMS has emerged as a significant boon in the realm of plant breeding and hybrid technology, facilitating enhanced yield stability, reduced costs in seed production, improved adaptability in gynoecious plants, and the development of better agronomic traits (Mao et al., 1998; Gupta et al., 2012; Budar and Pelletier, 2001). This review article explores the application and future prospects of Cytoplasmic Male Sterility (CMS), examining its implications across diverse crops, genetic mechanisms, breeding strategies, and future prospects of CMS on crop productivity and hybrid development.

2. Methodology

This paper emphasizes the application and future potential of cytoplasmic male sterility (CMS) in agriculture. To do so, a comprehensive literature review was conducted utilizing Google Scholar, Scopus, ScienceDirect from Elsevier, and ResearchGate to acquire an in-depth understanding of the subject under review. Various research papers outlining the significance of cytoplasmic male sterility, its application, its advancements in biotechnology, molecular mechanisms, and future prospects were studied.

3. Discussion

3.1 Mechanism

Despite the practical success and progress made in the application of cytoplasmic male sterility (CMS) and restoration fertility (Rf) in genetics and plant breeding, the mechanisms governing these processes remain unclear in scientific research. Numerous research findings indicate that genes linked to cytoplasmic male sterility (CMS) are situated within the mitochondrial genome and are responsible for impairments of pollens, while restorer fertility (Rf) genes, crucial for restoring fertility in F1 hybrids, originate from the nuclear genome (Tang et al., 2017; Budar et al., 2003). Cytoplasmic male sterility mechanism varies from plant to plant. If we look at the deteriorating anthers of a few crops like rice, corn, beets, and radish, practically no proline was present (Ogura, 1968). If there is an insufficient supply of sugars to use the ammonia produced by protein at the time of respiration during the early archesporial stage then the production of asparagine or alanine occurs which disbalances the equilibrium of amino acids, as a result, degeneration and destruction of pollen viability occur (Pearson, 1981). Some researchers have established the genetic foundation of CMS where they stated that interaction between the male sterile cytoplasm and homozygous recessive nuclear gene induce pollen sterility and as a result normal cytoplasm (male fertile) becomes ineffective (Bateson and Gairdner, 1921; Islam et al., 2014). In mitochondrial-encoded CMS systems, male sterility is frequently found to be caused due to programmed cell death of tapetum cells, and increasing the number of mitochondria will be unable to achieve the energy production at the time of pollen formation (Balk and Leaver, 2001). Ultimately, the central concept revolves around the imbalance between the mitochondrial and nuclear genomes, particularly driven by the expression of specific mitochondrial open reading frames (ORFs) genes.

3.2 Application

3.2.1 Hybrid Seed

Seeds produced from cytoplasmic male sterility (CMS) are genetically pure compared to seeds produced through open pollination (Singh et al., 2023). In agriculture, hybrid seed is a vital resource for production enhancements; hybrid vigor or heterosis enhances the tolerating ability of the plant to abiotic and biotic challenges. Along with contributing to higher production, heterosis enables stress tolerance against low soil fertility, drought conditions pests, and diseases (Duvick, 2001). A study conducted on hybrid seed production in wheat (*Triticum aestivum* L.) also shows that the mean grain yield of the CMS-based hybrids was greater than that of the CHA-based hybrids (Adugna et al., 2006). CMS hybrids exhibit a "female advantage" due to either enhanced female reproductive capacity resulting from resource allocation away from male functions or improved seed vitality by reducing self-pollination (Budar et al., 2003).

Cytoplasmic male sterility (CMS) was utilized as the first male sterility system in onion (*Allium cepa*) to enable the production of hybrid varieties (Jones, 1943). The 1st T-Cytoplasm(CMS-7) trait was seen in the Golden June line of Maize in Texas which allowed the use of CMS in maize hybrid production (Rogers and Edwardson, 1952). CMS removed the cost of emasculation for the hybrid seed production. The CMS line is crossed with the nuclear fertility restorer line (rf) for hybrid seed production. In hybrid seed production, the presence of fertility restorer genes is crucial. As a result, the discovery of restoration-of-fertility (Rf) genes in various crops has progressed at a rate similar to the discovery of sterile cytoplasm. It maintains the genomic equilibrium between cytoplasmic (mitochondrial and plastid genomes) and nuclear genomes and enables functional pollen production (Dahan and Mireau, 2013).

Cytoplasmic male sterility (CMS) has been extensively employed in hybrid rice production (Huang et al., 2014). From Studies, it has been concluded that hybrid rice has (15-20%) higher yield as compared to the traditional cultivars (Toriyama and Kazama, 2016). In plants where the effective three-line system can be maintained, Cytoplasmic Male Sterility can be widely used for hybrid seed production. The three-line system represents the A-line, B-line, and R-line which represent seed parents with male sterile genotype, the maintainer line with male fertile genotype, and the restorer line to recover male fertility in hybrid respectively (Jordan et al., 2011). In three-line indica hybrids, the wild abortive (WA) CMS from the wild rice species *Oryza rufipogon Griff*. is frequently used for hybrid production (Guo et al., 2022).

3.2.2 Enhance Agronomic Character and Yield Traits

CMS facilitates the development of improved varieties through controlled breeding. Eventually, it results in an improvement in agronomic traits. CMS maize shows slow senescence of silk. The decelerated senescence of silk in CMS maize cultivars could extend the duration of pollen shedding, thereby augmenting the probability of effective pollination and subsequent seed development (Stamp et al., 2000). Consequently, this phenomenon has the potential to significantly enhance overall crop yield. Even under stress, such as lack of water and nitrogen and high sowing density CMS hybrid maize shows greater yield as compared to conventional variety which shows that CMS can be widely exploited to enhance the growth and yield even in the stressed condition (Bruce et al., 1966; Duvick, 1965). In the case of sunflower, hybrids produced from the cross of the CMS line (CMS-XA × P100R, CMS-ARG-2A × P100R) give better seed and CMS line CMS-E002-91A × P124R, CMS-E002-91A × P100R, and CMS-40A× P124R were the best combiners as for oil content as compared to conventional variety (Tyagi et al., 2018). In wheat (*Triticum aestivum* L.), lines with cytoplasmic male sterility (CMS) showed nearly double the outcrossing percentage rate compared to lines treated with (CHA) chemical hybridizing agents (Singh et al., 2021). In comparison to both the CHA-treated lines, the weight of 1,000 grains weight of cross seeds in the CMS lines was higher. Viable crossbred seeds from CMS lines showed three times higher viability compared to CHA-treated lines, with double the average germination rate. (Adugna et al., 2004). This shows the contribution of CMS in enhancing the agronomic trait of crops as compared to that of chemically hybridized crop lines.

Plus hybrid method is being used in the world which uses cytoplasmic male sterility and xenia i.e. allo-pollination (Vulchinkov et al., 2014). The practice of planting high-yielding CMS-hybrids (90%) along with pollinators (10%) containing genes for larger embryo development has significantly increased both the quantity and quality of oil and protein in maize (Thomison and Geyer, 1999). Incorporating CMS with other agricultural practices holds great promise for enhancing both the quality and quantity of yield. Due to its larger root mass and depth, 1st commercial CMS-based pigeon pea hybrid ICPH 2671 (profuse branching and maturing between 164-184days) quickly bounces back from temporary droughts and shows (88%) survivability against waterlogging conditions (Saxena and Sharma, 2018). Breeding practices should be focused on the manipulation of traits shown by ICPH 2671 to other varieties for better agronomic practices. Maternally inherited CMS doesn't show segregation so uniformity of population can be maintained in simple cross-pollination in a closed system (Singh et al., 2021). CMS lines have been employed to enhance the purity of hybrid cultivars. By employing the CMS line in the breeding program, the purity of the commercial cabbage cultivars "Jingfeng No. 1" and "Qingfeng" was raised by 5-7% in comparison to the self-incompatible lines (Ding and Jian, 2006).

3.2.3 Control Pollination

Before the middle of the 20th century, emasculation was essential for producing hybrid seeds in self-pollinating plants to prevent self-pollination. However, this process required manual labor, machinery, or chemical treatments, making it expensive, inefficient, and potentially harmful to the environment. The issues of self-pollination and emasculation have been resolved with the advancement of CMS technology (Chen and Liu, 2014). Cytoplasmic Male Sterility is an effective tool to control Self-fertilization and allow breeders to enhance hybridization and increase productivity (Singh et al., 2015). CMS removes the cost of detasseling in males (Levings 3rd, 1993). CMS induces male sterility and the Restorer (Rf) gene can restore the fertility of pollen in an F1 hybrid so CMS has been widely used to control pollination and used for producing a hybrid of pearl millet, sunflower, sorghum, sugar beet, wheat, rice and corn more extensively (Williams, 1995). In the case of Brassicaceae, natural sporophytic (SI) has been replaced by the CGMS to control pollination and hybrid production because of the occurrence of self-pollination in some cases (Singh et al., 2013).In the case of carrots, Petaloid CMS is widely used to control self-pollination (Kalia et al., 2019). In conclusion, cytoplasmic male sterility (CMS) plays a crucial role in enabling breeders to effectively control self-pollination, facilitating the development of hybrid plants with desired traits in various crop species.

Table 1: CMS Hybrid Varieties

S.N.	Crop	Predominant Cytoplasm	Hybrid variety	References
1	Rapeseed/Canola (*Brassica napus*)	tour CMS pol CMS mori CMS Ogu CMS	PGSH 51 (1994) Hyola 401 (2000) NRCHB-506 (2008) PAC-432 (2009)	(Chand et al., 2021)
2	Pigeon Pea (*Cajanus cajan* (L.) Millsp.)	………. A2 Cytoplasm A4 Cytoplasm	ICPH 2671 ICPH 3762 (2014) ICPH 2740 (2015) GTH 1 Pusa Ageti, ICPH 2433, ICPH 2438, and ICPH 2383	(Saxena and Sharma, 2018)
3	Mustard (*Brassica juncea*)	126-1 CMS	DMH-1 (2008)	(Sodhi et al., 2006)
4	Cabbage *(Brassica oleracea)*	Ogura CMS -----	Chinese Cabbage (*Brassica rapa sps. pekinensis*) KCH-5 Qiugan No.1	(Dong et al., 2013) (Dhall, 2010) (Dhall, 2010)
5	Wheat *(Triticum aestivum)*	K-CMS	Xinong-901	(Rathburn et al., 1993)
6	Pearl Millet *Pennisetum glaucum*	A1CMS (Tift 23A)	Hybrid Bajra-1 (HB 1), HGM 100 & Tifgrain-102	(Srivastava et al., 2020)
7	Rice *Oryza sativa*	WA-CMS HL-CMS ……	Nanyou-2 & Weiyou 6 Honglianyou6 & Yueyou 938 Pusa RH-10	(Li et al., 2007) (Siddiq et al., 2012)
8	Chilli *Capsicum annuum*	….	Kashi Surkh (CCH-2) &Kashi Early (CCH-3)	(Dhall, 2010)

3.2.4 Disease Resistance

By introducing a dominant gene into a single-parent cytoplasmic male sterility system, researchers have enabled the restoration of genes, thereby allowing the induction of resistance against downy mildew caused by *P. halstedii* in the American Red River race of Sunflower (Zimmer and Kinman, 1972). The parental line CMS E002-92, R-5, and R-77-2 III of Sunflower (*Helianthus annum* L.) were found to be resistant to powdery mildew caused by *Erysiphe cichoracearum* (Nandini et al., 2016). World 1st CMS-based legume hybrid ICPH 2671 is found to be resistant to wilt and sterility mosaic disease (Saxena and Sharma, 2018). *S. stoloniferum* (W/γ-CMS) and *S. demissum* (D-CMS) were obtained from maternal parental accessions of Mexican polyploid species which are widely used in the breeding program as a source of resistance for the Late blight caused by (*Phytopthora infestans*) and Potato virus Y. (Sanetomo and Gebhardt, 2015; Ross and Hunnius, 1986).

CMS aids in facilitating the incorporation of disease-resistance traits into cropsR1-CMS provides pollen control, streamlining the incorporation of disease-resistant genes through backcrossing CMS plants with recurrent plants. This concept has been introduced in CMS Brassica campestris L. germplasms, rendering resistance to Turnip Mosaic Virus (TMV), Downy Mildew, and Clubroot (Leung and Williams, 2022). Through the implementation of Marker-Assisted Selection (MAS), resistance to Bacterial Leaf Blight (BLB) has been incorporated into the parental line (Pusa Sugandh 2 × CMS Line Pusa 6A) of Pusa RH10 (Basavaraj et al., 2010). However, with the scarcity of CMS lines, diversification of both CMS and inbreeding lines is pivotal through diverse crossing programs to mitigate genetic risks associated with reliance on a single cytoplasm. This diversity helps prevent vulnerability to specific stresses, reducing the potential for outbreaks of epidemic diseases.

In conclusion, while cytoplasmic male sterility (CMS) itself does not directly grant disease resistance in crops, its integration into hybrid breeding programs offers a pathway to develop hybrids endowed with both male sterility and disease resistance. Through strategic crossings between CMS lines and disease-resistant varieties, breeders can leverage the genetic mechanisms governed by CMS to enhance traits associated with disease resistance.

Table 2: CMS Hybrid Varieties with Disease Resistance

S.N.	Crop	CMS hybrid	Resistance	References
1	Cabbage	FRCRC	Club root	(Ren et al., 2020)
2	Pigeon Pea	ICPH 2671, IPCH 3672	Wilt and sterility mosaic disease	(Saxena and Sharma, 2018)
3	Sunflower	CMS DV-10 x R-393Br CMS DV-10 x R-5(77A x 72B) x (R-393Br)	Powdery mildew (*Erysiphe cichoracearum* DC.)	(Jondhale and Goud, 2015)
		PRUN 29 CMS Line	Powdery mildew	(Nandini et al., 2016)
4	Pearl Millet	PHB- 10 & PHB-14	Downy Mildew	(Srivastava et al., 2020)
		Tifgrain-102	Root knot nematode and rust resistance.	(Gulia et al., 2007)
5	Maize	ZM477	Maize lethal necrosis (MLN)	(Boddupalli et al., 2020)

PHB-10 and PHB-14 are also known as HB-60 &HB-7 respectively.

3.2.5 Simplified Breeding System

The breeding system has changed a lot with new methods like cytoplasmic male sterility (CMS), making it simpler. Cytoplasmic male sterility has a great prospect in the case of forage plants and even the cost of fertility restoration isn't required because the major concern of breeding in the forage plant is the production of biomass (Ruge et al., 2003). Gahi-3 (Tift 23DA1 × Tift 186) was the 1st CMS-based forage hybrid released in 1972 A.D. after the discovery of A1 CMS in pearl millet (Serba et al., 2017). However, as the breeding system prioritizes increasing biomass, there is a consequential loss of seeds, leading to the imminent extinction of landrace varieties.CMS was introduced via wide hybridization in diverse species including cruciferous crops and perennial ryegrass (Wit, 1974). Ogura CMS is extensively utilized in cruciferous crop breeding owing to its notable benefits, such as complete pollen abortion and a high progeny sterility rate, reaching up to 100% (Ren et al., 2022). For crops where seed production doesn't play a vital role, CMS-based breeding can be quite effective for that process. In Cauliflower (*Brassica oleracea)* it is not necessary to maintain a restorer line (Rf) because the curd (intermediate stage) in this situation is an edible component (Kumar et al., 2020). Thus, the cytoplasmic male sterility mechanism works best for the hybrid development of cauliflower. CMS makes breeding easier by providing hybrid seeds in self-pollinating crops, saving money on emasculation and reducing the time needed for good yields.

4. CMS Limitation and Instability

CMS is highly useful for plant breeders, yet it's crucial to recognize its limitations. The commercial viability of a CMS system depends on how consistently male sterility is maintained across different genetic backgrounds and environmental conditions (Yadav and Rai, 2013). Environmental factors such as high humidity in pearl millet, cold weather during panicle development in maize and pigeon pea, and scorching temperatures of 42°C and higher in sorghum all affect CMS-S's fertility stability (Reddy et al., 2004). The instability of CMS is regulated by multiple minor quantitative trait loci (QTLs), and the complete molecular mechanism behind this genetic modification is still totally understood (Su et al., 2016). Research is needed to fully understand the interaction between environmental factors and genetics in CMS-based hybrid production.

Not all crop species naturally possess CMS mutations or the necessary fertility restoration genes. There are limited alternatives for fertility-restoring genes across various crops (Liu et al., 2002) which limits the possibility for the screening of strong heterosis combinations (Guo et al., 2006). In the absence of a fertility restorer gene, the flower of the cytoplasmic sterile parent must attract pollen vectors or possess highly adhesive stigmas to catch wind-carried pollen for fertilization. Currently, PET1 (CMS Source) and Rf1 are the only fertility restoration (Rf1) genes used in commercial hybrid seed production in sunflowers that result in a lack of genetic diversity. This makes commercial sunflower seed production susceptible to environmental challenges and diseases (Talukder et al., 2019). In the 1970s, breeders heavily depended on "CMS-T" cytoplasm for maize hybrid seed production. However, a natural mutation in a fungus led to Southern Corn Leaf Blight, affecting CMS-T maize and resulting in the destruction of 80% yield loss, costing US$1.0 billion (Bruns, 2017). Events like this underscored the narrow genetic base, emphasizing the need to prioritize the development of a stable and resilient CMS.

For CMS hybrid development, the parental lines should be kept as genetically pure as possible (Saxena and Sharma, 2018). Though CMS hybrids of soybeans have been developed large-scale hybrid seed production is difficult due to poor pollen migration from the male to the female (Palmer et al., 2011). Claviceps fungus particularly hinders the development of pollen tubes through the stigma. (Tenberge, 1999). 2/3rd of the hybrid cultivars of rye grown in Germany exhibit the Pampa-type CMS and it is hard to restore pollen fertility genes in Pampa-type CMS as a result commercial CMS hybrids are more susceptible to ergot than pollinated varieties because the absence of pollen results in a prolonged duration of a flower opening (Geiger and Schnell, 1970; Miedaner et al., 2010). This leads to increased secondary growth of the pistil, thereby elevating levels of Ergot infection. Nevertheless, it cannot be denied that the prospects for CMS in breeding are promising.

5. CMS Advancements and Future Prospects

Cytoplasmic male sterility (CMS) holds significant promise as a breeding tool in modern agriculture. Modern omics methods like genomics, proteomics, and metabolomics offer vast potential for enhancing CMS-based hybrid breeding and advancing hybridization techniques (Mahmood et al., 2022). Due to restoration and maintenance of fertility, cytoplasmic male sterilities (CMS) are preferable to mendelian male sterilities to create hybrid seeds. Furthermore, CMS that have been naturally chosen are less likely to exhibit characteristics that impair seed production (Budar and Pelletier, 2001). Thus, it can be concluded that CMS will play a significant role in hybrid seed production soon.

Biotechnological advancements, particularly protoplast fusion, are extensively utilized to develop new cytoplasmic male sterility and enhance the performance of existing CMS. CMS can be transferred from one species to another species through protoplast fusion and it is already reported in petunia and nicotiana (Izhar and Power, 1979; Zelcer et al., 1978). Similarly, protoplast fusion has been employed in Brassica species to induce cytoplasmic sterility, such as transferring B. napus polima CMS into B. oleracea, sterile cytoplasm of *Diplotaxis muralis* in B. juncea (Yarrow et al., 1990; Chatterjee et al., 1988). Protoplast fusion has the potential to create new alloplasmic male sterile lines and cybrids which enhance the transfer of CMS from one source to superior breeding lines (Khan et al., 2015). This allows for the transfer of male sterility traits between plant species, facilitating the development of novel breeding lines with desired traits. Examples include regenerating rice plants from both indica and japonica protoplasts, utilizing protoplast fusion to generate cytoplasmic hybrids (cybrids), and transferring cytoplasmic male sterility (CMS) into elite breeding lines (Brar et al., 1994).

Genetically diverse CMS sources have been identified, such as CMS-ARC and CMS-Oryza perennis at IRRI, and CMS-Kalinga in India aimed at overcoming potential genetic vulnerabilities in hybrids but breeders should prioritize diversifying the sources of CMS across various crops (Virmani, 1994). Breeders must diversify the sources of CMS to mitigate the risk of abiotic stress, biotic stress catastrophic losses, such as those experienced in the 1970s due to *bipolar maydis*. Although *T. timopheevi* cytoplasm can induce male sterility in wheat, commercially available CMS-based wheat hybrids are not yet available, although research in this area is ongoing. Nevertheless, the identification of restorer genes like Rf1 and

Rf3 holds promise for overcoming these challenges and maximizing the potential gains of F1 hybrids (Brownfield, 2021).

According to UNO global population is likely to from which is expected to reach between 8.3 to 10.9 billion by 2050 (Falcon et al., 2022). As the population continues to grow at an alarming rate, the demand for food increases accordingly, so breeding programs should primarily focus on creating enhanced varieties with superior qualitative and quantitative characteristics. Therefore, it's imperative to evaluate various cytoplasmic male sterile (CMS) lines and restorers for their combining ability to harness heterosis in crops like wheat, maize, rice, etc. Scientists have investigated novel approaches, such as employing synthetic CMS genes, to generate third-generation hybrid rice varieties (Song et al., 2021). This aims to increase the efficiency of hybrid seed production and further explore the phenomenon of heterosis. CMS genes induce male sterility in hermaphroditic plants within gynodioecious populations, causing these plants to develop as females while rendering hermaphrodites incapable of producing viable male reproductive structures (LEWIS, 1941). Utilizing CMS genes to produce female-only plants in hermaphroditic species will simplify production and enhance yield in fruit-focused crops.

Wild abortive-type CMS (WA-CMS) is extensively utilized in China for producing a significant proportion of three-line rice hybrids. Development of all three lines (A Line, B Line, and R Line) takes a lot of time, money, and effort. To rectify the cytoplasmic abnormality of the A-line, the Perfect R line is essential for restoring fertility in hybrid plants so a two-line breeding system is currently being explored all over the world (Jordan et al., 2011). Using NMS two-line system hybrid has been explored in rice (Chang et al., 2016). Compared to hybrids produced using the CMS three-line breeding method, those created with the 2-line approach exhibit a 5–10% higher grain yield (Kim and Zhang, 2018). The two-line breeding system, employing NMS (Nuclear Male Sterility), is poised to supersede the three-line system as it eliminates the need for male sterile cytoplasm, thereby averting cytoplasm homogeneity issues and disease outbreaks during hybrid production (Chen et al., 2019).

There is immense potential for CMS to develop crops that are more resilient to climate change, pests, and diseases. By incorporating multiple parents, such as A-line, B-line, and R-line, each with resistance to various stresses and diseases, along with agronomic trait enhancements like deeper roots and wider coverage, crops can better adapt to climate stress, drought, acidity, and salinity. While cytoplasmic male sterility (CMS) is being investigated, many crops lack usable CMS and fertility restoration systems for hybrid variety development. In the future, it's essential to develop functional male sterile lines through genetic engineering. In such instances, techniques like heterologous gene expression or CRISPR-Cas gene editing can be utilized to manipulate genes responsible for male gamete development, thereby creating male sterile lines.

6. Conclusion

CMS provides a significant advantage to breeding efforts. CMS revolutionized hybrid seed production in various crops, enhancing yield, agronomic traits, disease resistance, and breeding efficiency. However, limitations like instability and genetic vulnerability persist. Future prospects lie in leveraging omics technologies, and CRISPR-Cas gene editing techniques to develop stable CMS hybrids. Conduct multilocation, multiyear experiments for robust performance assessment across diverse environmental conditions. Diverse CMS sources with adopting novel breeding approaches are the global demand to address food security challenges amidst climate change. Further research and comprehensive studies should be conducted to fully explore and exploit the potential of cytoplasmic male sterility, thereby paving the way for its widespread application. In conclusion, cytoplasmic male sterility holds immense promise in revolutionizing agricultural practices and crop breeding, offering unparalleled opportunities for enhanced yield, hybrid vigor, and sustainable food security in the face of escalating global challenges.

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